A longitudinal study of long-term *Escherichia coli* colonisation of the elderly bladder

Lauren Kathryn Letitia Drage (BSc, MRes)

Thesis submitted for the degree of Doctor of Philosophy

Institute for Cell and Molecular Biosciences

Faculty of Medical Sciences

Newcastle University

December 2016

Abstract

Urinary tract infections (UTIs) are the second most common cause of infection worldwide, making them a huge economic burden. People can carry diagnostic loads of bacteria within the bladder without experiencing any symptoms, this is asymptomatic bacteriuria (ABU). ABU prevalence increases with age, with around 19% of those over 60 being susceptible. ABU can reactivate sporadically, causing symptomatic episodes, known as recurrence. Current treatment is with antibiotics, either short courses or prophylaxis. Guidelines state to only treat symptomatic episodes. However, UTI symptoms are often diffuse and unclear, especially in older people. Thus, ABU is inappropriately treated in up to 52% of cases, encouraging antibiotic resistance. Better ways of discriminating between symptomatic and asymptomatic cases are needed. This would allow for more efficient treatment and patient management.

To improve understanding of ABU, a longitudinal clinical pilot study was performed. For 6 months, every 2 weeks a urine sample and symptom questionnaire was collected from 30 patients over 65 who were clinically diagnosed with recurrent UTIs. The aim was to analyse potential changes in the host response and the colonising bacteria around periods of symptoms to see if distinct urinary profiles could be seen between symptomatic and asymptomatic states. Allowing for analysis into potential predictive biomarkers for symptoms. Uropathogenic *Escherichia coli* (UPEC) is known to cause the majority of UTIs, thus was the bacterial focus for this project.

The study firstly allowed us to test the feasibility of such a demanding study design. It was possible to fully recruit to the study with all 30 patients completing its entirety, thus suggesting such a design could be repeated or scaled up in the future. The study produced a large database of bacterial isolates as well as urine samples. Urines were measured for a wide range of immune response proteins. Despite varying levels of immune activation, UPEC was able to evade host-defences and thrive in the bladder long-term. The study showed that ABU patients can carry significant bacterial loads of UPEC, even with antibiotics, questioning the advantages of prophylactic treatment of ABU patients. No distinct host or bacterial profile was identified between symptomatic states. No biomarkers could be seen to predict symptomatic episodes in these patients. However, what was observed was an unexpected level of dynamic variability within individuals and across the patient cohort.

Acknowledgements

This project has been very interesting and kept me very busy for the last few years. Its success and smooth running is in no small part down to the collaborative effort of an incredible team of people who have taken their time to make it possible. This includes the urology research team at the Freeman Hospital, who guided me during every stage of the study. A huge thank you must go to all the study participants who went to incredible efforts to take part in this demanding research study. Their kindness and eagerness to help in the name of research was inspiring and without them this whole project would not have been possible. Thanks must also go to my supervisory team, Rob Pickard and Judith Hall, whose expertise and experience in different fields of research gave me great support for all aspects of the project and analysis. Special thanks goes to my main supervisor, Phil Aldridge, for being a patient and invaluable mentor and friend throughout this PhD.

I would like to thank my incredible network of colleagues, friends and family, who have provided ongoing support and fun, making the last few years an absolute pleasure. Special thanks go to Tom and David P whose absolute insanity kept me sane in the lab. Thanks to David W, David F, Calum, Charles, Caitlin, Amber, Adam, Anthony, Anna and Jonny who have kept me in Pinot Grigio and top quality banter over the years and have the amazing ability to make me smile during the best and worst times, especially during the writing of this thesis. I feel honoured to have made such amazing friends. Huge thanks to Scott who has provided unfaltering support and patience for the entire journey and more. Finally, I want to thank my parents who are two amazing people who have been my absolute rocks for the last 27 years and are truly responsible for getting me to where I am today.

All of these people and many more I don't have room to mention are responsible for making the entire experience a great pleasure, which I will truly treasure forever. Thank you all, so much.

Contents Page

List of Figures

List of Tables

List of Abbreviations

ABU	Asymptomatic Bacteriuria
ACB	Antibody-Coated Bacteria
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
CFU	Colony Forming Units
CRF	Case Report Form
DAEC	Diffusely Adherent *E. coli*
DNA	Deoxyribonucleic Acid
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
ELISA	Enzyme-Linked Immunosorbent Assay
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
ExPEC	Extraintestnal *E. coli*
GCP	Good Clinical Practice
H_2SO_4	Sulphuric Acid
HCP	Health Care Professional
HRP	Horseradish Peroxidase
IBC	Intracellular Bacterial Community
IFNγ	Interferon Gamma
IL	Interleukin
JRO	Joint Research Office
kDa	Kilodaltons
LPS	Lipopolysaccharide
LUTS	Lower Urinary Tract Symptoms
MALDI-TOF	Matrix-Assisted Laser Desorption/Ionization Time-of-Flight

MLST	Multi Locus Sequence Typing
NCBI	National Center for Biotechnology Information
NF-κb	Nuclear Factor-Kappa B
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMEC	Neonatal Meningitis-associated *E. coli*
NuTH	Newcastle upon Tyne Hospitals
PAMP	Pathogen-Associated Molecular Pattern
PCR	Polymerase Chain Reactions
PFGE	Pulsed-Field Gel Electrophoresis
PRR	Pattern Recognition Receptor
R&D	Research and Development
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
ST	Sequence Type
TLR	Toll-Like Receptor
TMB	3,3′,5,5′-Tetramethylbenzidine
TNFα	Tumor Necrosis Factor Alpha
UPEC	Uropathogenic *E. coli*
UTIs	Urinary Tract Infections

Chapter 1. Introduction

Infectious diseases are one of the largest threats to human survival. With an uncertain future surrounding antibiotic resistance, it is now more than ever imperative to understand the causes and effects of such infections. Better understanding of the pathogens causing infection; how they are interacting with the human body and the mechanisms they employ to survive can allow us to develop better tools to manage such infections. This may be in the form of more personalised treatment regimes, shorter or more effective treatment options and possibly a more sustainable way of treating the underlying disease and not just the appearance of symptoms.

Urinary tract infections (UTIs) are the second most common form of infectious disease in humans and can be fatal [1]. This places a huge economic and social burden on health care providers and funding bodies. UTIs are estimated to cost the UK alone up to £70 million annually [2]. Antibiotic treatment associated with UTIs is therefore responsible for a huge number of prescriptions. This has led to the development of extremely dangerous multi-drug resistant organisms which are almost impossible to treat with the treatment options currently available [3]. This pool of hard-to-treat pathogens is growing and will continue to do so, making it imperative to develop a better understanding of the mechanisms behind this type of infection so as to develop better and more efficient methods of treatment and hopefully eradication.

1.1. Urinary Tract

The function of the urinary tract in mammals is collection, storage and periodic release of urine. This leads to waste metabolites and toxic products produced by the body being eliminated in an efficient and co-ordinated manner [4,5].

1.1.1. Physiology

The urinary tract can be split into two main regions, the upper and lower urinary tract. The upper urinary tract comprises of the kidneys and the ureters, a healthy individual will have two of each. These lead into the lower urinary tract, which is made up of the urinary bladder and urethra (Figure 1A). The kidneys filter the blood continuously, removing any toxic metabolites and excess water, in order to maintain homeostasis [6] (Figure 2). Homeostasis is a

mechanisms the body uses to monitor the body's internal status and make adjustments via a series of feedback mechanisms in order to maintain optimal equilibrium and preserve stability, this occurs on the organ level all the way down to the cellular level [7,8]. The blood undergoes glomerular filtration in the Bowman's capsule, retaining any molecules greater than 68 kDa in size (Figure 2). This will leave the main components of urine, which include uric acid, calcium oxalate, calcium phosphate and myoglobin, in the filtrate [9]. The filtrate then goes through homeostatic reabsorption and secretion steps in the proximal and distal tubules of the kidney, dictated by the hydration status of the individual. The urine then drains out of the kidney via the ureters into the urinary bladder [9] (Figure 2).

The urinary bladder is a sack-like organ which can expand to store the urine as it drains in. The average bladder can hold a maximum volume of about 300-400ml [10,11]. Upon filling the bladder will trigger a sequence of signals which alerts the host to urinate. These signals cause the host to void the bladder by micturition via the urethra [12]. There is always some urine left in the bladder after voiding, this is known as post-void residual volume, this is usually around 50ml in healthy people [13].

Normal and Abnormal Urinary Tracts

The normal physiology of the urinary tract can be compromised in some individuals. These are known as abnormal urinary tracts and can be structural or functional abnormalities, both of which can have associations with UTIs [14]. Structural abnormalities could include pelvic-organ prolapse and prostatic enlargement. Vaginal prolapse, for example, is when the wall of the vagina and base of the bladder descend within the patients pelvis, this can be a result of damage to the supporting connective tissue [12]. The prostate is the organ responsible for semen production in males (Figure 1C). This can often become enlarged over time as a man ages [15]. Neurogenic bladder and vesicoureteral reflux are examples of functional urinary tract abnormalities [12,16]. Incidence of many urinary tract abnormalities increases with age [12].

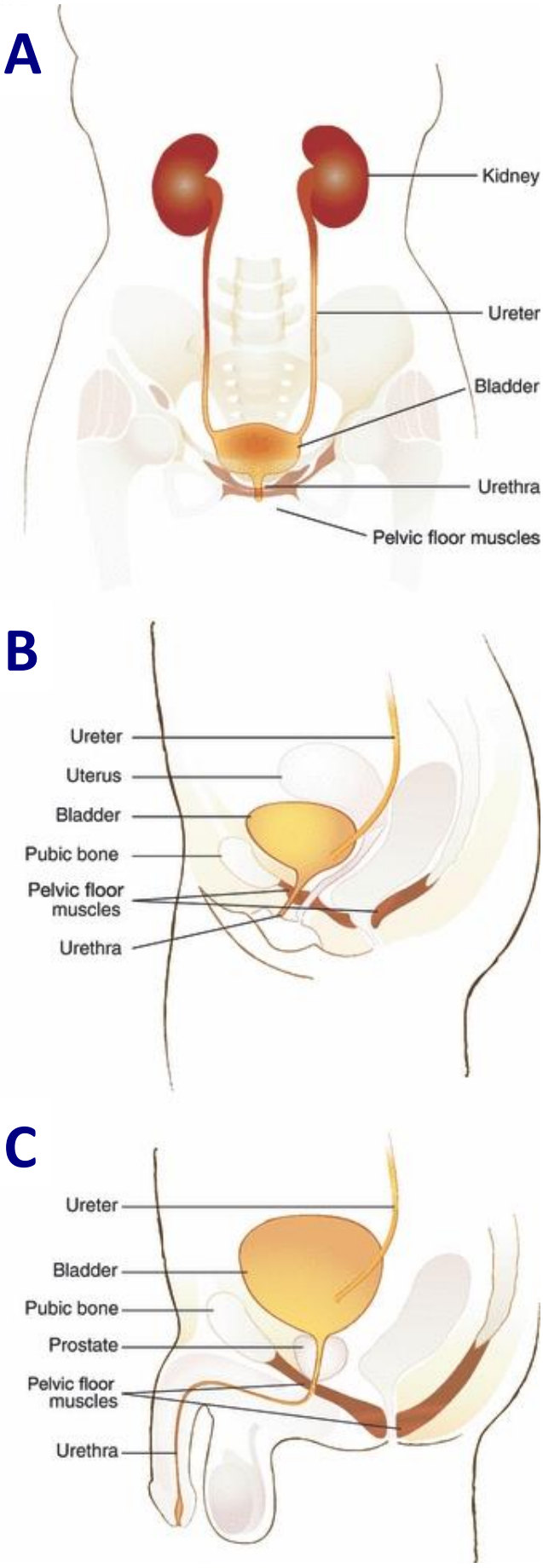


Figure 1. Physiology of the human urinary tract in front view (A). Lateral views of the urinary tract of a female (B) and a male (C). Figure from Lukacz *et al* (2011)[17].

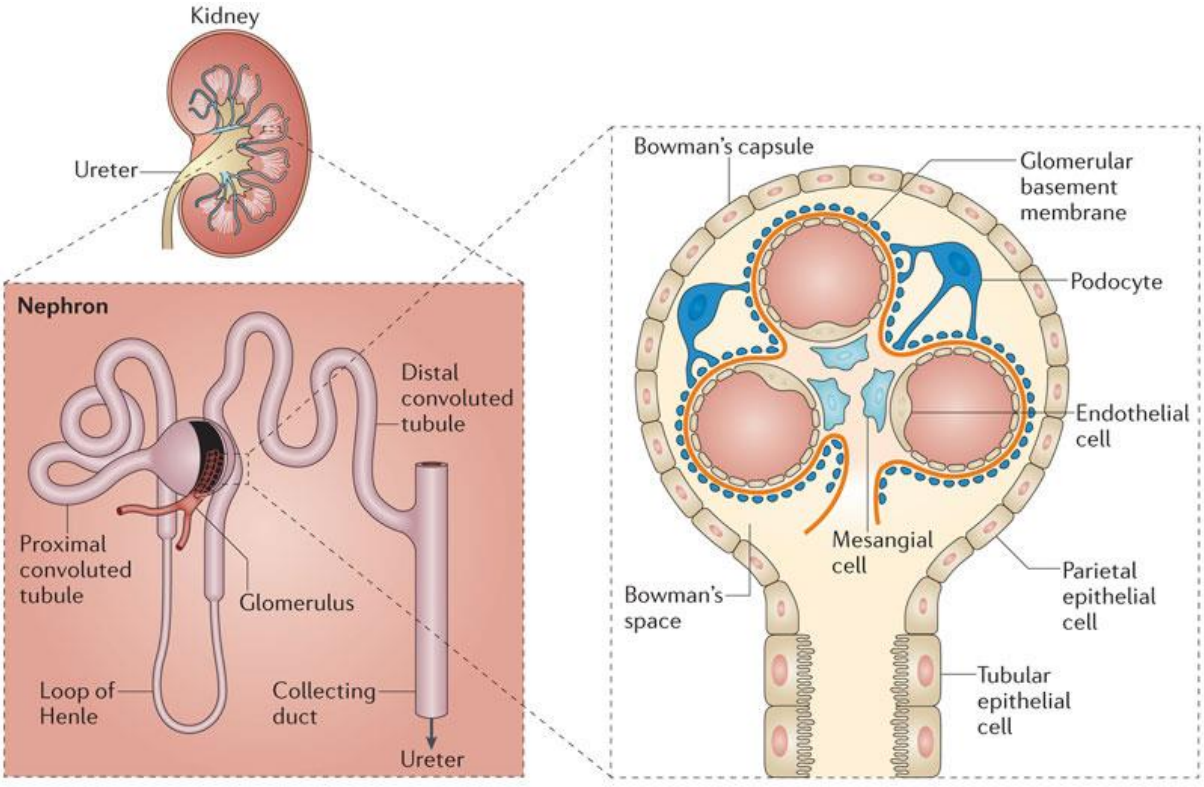


Figure 2. Physiology of the human kidney at varying magnifications [9].

1.1.2. Disorders and diseases

The wall of the bladder contains smooth muscle tissue which aids with urine voiding. As with any muscle, this can weaken as a person ages, this weakening is a common cause for lower urinary tract symptoms (LUTS) [17,18]. LUTS can include higher frequency of urination, more urgency to pass urine and passage of a lot more urine in the night than usual [19]. Other common urinary tract symptoms which can be difficult to diagnose include pain when passing urine (dysuria) and blood in the urine (haematuria), which can be signs of infection or a far more serious underlying urinary condition, such as bladder cancer [1,20].

The majority of painful urinary symptoms are associated with inflammation of the urinary tract. Inflammation of the kidneys is known as pyelonephritis and inflammation of the bladder is known as cystitis [21]. One of the main causes for pyelonephritis and cystitis is infection, which will be discussed in detail later. However, there are other causes of urinary tract inflammation that are non-infectious. For example, interstitial cystitis is defined as more than 6 weeks of pain or pressure felt to be related to the bladder, in the absence of an infection [17]. Diagnosis of non-infectious cystitis, can often be very difficult due to confusion around the definition. However, it is estimated to occur in up to 2.7% of women and 1.2% of men over 30 years of age [17]. Some causes can include medication, radiation and issues with the autoimmune response [22].

1.1.3. Microbiome

The bladder has been widely accepted to be sterile until relatively recently [23]. This was due to the lack of sensitivity in the culture method of isolating bacteria. Recent developments in sequencing technologies, such as 16s rRNA sequencing and metagenomic sequencing, have provided much more sensitive means to identify pathogens within the urine [24,25], these will be discussed in more detail later. It is now known that there are a number of organisms which make up the normal microbiome of the bladder in healthy individuals, including several *Lactobacillus*, *Prevotella* and *Corynebacterium* species. The identified microbes have covered a wide range of bacterial phyla, including *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. These organisms reside in the bladders of healthy people with no urinary symptoms, thus are able to survive in the urine without triggering an immune response [24,25].

1.1.4. Infections

UTI occurs when a pathogen, which is not usually part of the normal bladder microflora, establishes itself within the urinary tract. The location of the pathogen in the urinary tract defines its classification. As discussed cystitis and pyelonephritis are the terms given for infection of the bladder and kidneys, respectively. In general, the higher up the urinary tract the infection is found the more serious the disease, because the infection may be causing damage to vital organs. If left untreated or unsuccessfully treated the pathogen can ascend the urinary tract from the bladder to the kidneys and in some serious cases it can reach the blood, this is known as bacteraemia [26,27]. Bacteraemia is a serious problem, especially in older people, in whom 30% of cases are fatal [1].

Upon infection, the normal mechanism of action would be for the invading pathogen to be recognised and an immune response raised in order to clear it from the bladder. This will be discussed later on in more detail. The innate immune response leads to inflammation, which is the main cause of most UTI symptoms.

Symptoms of UTIs

UTIs can be very uncomfortable for a sufferer and have significant impact on their quality of life. The most common UTI symptoms include dysuria, frequency and urgency [18,28]. Several of the previously mentioned LUTS are also symptoms of urinary tract infection, which can make distinguishing between the symptoms of normal ageing and infection complicated, especially in older people [18,29]. In addition to LUTS, other potentially misleading symptoms which may or may not be UTI-related in the elderly include fever, incontinence, confusion and lower back pain [1,20,30]. However, the root cause of symptoms in infection is different to the causes of LUTS, which are caused by the general weakening of the urinary tract [18]. UTI symptoms are caused by the host producing immune response molecules in reaction to recognising the infection leading to inflammation of the urinary tract, as previously discussed.

Asymptomatic Bacteriuria

Some infections are able to persist in the bladder for long periods of time. In some cases, this will cause ongoing UTI symptoms, this is the cause of acute cystitis for example. These patients will require regular antibiotic treatment, possibly even prophylactic treatment in order to clear the infection and keep the inflammatory symptoms away. However, in some patients the symptoms can clear without the clearance of the bacteria from the bladder. A

patient who has a significant load of bacteria in the urine but no current symptoms of a UTI is known to have asymptomatic bacteriuria (ABU) [26,31]. ABU is a commensal-like dormant state of the infection, where the bacteria is able to thrive in the urine, seemingly without causing any ill effect to the host. ABU is estimated to occur in 3.5% of the population worldwide and this increases to 19% in the elderly [1,26].

Recurrence of UTIs

In some cases, ABU patients will suffer what is known as recurrence. Recurrence is when the infection sporadically triggers episodes of symptomatic infection. The definition of recurrent UTI is a patient who has suffered from at least 2 episodes of symptomatic infection in 6 months, or 3 episodes in 12 months [13]. Recurrence of cystitis within 6 months is also estimated to occur in around 25% of cases [32]. This is understood to be either caused by the bacteria persisting in the bladder and reactivating sporadically or by infection with a new pathogen [26,33]. These episodes can be very frequent in some patients, however in others, they can be many months apart. The current course of treatment is to simply treat these episodes as and when they occur. It is not understood what causes the switch from ABU to these symptomatic episodes of infection.

Treatment of UTIs

The current treatment for symptomatic UTIs is with a course of antibiotics, either as a short course or as a long term prophylactic course aimed at preventing recurrence [34]. First line treatments for UTIs are nitrofurantoin and trimethoprim due to their narrow spectrum of action [28,35]. If these do not succeed susceptibility testing of the infecting pathogen is essential [28,35]. Trimethoprim is suitable for use in both upper and lower UTIs, whereas nitrofurantoin is not suitable for upper UTIs, as it does not reach sufficient concentrations in the kidneys [28,35]. Elderly patients also have a higher risk of toxicity from nitrofurantoin, so pivmecillinam is another option for patients with lower UTIs [28]. Co-amoxiclav is also suitable to treat upper UTIs, however the guidelines are moving away from recommending the use of co-amoxiclav to treat UTIs, as well as cephalosporins and quinolones, due to their broad spectrum modes of action increasing the risk of antibiotic resistant pathogens [28,35]. However, in cases of multi-drug resistant organisms, fosfomycin can be used. This has only relatively recently been licenced in the UK, and is kept for special cases where other drugs can be shown to be ineffective, inappropriate or unsafe, for example in pregnant women [28,36–38].

National Institute for Health and Care Excellence (NICE) guidelines state that ABU should not be treated with antibiotics, without symptoms treatment is not advised [28,39–41]. However, due to the condition being poorly understood it is often inappropriately treated with antibiotics [42].

Inappropriate treatment is usually due to the lack of reliable definitions for diagnosing a UTI. Significant bacteriuria causing a UTI is defined as $>10^5$ colony forming units (CFU) of bacteria per millilitre of urine [28,31]. However, this does not take into account whether the patient is actually suffering from symptoms. Thus, patients may go to the doctor with signs such a peculiar smell to their urine, and proceed to being given antibiotics based on a positive urine sample. This is even more of a problem in older patients as there is a high likelihood of finding bacteria in the urine of healthy elderly people and, as previously mentioned, older people often suffer diffuse UTI-like symptoms without UTI being the cause [1]. Thus, a positive urine sample lacks the necessary specificity to distinguish between ABU and symptomatic UTI [1].

This lack of understanding and inappropriate treatment is especially worrying given the growing concern over antibiotic resistance to the public health system. Antibiotic resistances reduce the available arsenal of defence a clinician can use to treat an infection when symptoms occur [43–46]. It is therefore essential that the accuracy of targeted treatment is increased. This is particularly important in the elderly, who can face many other complications during treatment. Treatment with antibiotics can in some cases have the opposite of the intended helpful effect. As well as the potential development of resistances, patients can suffer adverse reactions to antibiotics [30]. In addition, treatment with antibiotics lowers the host's natural defences and can remove body's natural microflora allowing for niches where opportunistic infections can take place [47]. For example, *Candida albicans*, the causative agents of thrush, can often colonise a patient being treated with antibiotics for a UTI [48,49]. Another argument against treating these patients is that the presence of asymptomatic bacteria in the urine can be protective [50]. Bacterial presence can prevent, or significantly delay superinfection by a new pathogen, compared to ABU patients who have treatment to eliminate the bacteria [31,51–54]. This suggests that guidelines need to be clearer about the management of ABU patients, however more knowledge about what is actually occurring in the bladders of these patients is needed.

Prevalence

With over 50% of women alone suffering from at least one in their life, UTIs are reported to be the second most common form of infection worldwide [14,55,56]. An estimated third of these women will go on to suffer a recurrent infection within a year [26,57]. It is far less common in men, with only 12% being affected [58]. This is due to the fact that bacteria travelling from the gut to the bladder via the urethra have much further to travel in order to reach the male bladder than the female bladder [59,60] (Figure 1B and C).

It is estimated that there are as many as 150 million cases every year of UTI reported in the UK alone [61]. UTIs are responsible for up to 3% of all GP visits in the UK [61]. Whilst everyone is at risk of contracting UTIs, there are subgroups in the population who are more susceptible, such as patients with diabetes, catheterised individuals, paraplegic patients and people over the age of 65 years of age [26,62]. The risk of UTI increases with age, which means that the associated mortality also increases with age. The risk is increased further if patients are living in nursing homes and extended care facilities or having to be hospitalised long-term [63]. Such high global prevalence makes it a huge economic burden of healthcare providers worldwide.

Cost impacts

The average cost of a patient visiting a health care professional (HCP) for a UTI complaint, whether an initial visit or a follow-up, is around £31.62 [2]. When scaling this up based on the reported estimate of ABU affecting 19% of the elderly population, this equates to costs of up to £70 million per year in the UK alone [1,26]. This information alongside the serious detriments to sufferers' quality of health demonstrates the huge social and economic burden of this disease on the country and health care providers. Days off work are less relevant within the elderly population, however, may still contribute to the economic stress for anyone in this age bracket still in employment [2].

1.2. Diagnosing UTIs

Over the years, several different techniques have been used to diagnose and aid the management of patients suffering from UTIs, both in the clinic and clinical laboratories. An overview of the advantages and disadvantages of several diagnostic microbial techniques is given in Table 1 (p.29) as a reference, a number of these will be discussed here in more detail. Diagnostic techniques have developed over time from the basic dipstick analysis, urine culture and blood analysis as technological advancements have been made, to techniques such as forms of mass spectrometry and strain sequencing. Due to lack of understanding

around ABU, much of the inappropriate antibiotic treatment is due to downfalls in the current diagnostic techniques and guidelines that surround UTIs.

1.2.1. Traditional Diagnostic Techniques

Despite technological advancements, several traditional diagnostic techniques are still used in clinical practice due to their sensitivity, cost-effectiveness and in some cases speed of outcome. The urinary dipstick is a very cheap and fast method of getting a general overview of a patient's urinary health. Urine culture is widely used and provides a relatively cheap and sensitive method of urinalysis (Table 1).

Dipstick

A large amount of basic information can be collected by performing a simple dipstick analysis on a urine sample. Dipsticks in use currently within health care settings can measure several urine parameters at once. For example, the National Health Service (NHS) use Multistix® 10SG (Siemens), which measure glucose, bilirubin, urobilinogen, specific gravity, ketones, protein, pH, nitrites, leukocytes and both haemolysed and non-haemolysed blood within the urine.

Glucose is found at low levels in normal healthy individuals (up to 25mg/dL), however increases above this can indicate elevated glucose levels within the plasma or impaired glucose reabsorption within the kidney. The most common reason for glucose within the urine above the normal level is due to poorly controlled diabetes mellitus [64].

Bilirubin and urobilinogen are markers of liver function and appearance within the urine can indicate abnormalities [65]. Specific gravity is an indication of a person's hydration status as well as the functioning health of their pituitary gland and kidney. Continuously elevated specific gravity could be caused by high levels of solute within the urine, potentially indicating impaired blood filtering due to kidney damage [65]. Ketones and protein within the urine can also be markers of impaired kidney heath and can also indicate the presence of bacteria in the urine [65].

Higher levels of nitrites, leukocytes and pH within the urine are all indicators of bacterial colonisation [65]. Presence of organisms which are able to break down urea within the urine can be responsible for an elevated urinary pH, such as *Proteus* species [65]. Bacteria present in the urine are able to reduce nitrate into nitrite, thus a positive nitrite outcome on a urinary dipstick usually suggests the presence of bacteria within the urine [66]. A positive leukocyte

outcome indicates the presence of leukocyte esterase within the urine. This is an enzyme produced by the host's white blood cells in response to inflammation, usually indicating infection within the urine [66]. The presence of both nitrites and leukocytes within the urine have been widely accepted to be highly indicative of a UTI [2,18,67,68], with a predictive power of around 80-92% [28,35].

Blood in the urine could suggest many different things, the patient may be suffering with calculi or tumours within the urinary tract, trauma of tract epithelium or a UTI [65]. Some urinary pathogens have haemolytic activity, such as some strains of *E. coli* and *Streptococcus* species [69,70]. Thus, a high level of haemolysed blood within the urine could suggest their presence within the bladder.

Urine culture

Laboratory analysis of cultured urine is the gold standard method of checking for UTIs. In the NHS this currently involves growth of the mid-stream urine sample on chromogenic agar to allow the growth of conventional aerobic pathogens [28]. Quantifying the load of pathogenic growth within urine is essential to deduce whether the bladder is colonised or the sample is merely contaminated. This differs from blood culture, for example, where the presence of bacteria at any load is a biomarker of infection due to the unlikely nature of blood sample contamination [71]. Whilst urine culture does provide a sensitive and relatively cheap method of urinalysis, it is not an especially fast technique (Table 1). Upon identification of uropathogens at diagnostically relevant loads, it is possible to complete antibiotic sensitivity testing [72]. In the NHS this is completed using the disc diffusion assay technique [73,74].

Blood Analysis

Clinicians will often use blood tests to gain information on a patient's general health. Certain blood parameters can be measured to better understand the functioning of the kidney and urinary tract, which cannot otherwise be deduced from the urine. Serum creatinine is a blood marker of kidney filtration. Elevated serum creatinine concentration can suggest impaired blood filtering by the kidney [75]. Other blood parameters shown to indicated kidney filtration function are sodium, potassium and urea [76–78]. Vitamin D is another marker which can be measured within the blood. Some evidence has shown that deficiency in vitamin D can lead to a higher risk of developing recurrent UTIs [79–81].

1.2.2. Modern Diagnostic Techniques

Advancement in technologies have allowed certain aspects of diagnostics to become more efficient and more targeted. Many techniques have also been streamlined to make them faster and in some cases more cost effective for healthcare providers. An example of such technology would be sequencing, which has become vastly cheaper and more accessible over the past 30 years.

MALDI-TOF Mass Spectrometry

Until relatively recently the proteomics field of strain differentiation was based mainly on time consuming gel-based assays. These are not ideal for the high throughput and time-critical nature of a clinical diagnostic environment. Mass spectrometry is a relatively new technique used for identifying ionised molecules to solve molecular compositions. However, this technique was limited to very small molecules, mainly chemicals [82]. The development of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry technique allowed for the identification of larger molecules, such as proteins [82]. The technique involves 'soft-ionisation' so as not to destroy or alter the sample. The technique can be used on whole organisms in order to identify them within a sample. MALDI-TOF is a technique used to differentiate different pathogens within the urine faster and more accurately than is possible on chromogenic identification plates[82]. It has been shown that despite different phenotypes and protein expression profiles caused by different growth conditions, the ability of MALDI-TOF to identify pathogens is not affected [83]. This is essential for urinalysis as the bladder environment and urine will be very different from person to person and will also change significantly over time. However, access to the necessary equipment is not always available (Table 1).

PCR and Sequencing

Originally microbiological techniques to identify different pathogens involved single polymerase chain reactions (PCRs) on strain specific genes or the use of restriction enzyme-based techniques, including restriction fragment length polymorphism (RFLP) and pulsed-field gel electrophoresis (PFGE) [84,85]. All of these are multi-step, time-consuming and generally low resolution techniques. Thus, they often lacked the speed and accuracy to be useful in a clinical setting [84]. Due to the subjective interpretation of these gel-based techniques, they were also often subject to larger analysis variability [86]. These techniques also

lack the ability to distinguish phylogeny and evolutionary relationships of the different species [82].

Some improvements in technology saw the development of 16s rRNA and multiplex-PCR techniques. These techniques improved accuracy, speed and some ability to define phylogeny, but still lacked the strain typing ability and resolution needed for clinical application (Table 1) [24,25,82]. Developments in sequencing technologies have allowed further advancements in the field. Sequencing techniques have allowed genomic annotation and bacterial strain identification with significantly higher accuracy and resolution than earlier techniques [87]. However, the cost of completion and analysis remains an issue (Table 1).

Whole genome sequencing is a relatively recent advancement in microbial research [74]. However, the technique still remains too expensive to be routinely used in diagnostic microbiology. The average cost of sequencing a full bacterial genome is around £40. In addition, this does not factor in the cost of the necessary skilled labour to run and analyse. Therefore, this is currently too expensive for healthcare providers to undertake regularly [74] (Table 1).

A cheaper alternative to whole genome sequencing for strain identification, is by using a technique known as multi locus sequence typing (MLST). This strain typing technique has been successfully applied to many different pathogens including *Salmonella enterica*, *Neisseria meningitis* and *E. coli* [85,88]. The technique works by sequencing housekeeping genes around the genome which are known to slowly accumulate variation within the population [85]. Thus, the allelic differences within these genes allows for high resolution discrimination between pathogenic strains [85]. MLST in *E. coli* involves the sequencing of 7 housekeeping genes around the genome. The sequences return 7 ID numbers, the combination of which give a specific sequence type number [88,89]. Whilst whole genome sequencing would understandably give even more genotypic information on the specific strain, MLST requires far simpler data interpretation and is currently significantly cheaper. Other advantages are that it does not have the same subjective analysis variability drawback of gel-based assays and still allows phylogenetic analysis [88–90]. Thus, it is widely used for *E. coli* strain identification and phylogenetic grouping analysis [88,90]. It was for these reasons MLST was selected as the method of identifying the *E. coli* strains in this project.

1.2.3. Current guidelines for the management of UTIs

If a patient attends a GP clinic to declare they feel they have a UTI, guidelines state that if the patient is suffering from UTI-related symptoms such as dysuria, frequency and urgency dipstick analysis should be performed on the patient's urine [18,28]. The issue of diffuse age-related urinary symptoms such as LUTS makes the diagnosis of a UTI even more difficult [91]. The dipstick therefore helps aid a HCP deduce whether the patient's symptoms are perhaps being caused by pathogens within the bladder [18,28]. A positive dipstick outcome for both nitrites and leukocytes as discussed give a high likelihood of a bacterial colonisation within the bladder [28,35]. Upon obtaining a positive dipstick outcome a HCP should then send a mid-stream urine sample to a hospital laboratory for urinalysis, using urine culture and possibly also MALDI-TOF if available, as has been discussed above [18,28].

Growth on chromogenic agar will give the laboratory information on the species of pathogen present as well as the bacterial load. There has been much debate over the cut-off for what a diagnostic pathogenic load should be defined as, but the current guidelines state that $>10^5$ CFU/ml of growth is indicative of significant bacteriuria [28,31,42,92–97]. It is reported that this definition has an 80% predictive power of a UTI when a single urine is tested, this increases to 95% when two samples are analysed [28]. Therefore, providing the sample is not contaminated, the HCP will then be provided with information on what pathogen is present in the bladder and a list of antibiotics to which it is sensitive to in order to guide treatment decisions [18,28,73,74].

The issue with this definition of significant bacteriuria is that it holds no information on whether the patient's symptoms are indeed being caused by the bacteria found to be present in the bladder. This can lead to the inappropriate treatment of UTIs, especially ABU, due to the lack of understanding of this disease state. It is estimated that ABU is inappropriately treated in as many as 52% of cases [42]. This is therefore an enormous economic burden as well as potentially promoting antibiotic resistance formation within the uropathogens [72,98]. This can also be problematic in elderly patients, who can have higher toxicity risks to antibiotic treatments, such as nitrofurantoin [28].

Detection method	Advantages	Disadvantages
Culture on microbiological media	Sensitive	Lengthy and time consuming process
	Inexpensive	Might require 24–48 h
Immunological-based methods	Faster than conventional methods	Not as specific, sensitive, and rapid as nucleic-acid based detection methods
	Can detect both contaminating organisms and their toxins	Require large amounts of antigen
		Developed for only a small number of microorganisms
Molecular based methods (Real-time PCR and Multiplex-PCR)	Culturing of the sample is not required	A highly precise thermal cycler is needed
	Specific, sensitive, rapid, and accurate	Trained laboratory personnel required for performing the test
	Closed-tube system reduces the risk of contamination	
	Can detect many pathogens simultaneously	
DNA sequencing	16S rDNA and 18S rDNA sequencing are the gold standards	Trained laboratory personnel and powerful interpretation softwares are required
	Can identify fastidious and uncultivable microorganisms	Expensive
		Not suitable for routine clinical use
Microarrays	Large scale screening system for simultaneous diagnosis and detection of many pathogens	Trained laboratory personnel and powerful interpretation softwares are required
		Expensive
		Trained laboratory personnel required
Metagenomic assay	Useful for random detection of pathogens	Data acquisition and data analysis is time consuming
		Trained laboratory personnel required
MALDI-TOF MS	Fast	High initial cost of the MALDI-TOF equipment
	Accurate	
	Less expensive than molecular and immunological-based detection methods	
	Trained laboratory personnel not required	

Table 1. Advantages and disadvantages of different diagnostic microbiological techniques. Table adapted from Singhal *et al* (2015) [82].

1.3. Escherichia coli

E. coli is a gram negative, rod-shaped, flagellated, γ-proteobacteria which has been extensively researched and documented. *E. coli* has been located in many parts of the human body, but is most commonly found in the gut. *E. coli* is usually a harmless, and often even helpful commensal bacteria, involved with maintaining equilibrium within the host. The most common example of this is in the gut, which is an organ that is well adapted to cope colonisation with high levels of bacteria in healthy individuals [99].

1.3.1. Pathogenesis

E. coli is also a pathogen capable of causing a range of diseases. This happens when certain pathogenic strains of *E. coli* are able to reach organs and niches that are not equipped to deal with such colonisation. These pathogenic strains have adapted their virulence gene repertoire and phenotypes in order to survive in different conditions within the body [100]. To distinguish between these different types of pathogenic *E. coli*, they are named based on the location from which they are usually isolated. It is widely accepted that there are 8 different groups of pathogenic *E. coli* [100]. These are split into intestinal *E. coli* (enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC)) and extraintestnal *E. coli* (ExPEC). Uropathogenic *E. coli* (UPEC) and neonatal meningitis-associated *E. coli* (NMEC) are both types of ExPEC [100].

UPEC

The *E. coli* type being focussed on in this project is UPEC as this is the pathogen responsible for causing the majority of UTIs [100]. UPEC is estimated to be responsible for 70-95% of all UTIs [101]. It is generally accepted that the main source of bacteria in UTIs is from the gut. Bacteria travel from the gut to the urinary tract via the perineum where they can ascend the urethra into the bladder [59,60] (Figure 1B and C, p.17). UPEC is adapted to survive and grow in the bladder, using urine as its growth medium.

It has been shown in mice that UPEC are able to temporarily enter intracellular vesicles within the bladder in order to avoid being cleared by micturition. These vesicles are one of the methods thought to be utilised by the bacteria to persist long-term in the bladder [102]. It could be dormant intracellular bacteria that reactivate sporadically after long periods of dormancy to cause recurrent symptomatic infection in some patients.

In addition to flagella, UPEC possess a wide range of other virulence factors which can help them establish and successfully colonise the urinary tract. One such example are bacterial fimbriae, which are found in relative abundance in UPEC strains than other commensal *E. coli* strains [103]. Fimbriae are extracellular structures around 3µm in length which allow the bacteria to adhere to and invade the bladder epithelial cells [104–106]. The main types of fimbriae expressed by UPEC are P fimbriae and type 1 fimbriae, however, there are others including S pili and Dr family adhesins [107]. Fimbriae differ by the adhesin found at the end of the fimbrial rod, with P fimbriae possessing the PapG adhesin [108] and type 1 fimbriae possessing the FimH adhesin [109]. Bacteria able to colonise the kidney have found to mainly possess P fimbriae whereas bladder colonisers tend to have type 1 fimbriae [110]. This is due to the different substrates each of these ahdesisn binds being found on the epithelium of the different parts of the urinary tract. PapG is able to bind glycosphingolipid receptors on the renal epithelium [111] and FimH binds mannose-containing receptors on the bladder epithelium [112]. Thus, the different expression of these virulence factors can determine potential disease severity. Deletion of *fimH* has been shown to stop UPEC from being able to colonise the urinary tract, thus highlighting fimbrial importance in the successful uropathogen [113,114].

Efficient iron-acquisition can provide a huge survival advantage to uropathogens, due to the limited nutrient availability within the urine. Some UPEC possess the ability to actively transport iron into the cell via siderophores. There is evidence to suggest UPEC express more siderophores and that this could allow for more successful urinary tract colonisation [115]. Another virulence factor utilised by UPEC strains is the expression of the toxin hemolysin, which aids bacterial survival by destroying components of the host response in order to prevent immune clearance [116]. The virulence factors mentioned here among several others all work to confer survival advantages to UPEC strains within the urinary tract.

Pathogens have been shown to exchange and vary the expression of the genes involved with these virulence factors by a process known as horizontal gene transfer [115,117]. This is the process of homologous recombination of genes within and even between different species of microorganisms. The loss and acquisition of different genes by horizontal gene transfer can greatly aid adaptive evolution to different environmental niches [118,119]. Therefore, can play a large role in the success of pathogenic organisms to survive within different environments of the body, such as UPEC within the urinary tract [118].

1.3.2. Antibiotic Resistance in E. coli

As discussed antibiotic resistance is a huge burden on healthcare systems with growing social and economic impact. *E. coli* is commonly found in clinical settings to confer resistance to common antibiotics used to treat diseases, including UTIs [120]. There have been recent rises in the isolation of clinically isolated multi-drug resistant ExPEC strains worldwide. These resistances include the carriage of genes encoding extended-spectrum beta-lactamases (ESBLs) [121]. A pathogen carrying ESBL genes would likely be resistant to commonly prescribed drugs for UTIs, such as cephalexin and amoxycillin. Antibiotic resistance testing is therefore essential in a clinical setting in order to give patients the most effective choice to try and clear an infection.

1.3.3. E. coli Phylogenetic Groups

To better understand the complex variation among *E. coli*, sequence alignment and phylogenetic analysis if often used. This involves grouping the different *E. coli* strains by the degree of sequence matching, which can include virulence and housekeeping genes. These groups, or clades, often show trends with the niche in which the pathogen is commonly found [122]. For *E. coli* the main groups are known as clade A, B1, B2 and D, however there are others which are less represented such as clade E and F [123]. Clade A and B1 are mainly associated with commensal gut *E. coli* strains [123,124]. B2 is the clade is most commonly associated with virulent ExPEC strains, such as UPEC [90,119,125]. Clade D is also known to be associated with UPEC strains, to a lesser extent [125], this is known to be a sister group of B2 hence there is significant phenotypic overlap. To achieve this phylogenetic group alignment, techniques such as whole genome sequencing and MLST can be used [124]. MLST provides an accurate method of assigning different *E. coli* sequences to the different *E. coli* clades based on their sequence alignment [88]. MLST alignment is a method of identifying a specific *E. coli* strain's clade, in order to better identify it with respect to the literature [90].

1.4. The Immune Response

The innate immune response is the body's initial defence against unwelcome pathogens. Innate immune cells (both static epithelial and migratory inflammatory cells) continuously monitor the body's environment in order to raise a defensive response if it encounters invading pathogens. This response is intended to lead to removal of any molecules which threaten the equilibrium within the host [126].

1.4.1. Pathogen-Associated Molecular Patterns and Toll-Like Receptors

Pathogen-associated molecular patterns (PAMPs) are structures on pathogens that can be recognised by host cells, via pattern recognition receptors (PRRs). An example of a PRR are the membrane bound toll-like receptors (TLRs) [127]. There are 10 TLRs that have been described to date in humans, all of which recognise different microbial PAMPs which lead to the activation of signalling cascades resulting in the production of an immune response (Figure 3) [128,129]. Two of these TLRs most commonly described to have association with UTIs are TLR4 and TLR5 [130]. TLR4 recognise bacterial lipopolysaccharide on the cells surface and TLR5 recognises the bacterial flagella (Figure 3) [129,131]. Binding of TLR4 and TLR5 both cause a signalling cascade detailed in Figure 3, which ultimately leads to the movement of nuclear factor-kappa B (NF-κB) into the nucleus. This allows NF-κB to bind and up-regulate target genes, leading to an innate immune response aimed at clearing the infection (Figure 3) [132,133].

TLR11 has been implicated to have potential association with UTIs (Figure 3), its presence in the bladder has been found to have a protective effect in mice. However, this receptor has not yet been found in humans [134].

Flagella

An example of a PAMP is the bacterial flagellum, an external tail-like structure which allows the cell to swim to more favourable environments, for example towards nutrient sources [135]. Flagella are made up of thousands of monomeric proteins, called flagellin, which form a long filament protruding out of the cell by up to 10μm [136–138]. Due to its external location flagella are easily recognised by the human body's immune system [139]. Flagellin is recognised in the body by TLR5, which activates an innate immune response within the cell (Figure 3) [131,140]. TLR5 has recently been found to appear on bladder epithelial cells [Ali, Lanz, Pickard and Hall, unpublished data]. Flagella have been suggested to help bacteria ascend the urinary tract during infection [141,142]. Deleting a gene called *fliC* will remove the ability of the bacteria to produce flagella. This deletion has been shown to significantly decrease the capacity of the pathogen to travel up to the kidneys. This is consistent with the suggested role of flagella in early stages of UTI [141]. Thus, flagellation and motility appear to have an important role in a successful colonisation of the urinary tract.

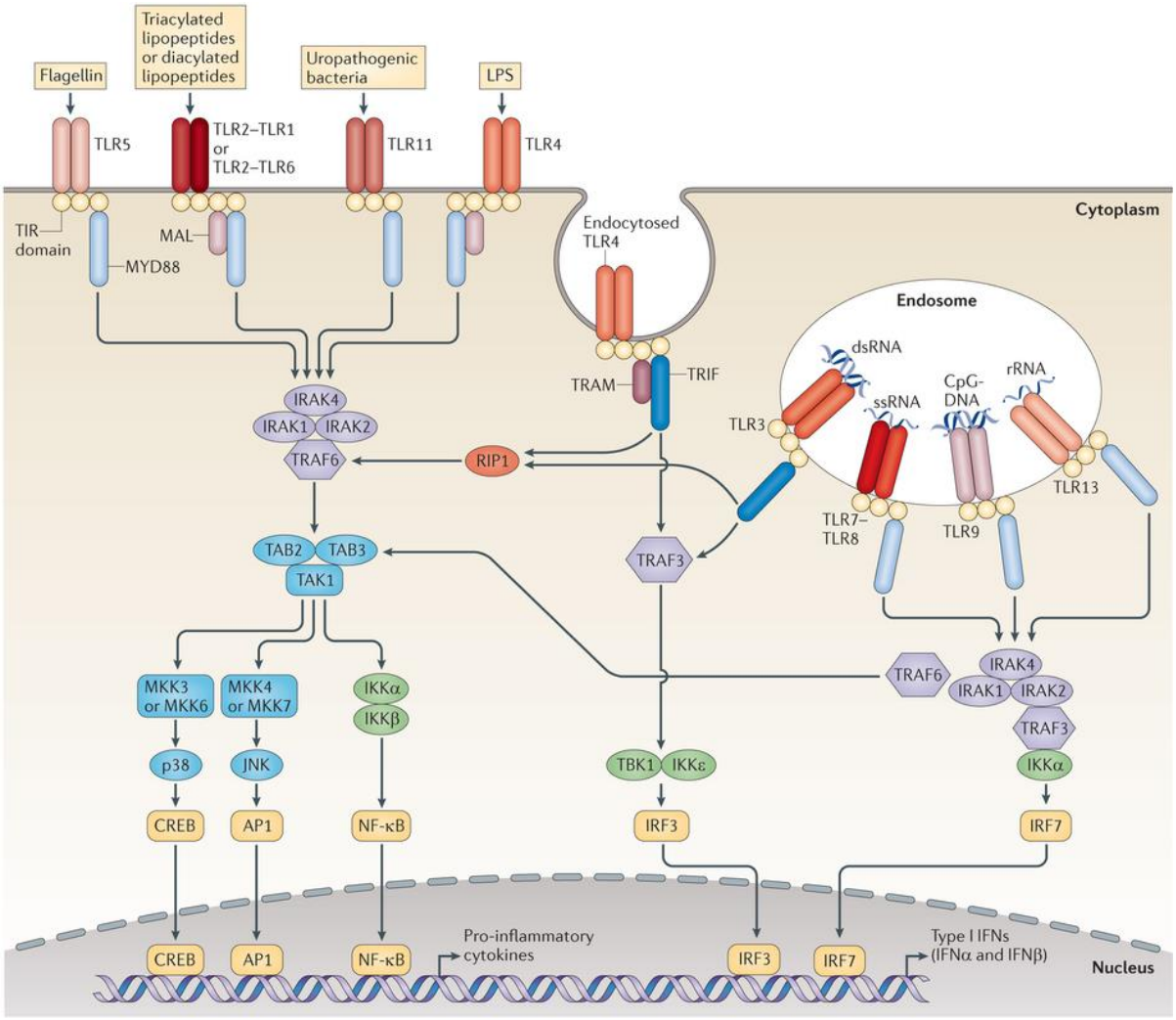


Figure 3. Toll-like receptor signalling pathways within mammalian cells. Figure from O'Neill *et al* (2013) [143].

1.4.2. Innate immune response molecules

The NF-κB dependant immune response involves the release of immune response molecules, such as cytokines and antimicrobial peptides [133,144,145]. Anti-microbial peptides will directly attack the bacterial cell and cytokines will recruit other innate immune cells in order to increase the defensive response (Figure 3) [146,147]. The aim of these response molecules is to clear the infection [148,149]. Concentrations of several of these cytokines have been shown to be associated with UTIs in some way. Ten of these response proteins have been chosen for close analysis in this project due to their involvement in UTIs and ability to be detected within the urine. These 10 proteins and their UTI reported involvement will be discussed here in detail.

Cytokines and Chemokines

Cytokine is a general term for a number of different types of immune response molecules. These include chemokines, which are proteins that have leukocyte activation and chemotactic activities. Interleukins (ILs) are cytokines produced by leukocytes and are generally involved with activation, proliferation and differentiation of other leukocytes. Monokines are cytokines produced by monocytes and lymphokines are cytokines produced by lymphocytes [147,150,151].

IL-1β is a pro-inflammatory cytokine produced by a range of different cell types including fibroblasts, macrophages and endothelial cells during infection, cell invasion and inflammation [147,150]. It has roles in promoting coagulation of endothelial cells by modulating the expression of adhesion molecules, as well as inducing the release of other cytokines such as IL-4, IL-5, IL6, IL-8, TNFα and IFNγ [152]. The protein has been shown to be found at increased levels in patients with bacterial cystitis compared to normal [153]. This increase has also been shown when compared to patients with interstitial cystitis [154]. Rodhe *et al* (2009) reported levels of IL-1β in the elderly bladder to be between 15.8 and 153 pg/mg creatinine (Table 2, p.39) [155].

IL-4 is an anti-inflammatory cytokine produced by lymphocytes and mast cells [147,150,153]. Anti-inflammatory cytokines regulate the host immune response by controlling the release and action of the pro-inflammatory cytokines [147]. IL-4 has regulatory roles on the differentiation and development of immune CD4+ T cells as well as promoting the cell cycle progression of B cells [156–158]. Production has been shown to be promoted in UTI patients in the presence of flagellin [159]. However, reported urinary concentrations of IL-4 are generally very low, with Davidoff *et al* (1997) reporting levels around just 0.04 pg/mg creatinine in cystitis patients (Table 2) [153].

IL-5 is another anti-inflammatory cytokine produced by lymphocytes and T cells [150,160]. It has a role in inducing production of and recruitment of additional immune cells [150].Early studies have suggested an involvement in UTIs due to elevated concentrations in the urine of mice with bacteraemia [161].

IL-6 has been shown to be produced by macrophages, fibroblasts, and epithelial cells amongst many others [150,162]. It is an important cytokine with mainly pro-inflammatory roles within the innate immune response, such as controlling fever and the early acute phase response to infection in order to clear infection [147,150]. However, it has also been shown to have some anti-inflammatory activities as well. IL-6 has been shown to suppress the production of IL-1β and tumor necrosis factor alpha (TNFα) [163,164]. Thus, IL-6 is a cytokine with an important central role of innate immune modulation. Concentrations of IL-6 have been reported to be significantly elevated in the urine of patients with cystitis [153,165]. Levels have also been shown to be higher in ABU patients when compared to pyelonephritis patients [94]. Rodhe *et al* (2009) reported levels of IL-6 in the elderly bladder to be between 7.1 and 37.4 pg/mg creatinine (Table 2) [155]. It has also shown some promise as a biomarker for distinguishing between ABU and symptomatic UTIs when used in conjunction with a positive HCP outcome from a urinary dipstick [155].

IL-8 is another very important pro-inflammatory chemokine involved with the acute phase response [166]. IL-8 has also been shown to be produced by a wide range of cells, including monocytes, fibroblasts and epithelial cells [150,151]. IL-8 falls under the classification of chemokine, due to its chemotactic activity [150]. IL-8 can also cause the intracellular storage organelles to release various enzymes involved with the immune response, such as lysozymes, collagenases, proteases and hydrolases, in order to try and clear and infection from the host, such as [167]. IL-8 concentrations have been reported to be higher in the bladders of patients with ABU and symptomatic UTI compared to healthy controls [155,168]. Rodhe *et al* (2009) reported a wide range of IL-8 concentrations within the elderly bladder of 6.5 all the way up to 809 pg/mg creatinine (Table 2) [155]. Attraction of neutrophils to the site of infection by IL-8 is key for aiding bacterial clearance within the urinary tract [169–171]. As would therefore be expected, mice without the ability to recognise IL-8 showed higher susceptibility to UTIs and poorer bacterial clearance from the urinary tract upon infection [172].

IL-10 is a key anti-inflammatory cytokine, produced by activated T cells, mast cells and lymphocytes [147,150,173]. It has several anti-inflammatory effects including inhibiting

antimicrobial effects of macrophages, downregulating cytokine receptor expression and inhibiting inflammatory cytokine secretion by various immune cells [147,150]. It can be responsible for repressing expression of IL-1β, IL-6 and TNFα [147]. IL-10 has been shown to be found at elevated concentrations within the bladders of patients with cystitis [174]. It has been suggested that it may play a role in long-term bacterial colonisation due to its strong anti-inflammatory roles [173]. Rodhe *et al* (2009) reported a concentrations of between 5.3 and 31.6 pg/mg creatinine within the elderly bladder (Table 2) [155].

IL-12 p70 is a pro-inflammatory cytokine produced by macrophages, B cells and other antigen presenting cells [150,175]. The protein has a directive role in the immune response by driving the production of other cytokines [150]. Studies have shown its importance in the immune response to bacterial infections [176,177]. Women prone to suffer from UTIs have shown elevated IL-12 production [178]. Rodhe *et al* (2009) report IL-12 concentrations of between 5.3 and 67.8 pg/mg creatinine within the bladders of the elderly (Table 2) [155].

IL-17A is produced by T cells and lymphocytes and has a pro-inflammatory role in the immune response. Increased levels can lead to the induction of IL-6 and IL-8 production [150]. It also has an innate-adaptive immunomodulatory role, whereby it can enhance the expression of adhesion molecules on the surface of human fibroblasts [150,159]. It has been suggested to have a role in the innate immune response raised to UTIs caused by UPEC [179].

TNFα is a pro-inflammatory cytokine produced by lymphocytes, neutrophils and endothelial cells amongst several other sources [147,150,180]. However, the main source is from release during late stage macrophage differentiation [153]. TNFα functions by modulating expression of other cytokines and their cell receptors, which has shown to have a key role in the immune response to infections [150]. Several of the cytokines discussed have overlapping and interlinking functions. For example, IL-1β and TNFα have very similar effects [150]. This overlap can be seen by IL-6 production being increased in response to elevations in both IL-1β and TNFα [181]. There have been reports showing elevated levels of TNFα within the bladder of cystitis patients compared to uninfected controls [153]. Rodhe *et al* (2009) reported levels of TNFα in the elderly bladder to be between 2.4 and 20 pg/mg creatinine (Table 2) [155].

The final immune protein chosen for analysis in this study was interferon gamma (IFNγ). This is a pro-inflammatory cytokine produced by T cells and natural killer cells [150,160]. It has roles in increasing immune cell expression and increasing macrophage activation in order to

promote pathogenic clearance during infection [150]. Increased concentrations of IFNγ have been found in the bladders of patients suffering from bacteriuria [54].

By analysing this library of immune response proteins within the bladder of infected patients, will provide an overview of the overall immune activation taking place. They provide a cross-section of the innate immune response with a wide range of pathways both pro- and anti-inflammatory being covered.

	Pro- or anti-inflammatory	Concnetration in young women (pg/mL) [A]	Concnetration within elderly urine (pg/mg creatinine) [B]	Concnetration within bladder infection patients (pg/mg creatinine)
IL-1β	Pro-inflammatory	0.04	15.8 - 153	53.76 [C]
IL-4	Anti-inflammatory	3.53		0.04 [C]
IL-5	Anti-inflammatory	0.04		
IL-6	Pro-inflammatory	0.2	7.1 - 37.4	10.5 [C]
IL-8	Pro-inflammatory	7.49	6.5 - 809	
IL-10	Anti-inflammatory	0.27	5.3 - 31.6	
IL-12 p70	Pro-inflammatory	0.18	5.3 - 67.8	
IL-17A	Pro-inflammatory			6.08 [D]
TNFα	Pro-inflammatory	0.09	2.4 - 20	4.86 [C]
IFNγ	Pro-inflammatory	0.23		

Table 2. Overview of the cytokines being studied within this project, including their inflammatory nature and some of the reported urinary concentrations from the literature. [A] indicates figures from Nobles *et al* (2015) looking at concentrations in women of reproductive age [182]. [B] indicates figures from Rodhe *et al* (2009) looking at concentrations within the elderly bladder [155]. [C] indicates figures from Davidoff *et al* (1997) looking at concentrations within cystitis patients [153]. [D] indicates figures from Helbig *et al* (2013) looking at concentrations within patients with *Candida* infection [183].

1.4.3. Adaptive immune response

The innate immune response is not specific to the type or species of organism which it recognises whereas an adaptive response tends to have far more organism specificity. Unlike the immediate action of the innate response, an adaptive response can take up to 10 days to establish [145,184]. Upon bacterial recognition by the host the activation of B cells by some cytokines can stimulate the production of antibodies [127]. It is widely accepted that the adaptive immune response plays a much smaller role in defence against UTIs than the innate response [130,185]. It has been noted that pathogens originating in the kidneys display antibody coating, which is not seen in pathogens colonising the bladder [186]. These antibodies are also detectable in the urine and serum of patients who are suffering from pyelonephritis but not cystitis [187–189]. Production of the anti-inflammatory cytokine, IL-10, was found to be specific to the bladder and not the kidneys, suggesting that this could be responsible for the lack of adaptive response seen within the bladder [173]. In support of this, mice unable to produce IL-10 showed the production of antibodies to bladder pathogens [173].

1.5. Biomarkers

Biomarkers have been used for many years in medicine for many different indicators, such as helping make decisions in the clinical setting, aiding drug development and evaluating new drugs coming to market. Due to recent advances in technology, such as human genome sequencing, microarrays and vastly improved imagining techniques, biomarkers have a new heightened importance in Research and Development (R&D) and in the clinic [190].

The accepted definition of a biomarker [191] is a characteristic that is objectively measured and evaluated as an indicator of one of the following:

1. A normal biological process
2. A pathogenic process
3. A pharmacological response to a therapeutic intervention

We are most interested in biomarkers involved with the first two processes. Biomarkers are being used more and more widely in decision making roles in the clinic due to ever more powerful analysis techniques on a broad number of possible markers [190,191]. Accessibility to a biomarker plays a larger role on whether it becomes adopted in regular clinical practice. Less

invasive methods of collection, such as urine or saliva, will be quicker for HCPs to obtain and generally preferred also by patients [192]. Qualitative biomarkers are those that are present only in a certain subset of patients, whereas quantitative biomarkers are present in varying quantities in all patients [192]. Thus, the cytokines, previously mentioned, being measured in this project are quantitative biomarkers as they have been reported to be found in the bladders of all patients to some degree [182]. As well as cytokines, bacteria within the urine are a quantitative biomarker of disease, as many have been found to be present in the normal bladder microflora as previously discussed. Low levels of bacteria in the urine can suggest gut contamination, therefore a threshold of $>10^5$ CFU/ml is used to define significant, thus making this a quantitative biomarker [28,31].

In order for biomarkers to be robust they should be put through extensive analysis to ensure they are 'fit-for-purpose'. For this analysis they must initially go through method validation followed by extensive biomarker qualification. Method validation is a process in which the assay designed to collect and quantify the biomarker of interest has been fully assessed. This includes outlining assay conditions, which will be needed to give accurate and reproducible outcomes [190,193]. Essentially the method of acquiring data must be suited for the biomarker's intended application [193]. Biomarker qualification (otherwise known as biomarker validation or evaluation [190]), is the process in which evidence is used to build up a link between the marker and the biological process of interest. This can be done relating to clinical end points [190,194]. Whilst there is little governing control for biomarker assay validation, new devices or clinical tests require substantial evidence to support their approval [190]. The four categories outlined in Figure 4 allow a good basis to logically and thoroughly undergo biomarker qualification [190,195].

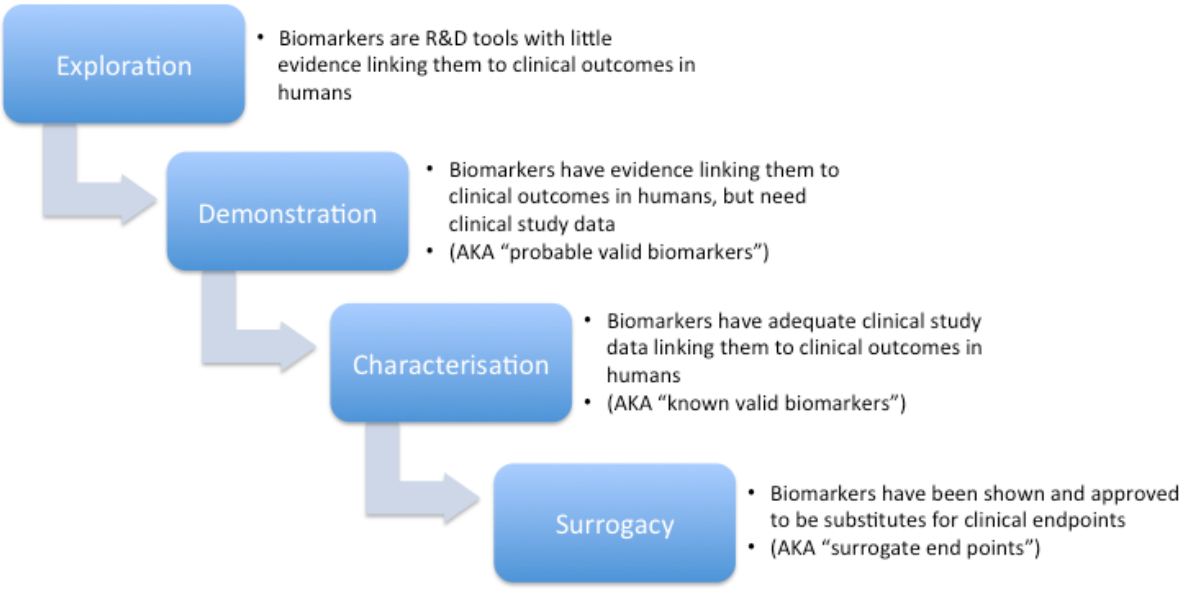


Figure 4. Fit-for-purpose categorisation of biomarkers. The more valid evidence provided for a certain biomarker allows it to progress from an 'Exploration Biomarker' towards a 'Surrogate Biomarker'. The evidence allows a link to be validated between the marker and the biological process of interest. Adapted from Wagner, J.A. *et al* [190].

1.5.1. Urine Tests Using Biomarkers

Some of the best tests exploring urinary problems are those involving a quick patient-side urine-based test, which can give a result in minutes. These tests are usually either a chemical reaction leading to a colour change or an enzyme-linked immunosorbent assay (ELISA) based technology. ELISAs involve an antibody that is attached to the surface of the test which, when urine is passed over, changes colour if its specific antigen is present to bind to it. One example of a urine test includes the pregnancy tests, which look for pregnancy-associated glycoproteins produced by the placenta. These will give a positive or negative pregnancy result in a very short time [196]. Another form of very successful patient-side urine test is the well-known diabetes diagnosis test, which simply shows if the patient has glucose in their urine. Glucose in the urine occurs in diabetic patients [64]. Glucose can be measured using a urinary dipstick, which is a tool used to measure several urinary biomarkers for urinary heath, as previous discussed. An example of urine tests developed to aid physicians treating UTIs is the antibody-coated bacteria (ACB) test. ACB are found in the urine once the infection reaches the kidney, thus a positive result to such a test could help differentiate between upper and lower UTI, which can assist with making further treatment decisions [186].

Basic microbiological investigation can also be a useful biomarker of urinary disease. As mentioned above, the presence of bacteria in the bladder over a given threshold is a biomarker of significant bacteriuria in a clinical setting. However, what is needed are better discriminatory tests to distinguish between ABU and symptomatic UTI.

1.5.2. Clinical studies into ABU biomarkers

Several studies have been undertaken to try and distinguish between ABU and symptomatic UTI and improve knowledge of the infected bladder. Nicolle *et al* (1993) measured IL-1β and IL-6 in the urine of institutionalised elderly patients. Their data suggested that increases in both of these cytokines could potentially discriminate between ABU and acute UTI this patients cohort [197]. However, this was only performed on 67 urine samples and there were no healthy control comparisons. Rodhe *et al* (2009) collected urine samples from elderly patients with both diagnosed acute UTI and ABU in order to try and find biomarker patterns which could distinguish between the two disease states. They measured a number of cytokines in the urine, including IL-1β, IL-6, IL-8, IL-10, IL-12 and TNF-α. They found that levels of IL-6 and IL-8 were significantly higher in patients with acute UTI than those with ABU, and both disease states showed higher levels than healthy controls [155]. This agreed with observations

previously made by Hedges *et al* (1992) in IL-6 concentrations in pyelonephritis patients compared to those with ABU [94]. However, a study undertaken by Sundvall *et al* (2014) was not able to establish a difference in IL-6 that was able to distinguish between patients with non-specific urinary symptoms, even when used in conjunction with the dipstick outcome [67]. The McNally group (Nottingham, UK) and collaborators completed a series of large scale genotypic and phenotypic analyses on up to 250 clinical urine cultures [198–200]. Toval *et al* (2014) ran genotype analysis of 265 *E. coli* isolates from UTI patients [124]. All of the above findings were based on single urine samples from each patient. Thus they only show a snapshot of information and do not take into account potentially important changes in symptoms or colonisation status around time of sampling.

There are some examples of repeated urine samples within the literature however. Hernández *et al* used repeat urine sampling to analyse the effect of intentional inoculation of patients with ABU *E. coli* strain 83972. Urine samples were collected monthly for host response analysis and to check the intervention had been successfully established within the bladders [185]. Sundén *et al* (2015) also sampled urine 4 weeks apart. This was to analyse changes in the host responses between ABU and symptomatic UTI patients over time. However, a total of just 2 samples were collected from patients, thus, lacks the longitudinal information of whether observations were fluctuations or normal for that patient [201]. In addition, both of these examples lacked resolution to obtain a detailed overview of changes in the patients' bladders over time. Therefore, highlighting the need for a high resolution longitudinal study of the bladder environment in the ABU patient cohort.

In 2009 Czaja *et al* reported their findings of a study very similar to the one undertaken in this project [202]. They collected daily urine samples from patients who suffer recurrent UTIs, these were refrigerated for up to 14 days and upon declaration of a symptomatic episode the previous 14 samples were prospectively analysed for bacterial presence and host response activity. Further analysis on these bacterial isolates looked into more detail of the genotypes of the uropathogens [203]. The study completed by Czaja *et al* was completed on a cohort women aged between 18 and 49, whereas this study is specifically looking into changes in the elderly bladder (65+ years). The study completed during this project aims to analyse fresh urine samples. A wider range of cytokines is analysed in this project compared to the Czaja *et al* study and age-similar controls will be used to provide a baseline for sample normalisation. One of the study downfalls discussed by Czaja *et al*, was their inability to see beyond the 3-

month time frame. Thus, the 6-month timeline of this project design should help address this issue.

As discussed here UTIs are a large social and economic burden worldwide. Many different clinical presentations make it a difficult disease to diagnose and therefore successfully treat. This is further complicated in elderly patients who often have misleading and ambiguous symptoms. There is a general lack of understanding around bacterial-host interactions within the bladder and how these are affected during long-term colonisation, as is seen in ABU patients. Better understanding will drive improved patient management, more successful disease treatment and increase patient awareness, in order to hopefully better control this disease on a global scale, especially in vulnerable populations. This thesis will describe the analysis of a longitudinal clinical study, which has been designed to address a number of questions raised from the UTI literature.

Chapter 2. Aims

UTIs are becoming increasingly well understood, however their treatment with ad hoc antibiotics is still very holistic. Some acute infections are simple to treat and clear. However, others provide a more complicated pathology. Patients who are able to carry bacteria for long periods of time appear to react very differently to this colonisation. It is widely accepted in treatment guidelines that colonisation of the urinary tract without symptoms should not be treated in otherwise healthy individuals. However, what remains unclear is what causes a person with ABU to transition from this harmless asymptomatic state to that of a full symptomatic acute infection. If there were ways of predicting such a transition, it may be that these patients can be more effectively and efficiently treated before symptoms arise, if at all. This could not only reduce patients suffering but also potentially alter antibiotic course length and overall usage. The latter would have obvious and direct implications on preventing antimicrobial resistances, which are becoming a huge global threat to public health.

In order to address this important issue a clinical study was designed in order to track patients who have a history of suffering from regular symptomatic UTIs. By monitoring these patient's urines and symptoms regularly for 6 months it would be possible to ask the data several questions. This would allow observation of changes in the urinary tract over time, with samples being collected from patients roughly 2 weeks apart. This sampling allowed for analysis of change over time in the urinary tract. This would mean that both the colonising bacteria and the host immune response could be analysed temporally. This analysis would create a large database of host and bacterial data. This database alongside data of patient's symptomatic state, collected from regular questionnaires and their medical records, would allow us to map changes in the urinary tract with periods of symptomatic infection.

The clinical study and the mapping of these various databases of analysis would provide a strong method of addressing the main aims of the project;

1. Testing the feasibility of this study design in this age group as a pilot for the potential of similar future studies, perhaps with a larger sample size.
2. Investigating the changes in the bladder over time of older patients with long-term bacterial colonisation from both a bacterial and host perspective.
3. Analysing any potential changes in the colonising bacterial phenotype and genotype during periods of changing UTI symptoms and treatment.

4. Analysing any changes in potential host immune urinary biomarkers around periods of symptoms, as these could provide a non-invasive method of predicting symptomatic episodes.

Chapter 3. Materials and Methods

3.1. Urine Sample Storage and Dip-Stick Analysis

Urine samples were all dipped within 4 hours of collection, using Multistix® 10SG (Siemens) based on availability. One aliquot of unfiltered urine was stored at -80°C. Urine was also filtered using a 0.45µm filter (GE Healthcare Life Sciences), 3 aliquots of this was taken and stored at -80°C. Finally, all urines were plated onto CPS ID 3 (CPS3) chromogenic agar plates (bioMérieux), each at 1µl and 10µl so that colony quantification could be made. Manufacture of CPS3 plates was discontinued during the study and replaced by an improved version, called CPSE plates. Plates were incubated at 37°C overnight.

The dip-stick allowed measurement of several parameters of the urine; glucose, ketone, specific gravity, blood, pH, protein, nitrite, leukocyte esterase including pH, nitrites and leukocytes. The 10SG sticks also measured urobilinogen and bilirubin. These were each read off a colour chart supplied with the sticks according to the manufacturer's instructions.

3.1.1. Dipstick Score

In order to run data analysis on the dipstick outcomes, a method of quantifying the parameters was devised (Table 3). Each of the 10 parameters measured on the dipstick was given a score from anywhere between 0 and 5. Zero indicated a normal dipstick outcome based on the most frequently returned result from the healthy controls, shown in Figure 30 (p.133). The score given to each outcome increased with its divergence from this healthy baseline. Continuous scales, such as glucose, ketone, specific gravity, blood, pH, protein and urobilinogen, all carry directly on from 0 up to 5. Non-continuous scales, such as bilirubin, nitrites and leukocytes all peak at 5 points and count back from there. The total of all 10 parameter scores gave the overall dipstick score for that sample. Essentially, a higher dipstick score indicated a less healthy urine sample.

Points	Glucose (mg/dL)	Bilirubin	Ketone (ml/dL)	Specific Gravity	Blood (Ery/µL)	pH	Protein (mg/dL)	Urobilinogen (mg/dL)	Nitrites	Leukocytes (Leu/µL)
2	n/a	n/a	n/a	1.000	~80 (Non-Haemolysed)	n/a	n/a	n/a	n/a	n/a
1	n/a	n/a	n/a	1.005	~10 (Non-Haemolysed)	5	n/a	n/a	n/a	n/a
0	- ve	- ve	- ve	1.010	- ve	6	- ve	0.2	- ve	- ve
1	100	n/a	5	1.015	~10 (Haemolysed)	6.5	Trace	1	n/a	n/a
2	250	n/a	15	1.020	~25 (Haemolysed)	7	30	2	n/a	~15
3	500	Small	40	1.025	~80 (Haemolysed)	7.5	100	4	n/a	~70
4	1000	Moderate	80	1.030	~200 (Haemolysed)	8	300	8	n/a	~125
5	2000+	Large	160+	n/a	n/a	8.5	2000+	n/a	Positive	~500

Table 3. Scoring system used to quantify outcomes from the dipstick analysis. A score of 0 (highlighted red) is achieved by what healthy urine should produce on the dipstick. The scores increased based on their deviation from what a healthy urine would produce. - ve indicates a Negative score on the dipstick.

3.2. Bacterial Isolation and Storage

CPS3 Plates were all photographed for referencing the different strains that had grown. Red colonies indicated the presence of *E. coli* in the urine. Red colonies were tested as an additional measure to identify them as *E. coli* using an indole test. Plates containing red colonies giving a positive indole test had one red colony picked and cultured overnight in LB. This was then frozen with 10% DMSO at -80°C the next day. All plates regardless of what had grown were washed down with LB and grown overnight. These too were frozen and stored at -80°C with 10% DMSO, this was so that at any time the complete culture could be back-referenced if necessary.

Bacteria needed to be reactivated would be streaked out onto LB plates and incubated at 37°C overnight. A single colony would then be taken from this plate and grown overnight in LB broth at 37°C.

3.3. Antibiotic Resistance Testing

E. coli isolates were reactivated on LB plates grown overnight at 37°C. From the plates colonies were scraped and resuspended in PBS to an equivalent of a 0.4-0.6 McFarland turbidity standard. A swab of this is then plated out evenly on Mueller-Hinton agar plates using a plate spinner to achieve a consistent and even lawn of bacterial growth. Discs containing pre-defined antibiotic concentrations are then placed onto the plate containing bacteria. The antibiotics tested were the first 6 front line antibiotics tested for all UTIs, as per the NHS laboratory protocol [204]. These were Amoxicillin, Amoxicillin-clavulanic acid, Cephalexin, Trimethoprim, Nitrofurantoin and Ciprofloxacin. Plates were then incubated at 37°C overnight before analysis. To measure the isolate's degree of sensitivity to the antibiotics, the zones of inhibition around each disc was measured manually. The zones of inhibition were then compared to well controlled European Committee on Antimicrobial Susceptibility Testing (EUCAST) defined breakpoints, defining an isolate as resistant or sensitive to the antibiotic in question [204].

3.4. Motility

Motility of isolates was measured by growing a single colony overnight in LB, 5µl of this was carefully placed onto a motility agar plate (1% Tryptone, 0.5% NaCl, 0.3% Agar). Plates were then incubated for 6 hours at 37°C. Pictures of each plate were then taken. The swarm created by the isolate was measured twice and an accurate measurement was obtained by using a ruler photographed with the plate to obtain the exact swarm size based on the pixels. Experiments were performed in triplicate to give a representative average motility.

3.5. Sequencing

In order to obtain an identification on the *E. coli* strains isolated during the study various genes were chosen for sequencing. The genes chosen to sequence were the well documented MLST genes for *E. coli* identification and the *fliC* gene for H-antigen serotyping [88]. These 2 methods would give a relatively accurate strain identification, which would allow differentiation in colonising strains over time if any changes were to occur. These methods of identification were chosen as they were comparably a lot cheaper than whole genome sequencing the isolates.

3.5.1. MLST and fliC Serotyping

Genome preparations were made of all isolates to be sequenced using a genomic DNA isolation kit (Sigma-Aldrich). PCR was then completed on each strain using 7 primer pairs to amplify the 7 different genes required for MLST (Table 4), primer sequences obtained from the University of Warwick Medical School website (http://mlst.warwick.ac.uk/mlst/). The *fliC* primers (Table 4) were designed and optimised previously by the Aldridge lab, to amplify a central region of the gene in order to obtain a reliable sequence via PCR. The PCR programme used was 2 minute at 95°C, 30 cycles of 1 minute at 95°C, 1 minute at the gene's annealing temp (detailed in Table 4), 2 minutes at 72°C, finally followed by 5 minutes at 72°C. The PCR reactions contained 50 ng of the prepared genomic DNA, 20pmol of each primer, 200µmol of the dNPTs, 10µl of 10x PCR buffer, 5 units of MolTaq polymerase (Molzym GmbH) and made up to a final volume of 50µl with filtered MilliQ water.

PCR products were run on a 0.8% agarose gel using a deoxyribonucleic acid (DNA) dye of SafeView Nucleic Acid Stain (NBS Biologicals) or Nancy-520 DNA Gel Stain (Sigma-

Aldrich) at 120V for ~45 minutes in order to see if the genes had been successfully amplified. Successful PCR products to be sequenced were cleaned up using a kit (Sigma-Aldrich) and sent, along with one of the primer pairs, to Source Biosciences (Nottingham, UK) for sequencing. Returned MLST sequences were then put into the dedicated PUB MLST database (Pubmlst.org - SequenceQuery), which allowed us to obtain an ID number for each of the 7 genes. It is the unique combination of gene ID numbers that give us the specific sequence type for the strain. Using the tools available on the University of Warwick Medical School website (http://mlst.warwick.ac.uk/mlst/) we were able to obtain the MLST of that strain from the 7 gene IDs.

The returned *fliC* sequences were aligned with all documented *E. coli fliC* serotype sequences. The alignments were all done using a National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) alignment search. The closest alignment gave the H-antigen serotype of the isolate.

3.5.2. MLST Strain Alignments

Full MLST sequences for the sequence types obtained were downloaded from the PUB MLST database (Pubmlst.org) as a concatenated virtual genome (~3kbp in size). These sequences were then aligned using ClustalX (Science Foundation of Ireland), which is multiple sequence alignment software [205]. This alignment was then transferred into Jalview (jalview.org), a free online tool designed for multiple sequence alignment editing, visualisation and analysis. This tool produces an alignment tree, from which the strain's clade can be deduced and visualised. The strains from this study were run alongside strains detailed in McNally *et al* (2013), which include *E. coli* from a wide range of pathogenic groups and disease areas (Figure 24, p.115) [90]. A detailed list of the names and sources of each of the reference strains utilised from the literature is given in Appendix 1 (p.209).

Gene	Forward primer	Reverse primer	Annealing temperature
adk	TCATCATCTGCACTTTCCGC	CCAGATCAGCGCGAACTTCA	54°C
fumC	TCACAGGTCGCCAGCGCTTC	TCCCGGCAGATAAGCTGTGG	54°C
gyrB	TCGGCGACACGGATGACGGC	GTCCATGTAGGCGTTCAGGG	60°C
icd	ATGGAAAGTAAAGTAGTTGTT CCGGCACA	GGACGCAGCAGGATCTGTT	54°C
mdh	AGCGCGTTCTGTTCAAATGC	CAGGTTCAGAACTCTCTCTGT	60°C
purA	TCGGTAACGGTGTTGTGCTG	CATACGGTAAGCCACGCAGA	54°C
recA	ACCTTTGTAGCTGTACCACG	AGCGTGAAGGTAAAACCTGTG	58°C
fliC	TCAACAACAACTTACAGCGT	GGTGTTGTTCAGGTTGGTGA	58°C

Table 4. Seven genes required for MLST and their corresponding primer pairs and PCR annealing temperatures (primer from University of Warwick Medical School website http://mlst.warwick.ac.uk/mlst/).

3.6. Enzyme Linked Immunosorbent Assays

The technique of sandwich ELISAs was chosen as a way of quantifying the different immune proteins chosen to analyse in the study urine samples. Ready-Set-Go!® ELISA kits (Affymetrix eBioscience) were used for the assays due to the repeatability of the similar protocols, allowing the completion of multiple protein assays at one time. This was essential to avoid multiple rounds of freeze-thawing of the urine samples, as it is known to deplete the levels of cytokines and chemokines each time [206].

Table 5 shows the proteins and the corresponding detectable range of each of the assays. All ELISAs were completed in Nunc MaxiSorp® flat-bottom 96 well plates (Affymetrix eBioscience). The manufacturers protocol was followed by for each of the kits. This involved coating the plate with the capture antibody and incubating this overnight at 4°C. After thorough washing and blocking for an hour at room temperature, the standards and diluted samples (1:2) were added to the wells. This was then incubated again overnight at 4°C. This allows the target protein to bind the capture antibody if present in the sample. The wells were then washed again before the biotinylated detection antibody was added, which attaches to any target protein which has bound to the plate via the capture antibody. After an hour incubation at room temperature and another wash step, the Avadin, conjugated with the detection enzyme, horseradish peroxidase (HRP) was added. This was incubated for 30 minutes at room temperature to allow this to bind the biotin on the detection antibody. After a final wash step the 3,3′,5,5′-Tetramethylbenzidine (TMB) solution is added to the wells for 15 minutes at room temperature. This will detect any HRP that is bound to the plate, yielding a blue colour change, which indicates the target protein was present in the sample or standard. Finally, the reaction is stopped by the addition of 2N sulphuric acid (H_2SO_4) which yields a yellow colour, the darker the colour the more protein of question there is present in the well.

The plates are then read at both 450 and 571nm using an Infinite® F50 / Robotic Absorbance Microplate Reader (Tecan Trading AG, Switzerland). The 571nm data was subtracted from the 450nm reads to eliminate the chemical colour change background before analysis took place. All plates were run with at least 2 blank wells, the average of which was deducted from all reads to eliminate the background of the assay, where antibody may have bound to the plate even in the absence of any target protein. The standard curve was then plotted

according to the manufacturer's instructions. The target protein concentration of the scaled up samples were read from the linear regions of these curves.

Healthy concentrations were calculated by taking the averages of all cytokine concentrations from the healthy control patients, except for 2 control subjects (6 samples). One of these was excluded due to their almost full load of *E. coli* throughout the sampling period and the other was excluded due to their positive HCP outcome on the dipstick throughout the sampling period. It was felt that these samples would therefore potentially skew the data as they were not normal healthy controls. This still left 39 healthy control samples from which to obtain the healthy averages. These averages were deducted from the cytokine concentrations obtained from the patient samples, in order to normalise them to the healthy baseline concentrations. These normalised concentrations are the figures reported and the figures used to calculate the fold-changes within each patient.

Protein	Standard Curve Range (pg/ml)
IL-1β	2 – 150
IL-4	2 – 200
IL-5	4 – 500
IL-6	2 – 200
IL-8	2 – 250
IL-10	2 – 300
IL-12 p70	4 – 500
IL-17A	4 – 500
TNFα	4 – 500
IFNγ	4 – 500

Table 5. All proteins analysed via ELISA in the study urine samples and the concentration ranges the assays were able to detect. Ranges show the upper and lower limit of the assays detection levels, indicting the assay's sensitivity.

3.7. Total Scores

In order to bring together statistical analysis from the many different study aspects it was necessary to normalise the data for each study sample. This would enable quantification of all areas of the study and make the samples comparable to one another as a cohort. This was done by assigning quantification to each factor of the study database from all aspects of the study analysis. The sum of these quantifications was referred to as the sample's 'total score. The method of quantification of the total scores is outlined in Table 6. The total score gave an overview of general sample health, with a higher score indicating more of a divergence from an expected healthy outcome. Once a total score had been calculated for each sample it became possible to break down the database and run statistical analysis on the various data sets.

3.8. Statistical Analysis

All statistical analysis and data visualisation was completed using GraphPad Prism® Version 6.01. Where 2 data sets are being analysed an unpaired t test with Welch's correction was used to calculate statistical difference between them. Welch's correction is a more reliable statistical tool when the samples being compared have unequal variances and unequal sample sizes. The samples being analysed here fulfilled these criteria. When comparing multiple data sets an ordinary one-way analysis of variance (ANOVA) was used to assess variation between the data.

Study Aspect	Factor	Scores
Blood (out of 5)	Abnormal serum creatinine	0 or 1
	Abnormal vitamin D	0 or 1
	Abnormal sodum	0 or 1
	Abnormal potassium	0 or 1
	Abnormal urea	0 or 1
Clinical (max 24)	Diabetic	Non-diabetic: 0, Pre-diabetic: 1, Diabetic: 2
	Most frequent UTI severity	None: 0, Mild: 1, Moderate: 2, Severe: 3
	Symptom score (out of 18)	Max 15
	Self-declared UTI	0 or 1
	Treated for a UTI within 3 days	0 or 1
	Urine sample for a UTI within 3 days	0 or 1
	Positive sample for a UTI within 3 days	0 or 1
Dipstick (max 22)	Dipstick score	Max 21
	HCP outcome	0 or 1
Cytokines (out of 10)	10 fold change in IL-1β	0 or 1
	10 fold change in IL-4	0 or 1
	10 fold change in IL-5	0 or 1
	10 fold change in IL-6	0 or 1
	10 fold change in IL-8	0 or 1
	10 fold change in IL-10	0 or 1
	10 fold change in IL-12 p70	0 or 1
	10 fold change in IL-17A	0 or 1
	10 fold change in TNFα	0 or 1
	10 fold change in IFNγ	0 or 1
Bacterial (out of 8)	Diagnostic levels of *E. coli*	0 or 1
	Motile	0 or 1
	Amoxycillin resistance	0 or 1
	Co-amoxyclav resistance	0 or 1
	Cephalexin resistance	0 or 1
	Trimethoprim resistance	0 or 1
	Nitrofurantoin resistance	0 or 1
	Ciprofloxacin resistance	0 or 1
Total Score		**Out of possible max: 69**

Table 6. Outline of sample total score calculation from all factors of the study. Performed on each study sample as individuals.

Chapter 4. Clinical Study Design and Setup

4.1. Study Design

Most previous studies into the field of ABU involve snapshot sampling. This is where a patient will give a single sample, usually at the time of symptom presentation. This, therefore, lacks the temporal aspect of what is occurring in the bladders of individual patients over time. It was for this reason that a longitudinal study was decided uopn, in order to follow changes in patients over time. The study was designed to collect urine samples from each patient roughly two weeks apart for 6 months. The time frame was chosen as it would give us good temporal resolution without over-burdening the patient and risking drop-outs in participation. This would result in a total of 12 urine samples from each patient.

After consultation with an independent statistician a minimum of 30 patients was chosen to power the study in order to infer statistical significance within the data set. This would therefore allow the study to become a pilot framework for the feasibility to explore a larger study framework within patients of this age group.

4.2. Ethical approval

In order to recruit patients through the NHS, identified via their medical records, we would need full Research Ethics Committee as well as NHS R&D approval. The study would also require sponsor from the Newcastle upon Tyne Hospitals (NuTH) NHS Foundation Trust. Mr Andrew Johnston from the Trust;s Joint Research Office (JRO) agreed to be the sponsor for the study, he agreed to sign all of the study documents for submission to the REC and R&D department. The necessary documentation was all produced and the online Integrated Research Approval System was used to submit this to the different approval bodies. All people involved with undertaking the study were required to have completed the necessary Good Clinical Practice (GCP) training.

The first submission to the 14-NE-0026 REC on 16TH January 2014, was rejected as they felt some of the study documents needed amendments. The design protocol and supporting documentation were all updated to reflect these amends. The second submission was approved with a favourable opinion from the REC on 12th March 2014. This approval allowed a ‘research passport’ to be obtained in order to allow access to the Urology

Department at the Freeman Hospital, Newcastle upon Tyne, as well as the medical records in order to begin patient recruitment.

4.3. Recruitment

Due to the high prevalence, difficulty in diagnosing and issues with successfully treating the older population, a lower age limit of 65 years was chosen. This would allow us to potentially see a range of patients in the upper age brackets, without limiting ourselves to those who are potentially less physical and able to undertake such a demanding study. It was essential that the patients were mentally capable to fully understand and give informed consent to participate in the study.

Potential patients were identified based on the lists of two UTI clinics that were held weekly in the Urology Department. Patients who fell into the correct age group were identified for screening. These patients were then screened for eligibility based on their medical records. Patients were selected for follow up based on them fulfilling the following inclusion criteria;

- Patients must be over the age of 65.
- Patients must have had 2 or more positive urine samples on their record within the last 6 months (alternatively 3 or more within 12 months). This is the clinical definition of recurrent UTI.
- Urine cultures must have been sent on due to a suspected uncomplicated UTI as opposed to an underlying medical condition or previous procedure (e.g. cystectomy, urinary conduit, bladder cancer etc.).
- Patients must not be catheterised (either permanent or intermittent self-catheterised).
- Patients must not have any mental or physical disability that could stop them giving informed consent or completing the study in a way that was safe or a burden on their quality of life.

If patients fulfilled these criteria, they would be contacted after their appointment on the day of the clinic for a quick meeting. At this meeting the study was explained and they were given all study information. After a minimum of 48 hours, patients were contacted by telephone to gauge their interest in taking part in the study. If they expressed interest a baseline visit was arranged at a time of their convenience.

4.4. Consent and Baseline

At the baseline visit the study was explained again and patients were given the chance to ask any questions they had or express any concerns. If they fully understood the undertaking of the study and were prepared to go ahead, informed consent was taken and witnessed by the research team member (Appendix 2, p.211). A Case Report Form (CRF) was then completed asking them basic questions about their personal details, urinary history and antibiotic usage (Appendix 3, p.213). A blood sample was taken by a trained NHS nurse, one vial was sent to the hospital laboratory for analysis for sodium, potassium and urea, and the second vial was stored in the study biobank within the university. The first urine sample was then collected and the first questionnaire was filled out (Appendix 4, p.217). Patients were then supplied with all paperwork and consumables they would require for the remainder of the study. A sampling method and schedule that suited them was arranged. A copy of the signed consent form was given to the patients upon the second urine sample (or sent via post to them) for their records (Appendix 2).

A total of 31 patients agreed to take part in the study, however one patient dropped out as the burden of the frequent sampling was too difficult. Figure 5 shows a full Consort diagram of the recruitment and undertaking of the study, including all reasons for refusal of participation. The main reason for non-participation was their inability to undertake the frequent sampling involved with the study.

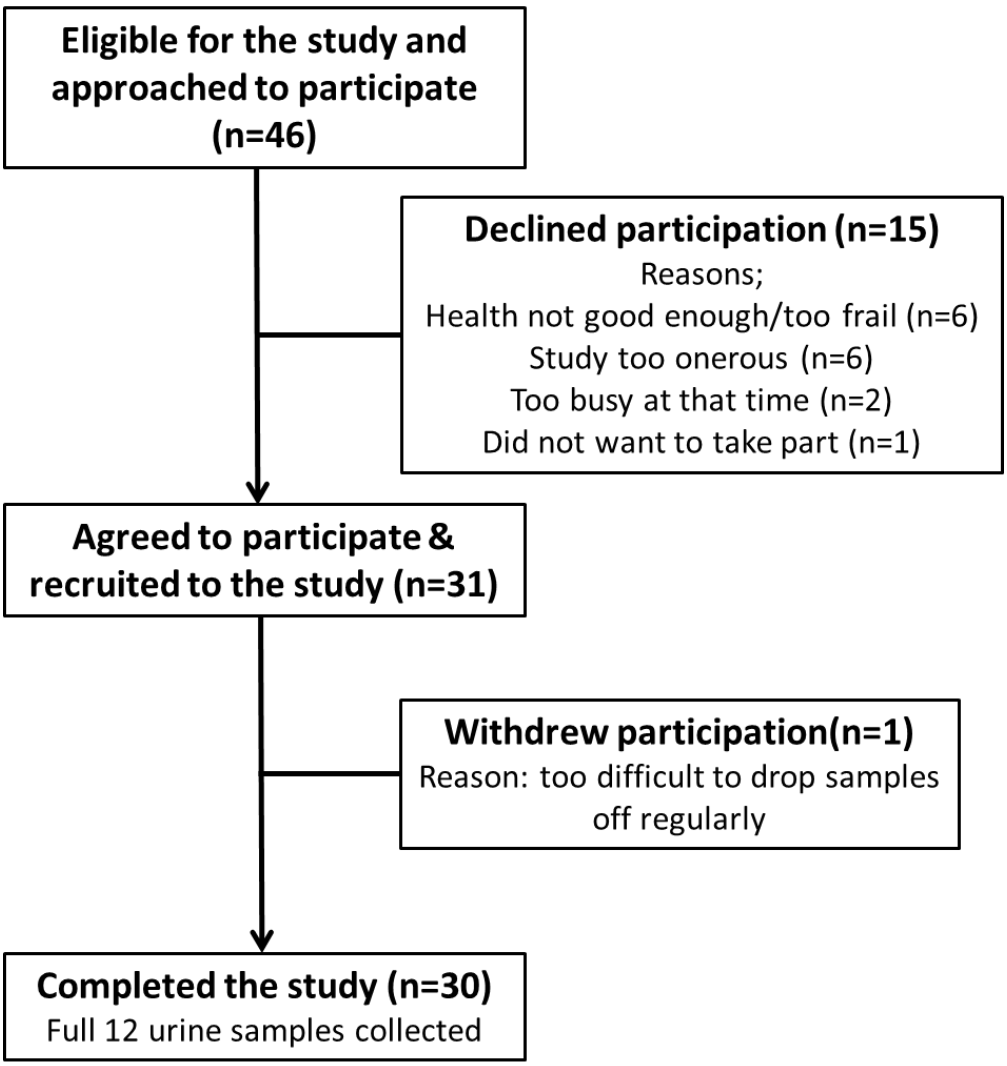


Figure 5. Consort diagram of recruitment of patients to the clinical study.

4.5. Sampling and Storage

For the remaining 11 visits a urine sample and corresponding questionnaire was collected from each patient. For 29 of the 30 patients, samples were collected in person. One patient sent their samples via post, using the Royal Mail pre-paid Safebox® system (containing no preservatives), as they lived too far away to deliver the samples by hand. Samples would be processed within 2 hours of collecting, samples stored for any longer than this were kept at 4°C until collection. However, these would be collected no longer than 4 hours after being stored.

Upon collection all urines were analysed with a dipstick as this is standard protocol within the NHS to identify infections in a sample [28]. However, for completeness and maximising data acquisition, outcomes of all aspects of the dipstick were collected, not just those that indicate infection. In addition, any potential outcomes of an analysis of dipstick data could be easily implementable in the NHS, as this was already a standard test done on these samples.

E. coli was chosen as the pathogen of focus for this project due to the fact it is estimated to be responsible for causing as many as 90% of all UTIs [100,101]. The decision to select only one colony of *E. coli* was made early on in the study design process. One reason was to keep the analysis to a manageable level, as higher than this would have been a minimum of 3 colonies which would have yielded over 500 isolates. This would have been too many to sequence within the time and budget of the project. Another reason was that the study design was intended to, again, mimic the current practice in the NHS as closely as possible for comparative reasons. NHS laboratories would also select one colony for further testing (e.g. antibiotic resistance) as normal practice. However, it has since been recognised within this study that the urine cultures are able to harbour more than one strain of *E. coli* at the same time, so perhaps in future studies the selection of multiple colonies would be advisable.

The first study sample was collected on 28th April 2014 and the last was on 17th November 2015. This meant the patient arm of the study ran for a total of 82 weeks (roughly 20.5 months). Figure 6 shows the different timelines from all 30 study patients. Week number 49 showed the most patient participation at any one time in the study, wherein 14 different urine samples were collected in that one week. Urine samples were collected on average 2.3 weeks apart. The average number of weeks the study ran for when looking at all the patient timelines was 26.7 weeks, the lowest being 21 weeks and the highest being 41 weeks. When looking at the study in terms of patient days, this study ran for a total of 5600 patient days.

The healthy arm of the study averaged 5 weeks and covered a total of 525 participant days. This appears to be higher than other longitudinal studies pf this kind reported in the literature, with the next highest observing 5289 patient days [202].

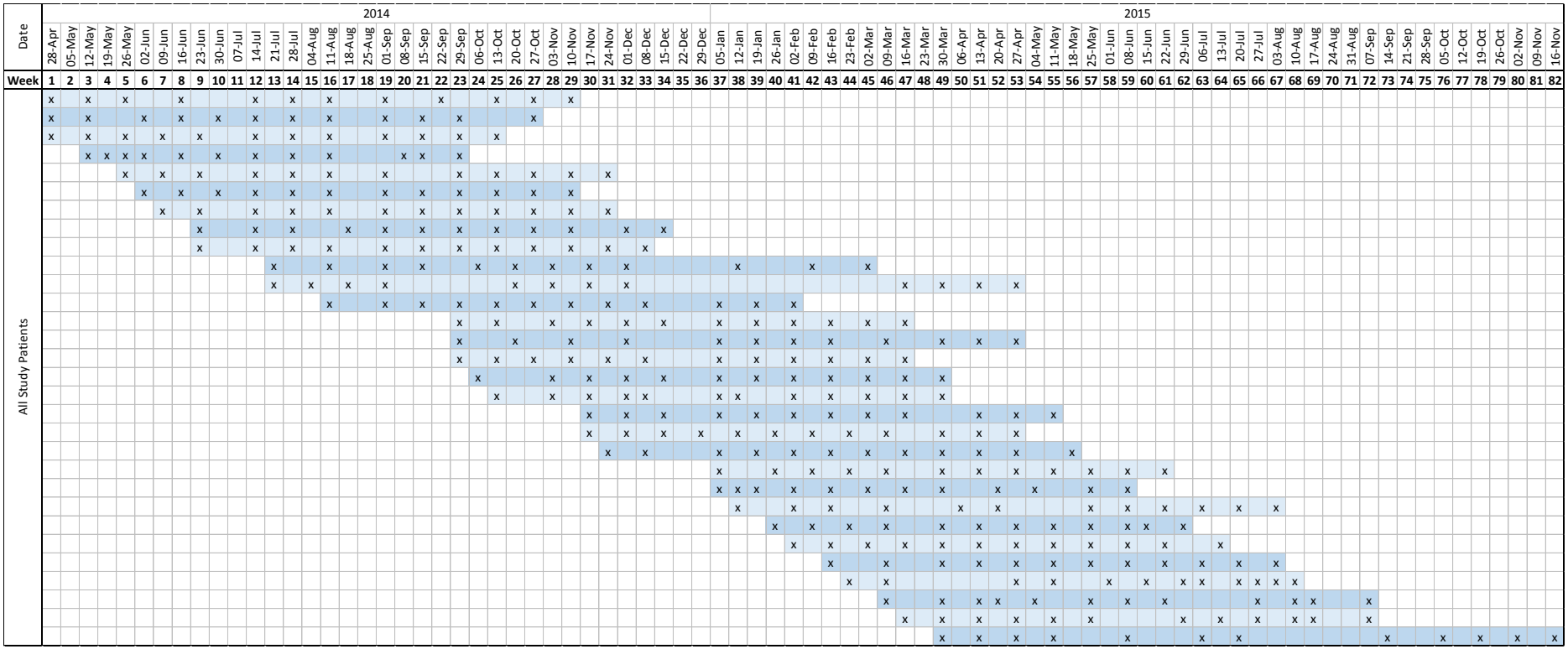


Figure 6. Timeline of all donations from all study patients. Each row represents the timeline of a different study patient. An 'x' indicates a urine donation from that patient. Patients were given chronological numbers for the duration of the study, these were then replaced with randomised patient numbers (used throughout this thesis) for anonymising the analysis. It is for this reason patient identifiers have been removed from this figure, in order to protect their anonymity as per the ethical codes in place.

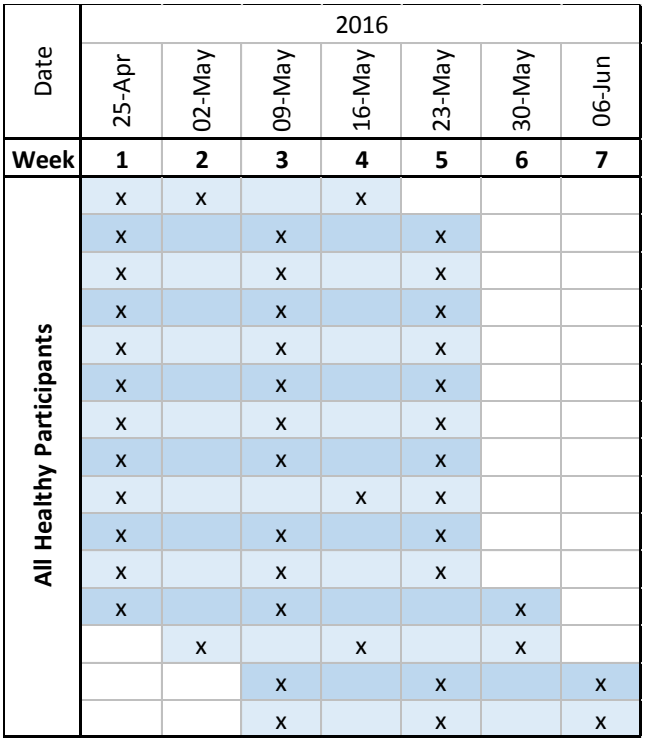
4.6. Healthy control participants

It was decided during the analysis of the patient urine that healthy age-similar control urine samples were needed for comparison. This comparison would provide 'normal' estimates of the proteins of interest in the urine so that the patient levels could be analysed more effectively. It cannot be assumed that the bladders of younger individuals behave in a similar way to the elderly bladder, both from a structurally functioning and immune perspective. Therefore, it was essential that the healthy controls must be a similar age to the patient cohort for comparability purposes. These samples would also allow for observation of the 'normal' flora in the otherwise healthy individual. Thus seeing if, for example, *E. coli* can also be isolated. A substantial amend to the ethics in place was submitted, as the changes involved a significant change to the study protocol. The request for an amendment was submitted on 25th February 2016 and a favourable ethical opinion was received on 15th March 2016 from REC 14-NE-0026.

We had the support of a local organisation called VOICENorth, which is a collection of volunteers of all ages willing to take part in research studies and trials. With the help of VOICENorth a list of 16 willing participants was produced. These were all contacted by either telephone or email, whereby the full study was explained. If they were still interested in taking part a meeting was organised. Of the 16 names provided, 1 was not contactable (incorrect email and unresponsive on email/telephone), however the remaining 15 all agreed to participate. The criteria for inclusion in the study were that participants had to be willing, able and available to participate in the study, that they were 65 years of age or more, had not suffered from a UTI in the past 5 years, had no history of diabetes and were not currently catheterised.

At the meeting they were given all study documentation and given a chance to ask any questions they had. If they fully understood and were agreeable to participate witnessed consent was taken. A CRF was then completed asking them basic questions about their personal details, urinary history and antibiotic usage. The first urine sample and questionnaire was then taken, and all paperwork and consumables needed was provided to the participant. Healthy patients gave a total of 3 urine samples 1-4 weeks apart depending on their availability. This allowed us to mirror the temporal aspect of the patient arm of the study without collecting an excessive amount of control samples. All urine samples were treated in the same way as patient urine samples in terms of collection, initial analysis and storage.

The first healthy control urine sample was collected on the 25th April 2016 and the last was on 6th June 2016, as detailed in Figure 7. This meant the control arm of the study ran for 7 weeks in total.



Date	2016						
	25-Apr	02-May	09-May	16-May	23-May	30-May	06-Jun
Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**
All Healthy Participants	x	x		x			
	x		x		x		
	x		x		x		
	x		x		x		
	x		x		x		
	x		x		x		
	x		x		x		
	x		x		x		
	x			x	x		
	x		x		x		
	x		x		x		
	x		x			x	
		x		x		x	
			x		x		x
			x		x		x

Figure 7. Timeline of all donations from all healthy control participants. Each row represents the timeline of a different participant. An 'x' indicates a urine donation from that participant. Healthy participants were given chronological numbers for the duration of the study, these were then replaced with randomised participant numbers (used throughout this thesis) for anonymising the analysis. It is for this reason participant identifiers have been removed from this figure, in order to protect their anonymity as per the ethical codes in place.

4.7. General study statistics

Upon completion of the study, the basic data collected from both patients and healthy controls could be reviewed. This made it possible to find out more about the populations being observed within the study.

4.7.1. Study Patient Demographics

The age range for the 30 patients who completed the study was 65-86 years and the average age was 75 years (Figure 8A). Twenty-three of the patients were women (76.7%) and the remaining 7 were male (23.3%) (Figure 8B). This is to be expected as UTIs are far more commonly seen in women than men.

4.7.2. Healthy Control Demographics

The age range for the 15 healthy controls was between 65-88 years of age, with the average age being 72 years (Figure 8A). There was no significant difference between the ages of patients and healthy participants (p=0.21), which means age will not be a variable when analysing data from the controls as a comparison to patient data. It must be noted that these significances are based on sample sizes of 30 patients and 15 healthy controls, but they are used to display the overall lack of difference within the cohorts with respect to age, with no inference to the wider population. The healthy control cohort was made up of 10 males and 5 females (Figure 8C). This ratio was not surprising, as it is known that UTIs are much more common in females. Therefore, finding healthy males with no recent history of UTIs is far more likely [26,207].

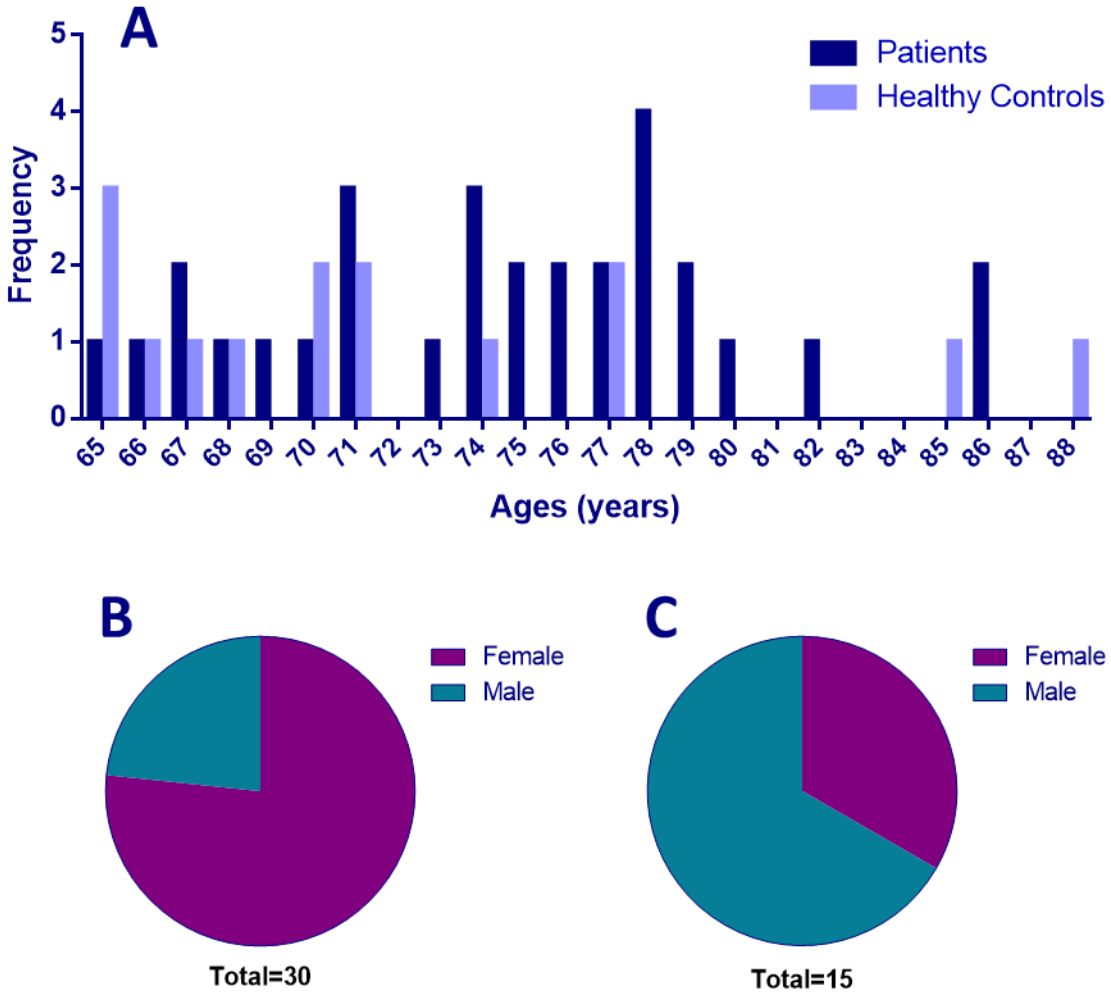


Figure 8. Basic study data of study patients and healthy control participants. 'A' shows a frequency histogram of the distribution of ages of study patients and healthy control participants at the time of recruitment. 'B' shows the split of sexes among the study patients. 'C' shows the split of sexes among the healthy control participants.

4.8. Urine and Bacterial Biobanks

One of the main outcomes of this project was the large volume of samples that have been produced as a result of the study. 30 patients were recruited to the study as a minimum number to power statistical inference within a pilot study. However, due to the frequent sampling from each of these patients it meant that a very large biobank of urines was produced. This would therefore allow for both analyses between individual patients as well amongst the large number of individual samples themselves.

A total of 360 urine samples were collected from the 30 study patients. From these urine samples, *E. coli* was isolated 184 times. This means that 51.1% of all the patient urine samples has some form of *E. coli* colonisation. All urine samples were frozen as an unfiltered and 3 filtered aliquots. This produced 360 unfiltered and 1080 filtered urine samples available for analysis. In addition to this the study yielded over 720 pictures of the plates and 360 plate washes, these would allow the future study of other potential uropathogens that were not in the direct remit of the project. Thirty blood samples were also frozen down into the study biobank.

The healthy database produced 45 unfiltered and 135 filtered urine samples. 90 plate pictures and 36 plate washes, this was lower than 45 because several of the healthy control samples were completely clear of any growth on the chromogenic identification plates. *E. coli* was isolated from 2 of the healthy control samples (8.9%).

4.9. Geographical Spread of Participants

All patients and healthy volunteers were recruited from the city of Newcastle upon Tyne and surrounding areas. Patients were recruited from weekly urology clinics at the Freeman Hospital. These clinics cover a vast catchment area of Newcastle upon Tyne and Northumberland. The next closest urology clinic is held at the Queen Elizabeth Hospital in Gateshead, which will likely explain why there are relatively few patients south of the River Tyne in our study (Figure 9). There are also clinics in North Tyneside and Northumberland which are further afield. Healthy Participants were recruited from Voice North whose members cover a large area of the north east of England (Figure 9). There was a reasonably even spread of locations among both patients and healthy participants. It might be noted that the healthy participants have a wider spread on the outskirts of the city centre than patients.

This may be due to the fact that healthy participants were only required to give 3 urine samples and a longer travel distance was less of a deterrent to take part in the study. This larger spread of healthy patients may also be due to VOICENorth having a larger footprint than that of the Freeman Hospital urology clinic. Whereas patients were required to provide 12 samples so those living far from the city centre or Freeman Hospital were more likely to decline participation in the study. This was often the reason given for declining the study based on it being too onerous (Figure 5, p.62).

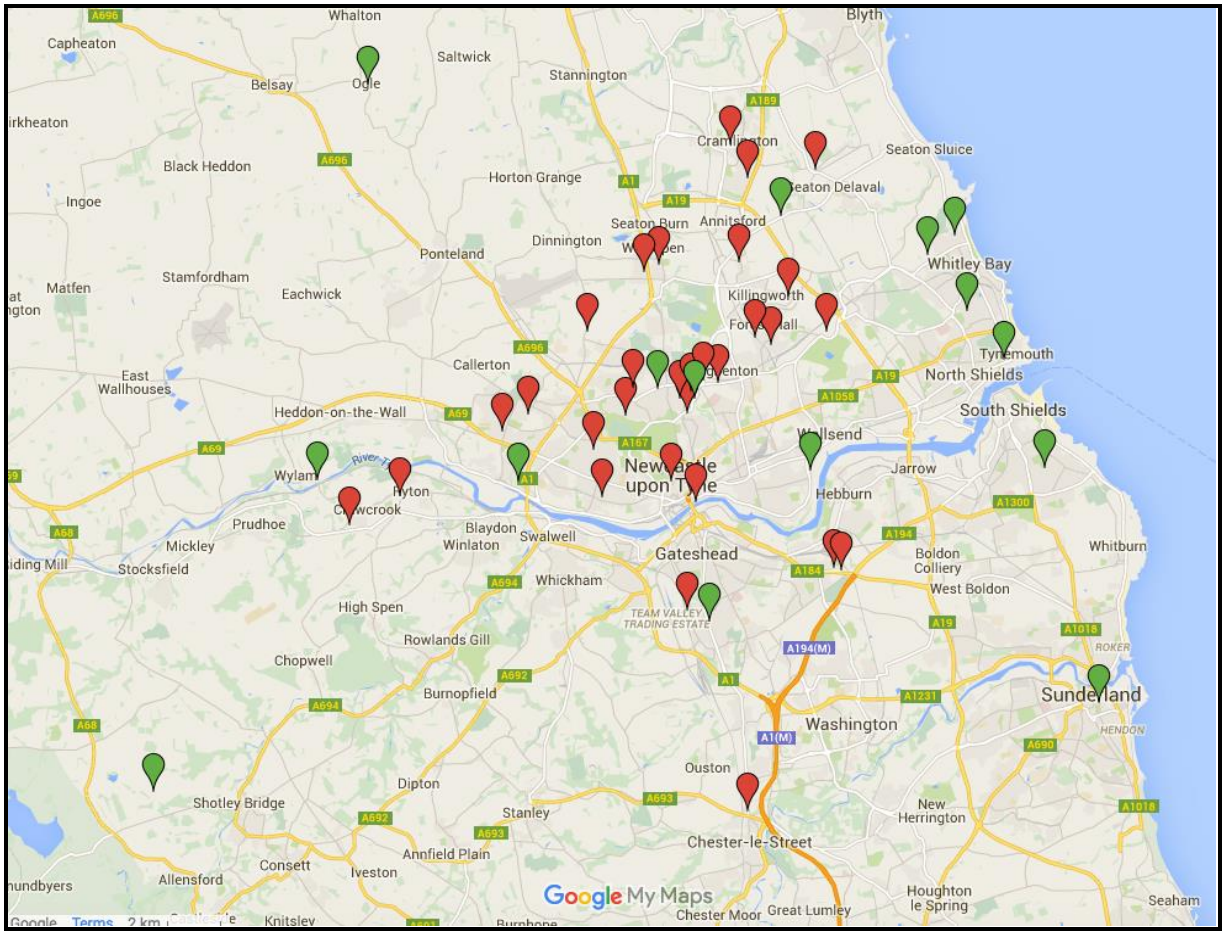


Figure 9. Geographical locations of all patients and healthy participants in the study. Patients' locations are marked with a red placeholder and healthy volunteers with green (Produced in Google Maps™).

4.10. Conclusions

Czaja *et al* (2009) showed that regular urine sampling was possible in women aged 18 to 49 for up to 3 months [202]. However, completion of this study has shown that such sampling over an even longer period of time is feasible within patients over the age of 65. Most participants were extremely willing to give up their time in order to complete the full study, with only one patient withdrawing. The study was made possible by a highly dedicated and well trained team of research nurses. The study required a lot of organisation and one-on-one time in order to run smoothly, this was carried out by both the chief investigator and research nurses. Thus, if this study is to be repeated or scaled up, a committed team would need to be in place. For the 30 patients who undertook this study it required 1 chief investigator and 3 research nurses. Therefore, proportionally more team members would be needed if more patients were required in order to ensure the correct resources were in place for smooth running of the study. The full study took 21.5 months to undertake, with a maximum of 14 patients participating at any one time. With a research team of this size, it is unlikely the number of participating patients could be increased by much without the need for a larger team and a longer timescale. The healthy control arm took just 6 weeks to complete. This was completed after the main patient study was finished due to the dynamic and results-directed style of analysis which took place in this project. However, the healthy control arm could have been undertaken overlapping the patient study, perhaps towards the end where the schedule was quieter. This would have taken place, however, the study design was amended to include healthy controls quite late into the project. This happened after completion of some of the patient analysis, which made it evident that control urines were required for baseline healthy measurements.

One of the most important outcomes of the whole project is the large biobank of samples which has been produced as a result. It is on this constructed biobank that the whole project's analyses will be run. This includes, from both the study patients and healthy control arms, a total of 1,215 filtered urine samples, 405 unfiltered urine samples, 188 *E. coli* isolates and 30 blood samples. These would allow for extensive analysis into the host immune response proteins within the urines and the phenotypic and genotypic changes of the bacterial isolates in order to address the aims of the project. In addition to the lab samples collected, the study also produced a large volume of data from the study questionnaires associated with each donation. Information was also collected from patients' medical notes on when they submitted urine samples for a suspected UTI to their GP and whether this came back positive

or negative. This information along with the questionnaire data would allow deduction of changes in disease state as well as any changes in symptoms. Importantly, this information would allow a definition of symptomatic episodes to be decided upon for analysis purposes. Thus giving the ability to analyse any changes in the host and bacterial data around periods of symptoms, potentially allowing for identification of predictive markers.

By not placing too many restrictions on the participation criteria, the study was able to give a more representative method of recruitment. Essentially the study would give a representative cross-section of the type of patients in this age group attending these clinics on a day-to-day basis. By not restricting participation the study was able to give a more realistic overview of the types of patients suffering from ABU and being seen by HCPs in a clinical setting. It also allows observation of the types of patients who are willing to participate in a study of this design, which may be useful to inform larger, longitudinal studies on similar patient cohorts.

Chapter 5. Patient and Clinical Data

The study not only provided the opportunity to analyse the urine samples which were collected, but also allowed observation of the patients' personal and clinical data. This included information provided by the patients themselves as well as relevant information that could be obtained from their medical records. By continuing to analyse the patient population, it becomes possible to further position the study within the patient population, allowing insight into the type of people suffering from recurrent UTIs in this age group.

Analysis of the collected clinical data from the patients included treatment data from the patients' medical records and symptom data from the study questionnaires (Appendix 4, p.217). It was included in the study set up that each patient would complete a short case report form (CRF) which would give some basic personal information as well as current treatments and urinary history (Appendix 3, p.213).

The questionnaire data was of vital importance to the study design as it would allow pairing of the laboratory analysis of the urine and bacteria with the human aspects of symptoms and treatment. Without such pairing the study would lack the ability of being able to retrospectively identify periods of change in urinary disease status.

5.1. CRF Data

CRFs are commonly used in clinical studies to gather basic personal and medical information on each of the study participants. The questions are usually geared towards enabling the analyses to be broken down by different patient attributes, such as sex and age. The CRF aimed to collect as much potentially relevant patient information which could be helpful during the analysis. It was also important that the questions were not too personal (e.g. sexual activity) as this would likely discourage participation, especially among older participants.

In addition to the basic patient information on age, sex and location, discussed in the previous chapter, the CRF also collected information on the patients' medical histories (Appendix 3, p.211). These included the diabetic status of the participants, any oestrogen supplementation in the female participants, an estimate on the number of UTIs they had suffered in the past year and their perceived symptom severity upon suffering from a UTI. This was complimented with questions collected from the patients' medical notes, such as the number

of positive urine samples submitted by each patient in the past year and the status of their urinary tract.

Some form of oestrogen supplementation was being used by 9 of the women (39.1%) (Figure 10A). Five of the patients in the study were diagnosed as diabetic (16.7%) and one as pre-diabetic (3.3%) (Figure 10B). None of the healthy controls were diabetic and none were taking oestrogen supplementation. However, this was not due to any restrictions placed on recruitment.

The majority of study patients had normal urinary tracts and just 36.7% had a tract status that was classified as abnormal. Of those that were abnormal, all were due to a structural defect, no patients had any functional defects in their urinary tract. The main reasons for patients having an abnormal urinary tract was due to a form of prolapse in the females and prostatic enlargement in the males (Figure 10C).

With regards to the most common symptoms seen in patients, the severities were broken down into mild, moderate and severe symptoms. Mild symptoms referred to patients who noticed smelly/cloudy urine, suffered from increased frequency of needing to urinate and feeling pain when passing urine. Moderate symptom severity was for patients who felt they tended to suffer with some systemic flu-like symptoms in addition to those seen in the mild symptom bracket. Severe symptoms involved patients suffering from fever/rigors and loin pain at the time of a UTI. One patient stated that they did not feel they suffered from this scale of symptoms, but had other ways of suspecting they had a UTI, unfortunately they did not give any more information on this. However, the vast majority of study patients (70.0%) stated that they tend to suffer from mild symptoms (Figure 10D).

By asking patients to estimate the number of UTIs they suffered from in the past year we were able to compare this to how many positive urine samples were submitted for NHS investigation. This would allow comparison of any disparity between what the patient sees and what the NHS sees (Figure 11). Only 4 of the 30 study patients stated the same number of UTIs as positive urine samples in the past year. In just 6 patients they underestimated the number of UTIs they had suffered in the past year, compared to how many positive urine samples the NHS labs had received. The majority of patients (66.7%) over-estimated the number of UTIs that they felt they had suffered from compared to the numbers seen by the NHS laboratories. This may indicate that patients feel they are suffering from symptoms more than they in fact are. They may perceive passing diffuse urinary symptoms with full

UTIs, which would explain why they estimate the number to be higher than those documented in their medical notes. It could however, alternatively, be an indication that some patients suffer from more UTIs but they do not always go to see a healthcare professional about them. This may suggest that some patients are better able to tolerate UTI or urinary symptoms than others. This variability in urinary symptomatic tolerance was also observed in the study undertaken by Czaja *et al* (2009) [202].

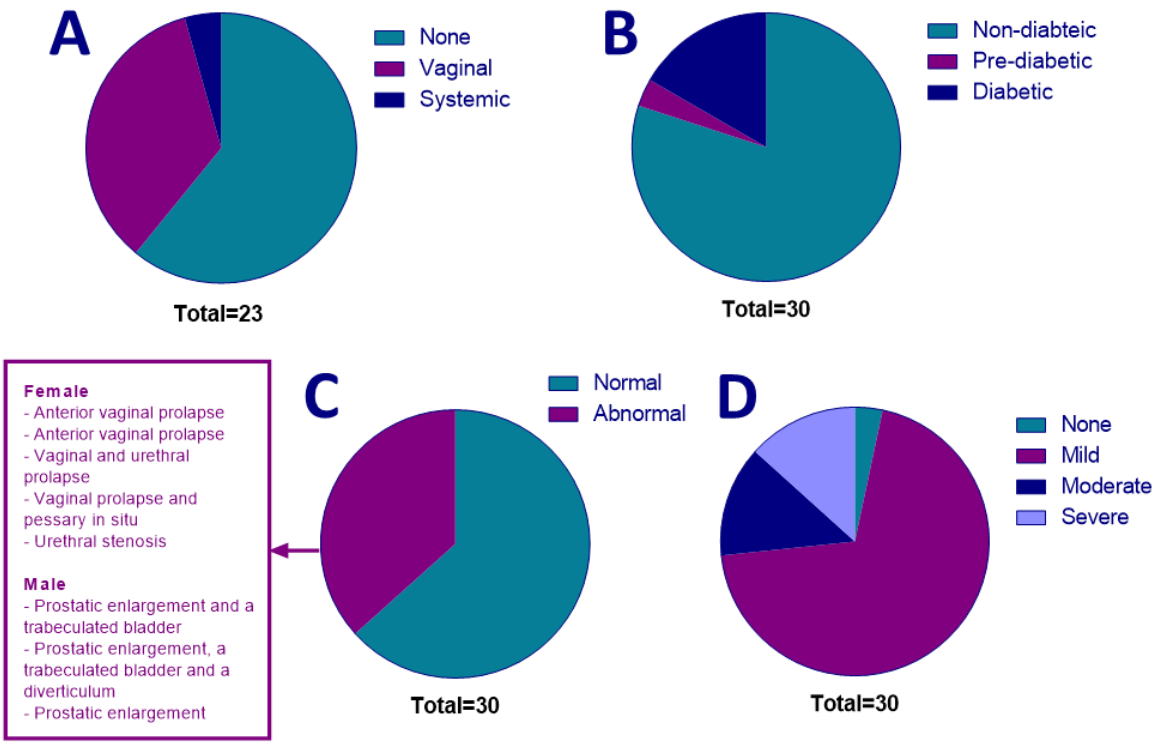


Figure 10. CRF data collected from study patients. 'A' shows the split of female study patients using different forms of oestrogen supplementation. 'B' shows the amount of study patients who were diagnosed as diabetic and pre-diabetic. 'C' shows the urinary tract status of study patients, the annotation gives details of the patients who had abnormal urinary tracts, split by females and males. 'D' shows the perceived level of UTI symptom severity they usually suffer from.

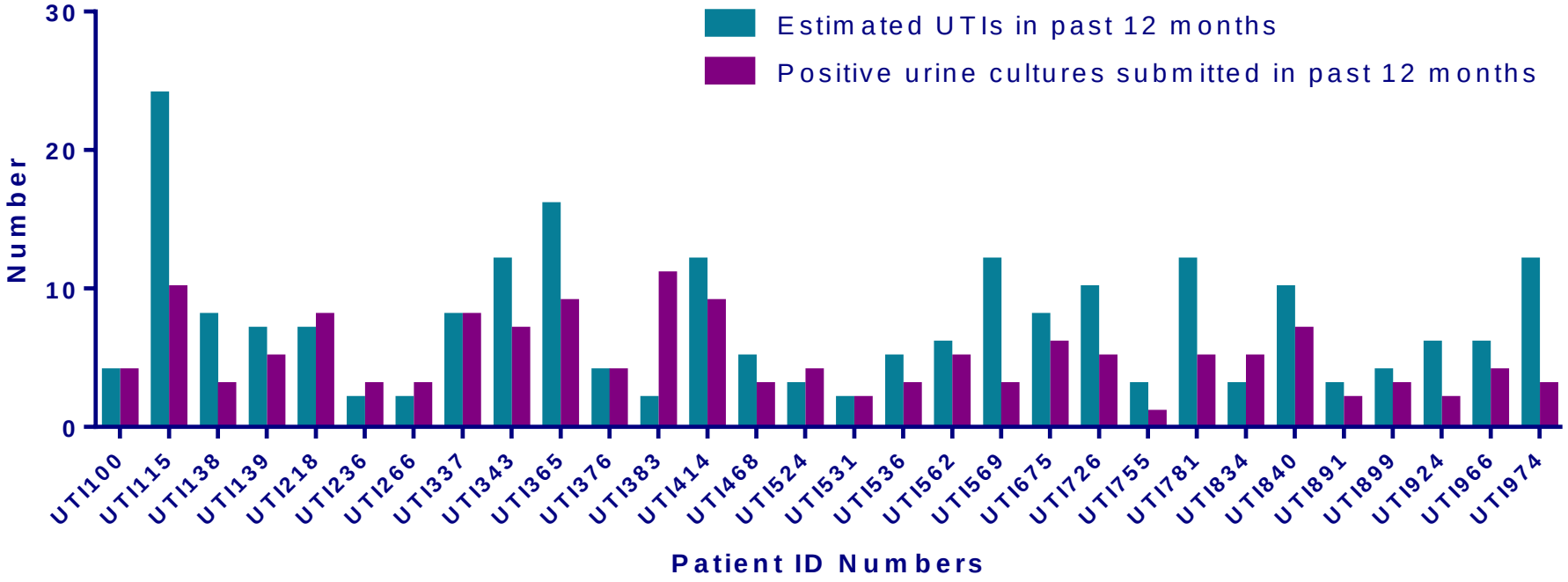


Figure 11. Comparison of the number of UTIs patients estimated they had suffered in the past 12 months, compared to the number of positive urine cultures submitted by the patient in the same time frame.

5.2. Symptom and Treatment Data

With each urine sample patients were asked to fill in a short questionnaire on their current symptomatic state with regards to their urinary tract (Appendix 4, p.217). The questionnaire also collected information about any antibiotics they had recently taken since their last donation, for both UTIs and any other infections. The questionnaire filled in with each urine donation asked patients about how they would rate their current urinary symptoms at the time of sampling. These were broken down into 6 questions;

1. Frequency of urination (going to the toilet very often)
2. Urgency of urine (a strong and uncontrollable urge to pass urine)
3. Pain or burning when passing urine
4. Not being able to empty your bladder completely/passing only small amounts of urine
5. Low back pain
6. Visible blood in your urine

Each question allowed patients to give an answer of '0: did not have', '1: mild', '2: moderate' or '3: severe' (Figure 12). During the course of the study the most frequently reported UTI symptom was frequency of urination with 63.1% of samples returning a score of mild to severe. This was followed by the urgency of urination symptom being mild to severe for 56.7% of all study samples. Frequency and urgency were the most common symptoms to be reported as severe, in 28.6% and 27.5% respectively. Pain when passing urine, incomplete voiding and lower back pain symptoms were all reported as absent in the vast majority of all study samples. Blood in the urine was only stated to be a symptom for 2 samples throughout the study, and even then it was only reported to be mild. Patients were significantly more likely to report UTI symptoms of frequency and urgency than any of the other listed symptoms ($p=0.03$). It is not surprising that the frequency and urgency were the most commonly reported symptoms in these patients, as these are very common symptoms in the older population. They do not necessarily indicate an infection and are often commonly misinterpreted [29]. This may mean that symptom scores are not the most reliable method of defining periods of symptoms for the study.

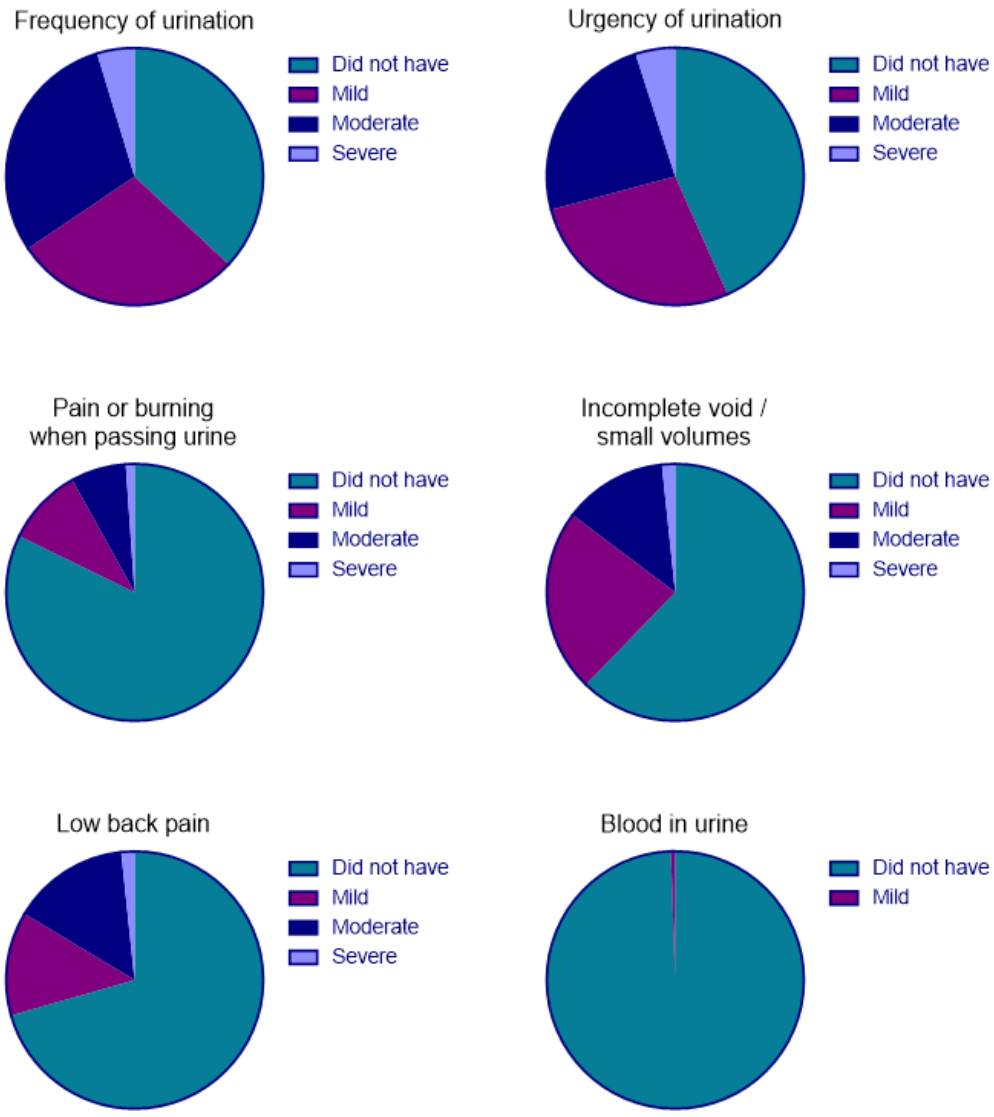


Figure 12. Frequency of each symptom being experienced throughout the study. Results from all n=360 urine samples from the study, taken from the responses given on the questionnaires at the time of collecting the study urine.

5.2.1. Changes in UTI Symptoms and Periods of Treatment

To compliment the symptom breakdown analysis, it was also possible to use patients' feelings, treatment and urine sample submissions as potential ways of deducing periods of symptoms within the study. Patients were asked on the study questionnaire, 'do you feel you are suffering now from symptoms of a urinary tract infection?' (Appendix 4, p.217). This was a simple yes or no question for the patients to answer at the time of sampling. This simple statement enabled collection of a black and white decision made by the patient on whether they felt they had a UTI at that time (Figure 13). They were allowed to then expand on their reason for selecting each option, the results of which will be discussed later in this chapter.

Patient suspicion was compared to the symptoms scores derived from Figure 12, antibiotic treatment and positive NHS urine samples (Figure 13). These data were collected from both the questionnaire data and the patients' medical notes. The comparison of these factors clearly shows that there is a large disparity between when patients felt like they had a UTI at the time of sampling and when they actually went to a physician to provide a urine sample (Figure 13). For example, in patients UTI218 D5, UTI365 D7, UTI899 D10 and several times in patient UTI781.

In addition, from the questionnaire it could be seen that 12 of the declared UTIs were based on the smell of the urine, which is not technically a symptom which would require treatment. Thus using the patient declared UTI information is not going to be the most reliable definition of a symptomatic state for the purpose of this project's analysis. The symptom scores are statistically higher when patients declare themselves as having a UTI ($p<0.0001$) (Figure 14A, p.88). This may be to be expected as the symptoms and self-declared UTI information was all taken together on the questionnaire, so a fairly strong correlation is to be expected. Having said this, it is also interesting to note that when patients give a high or higher than normal symptom score they do not always state they feel they currently have a UTI, which suggests the patients themselves may have some confusion as to what their symptoms mean. This can be seen, for example, in UTI138 D2, where a symptom score of 12 is obtained, one of the highest seen in this patient, however they do not declare that they feel they have a UTI at that time. UTI138 does declare a UTI later in the study but with far lower symptom scores. The same can be seen in UTI383 D1, UTI468 D3 and UTI675 D3. There were also several patients where relatively high symptom scores were given yet no UTIs were ever declared, including in patients UTI524, 531, 755, 924, 966 and 974. In addition to this,

patients UTI138 and 383 seem to self-declare they have a UTI for much of the study without providing a positive urine sample or seeking treatment, further highlighting what seems to be confusion on behalf of the patients with respect to their urinary symptoms. It should be noted that in UTI383 a positive urine sample was submitted 4 days after the first donation, this may explain why the patient felt they had a UTI after that. However, this doesn't explain the delay in them starting antibiotic treatment.

Samples which were given within 3 days of some form of antibiotic treatment for UTIs correlated with significantly higher symptom scores (Figure 14B). However, the treatment data also shows a lot of variation between when a positive urine sample was handed in and prescription of antibiotics (Figure 13). For short courses this suggests patients are being treated without having their urine checked for bacteria, which is not following the proper guidelines. Thus, these periods of treatment are likely not the best indicators of a symptomatic state. As well as this, 11 of the study patients were on prophylactic antibiotics at some point during the study. This means they may not have been symptomatic at the time of sampling, which is another reason treatment is also not going to be the most reliable definition of a symptomatic state. A couple of occasions can be observed where prophylactic treatment was able to keep patients from suffering with UTI symptoms, such as patients UTI365 and 524 (Figure 13). However, it can also be seen that long periods of antibiotic prophylactic treatment were not sufficient at avoiding increases in symptom scores and self-declared UTI episodes in several patients. For example, this can be seen in patients UTI100, 343, 383, 675 and 726. Therefore, this data may suggest that long courses of antibiotics are not necessarily appropriate for evading symptoms in these patients.

A positive urine sample, shown in Figure 13, indicates where a patient has provided a urine sample for a suspected UTI and it has been returned as a positive culture according to the NHS laboratory protocols within 3 days either side of collecting the study sample. Three days was selected as this would mean the patient's body was still reacting to the infection, which would be essential for the urinalysis aspect of this project. The majority of positive urine samples (63.6%) was accompanied by treatment within 3 days of the study sample or by the next study sample. However, this does leave 8 positive urine samples which remained untreated. These were in patients UTI343, 365, 562, 569, 675, 840, 891 and 924. This means that the patient did not receive a follow up course of antibiotics, perhaps suggesting the symptoms resolved on their own. This may explain why there was no significant difference seen in symptom scores between samples that were accompanied with a positive urine sample

within 3 days and those that were not (Figure 14C). Regardless, a positive urine sample is likely to be the most reliable definition of a symptomatic episode for the purpose of this study, from the perspective of the NHS. As in order for the patient to have submitted a positive urine sample it would mean that they have felt symptomatic, made an effort to go and see a HCP, the HCP has deemed their symptoms to be fitting of a potential UTI and sent a urine sample to a hospital laboratory. This decision may or may not be aided by the use of urine dipstick analysis. Finally, that the sample fits the NHS' microbiological guidelines for a defined UTI. However, it should be noted that defining a symptomatic state is not straight forward due to confusion amongst patients, and untreated urine submissions.

In addition to this, there were 14 occasions during the study in which negative urine samples were submitted within 3 days of a study sample (data not shown). Below is a list of the patients and the corresponding donation (D) numbers that these took place in the study;

- UTI337 D11
- UTI365 D2
- UTI414 D7
- UTI524 D2
- UTI524 D3
- UTI524 D4
- UTI726 D3
- UTI726 D8
- UTI755 D4
- UTI755 D5
- UTI834 D2
- UTI834 D6
- UTI840 D2
- UTI840 D3

Whilst these may not be of importance to the NHS, it still implies the patient and HCP felt that the symptoms indicated a possible UTI. Only 3 of these negative samples correspond to occasions where patients felt they were suffering from UTIs, these were in UTI337 D11, UTI726 D3 and UTI840 D2. Interestingly, 9 of these 14 negative samples still correlate with antibiotic treatment being started at that donation or by the next study sample. For example, in patients UTI337 D11, UTI726 D8 and UTI840 D2. This could potentially mean that HCPs are initiating antibiotic treatment before awaiting a positive result from the urine sample analysis, a possible flaw in the current treatment pipeline for these patients.

Patients UTI337 and 343 and 569 are all examples of patients who seemed to suffer greatly with urinary complaints during the course of the study and clearly worked with their HCP to rectify the situations with sending off regular urine samples and initiating antibiotic treatment. It is clear here that the clinical outcomes are in agreement with the patient's

perspective. Similarly, in patients UTI218, 236 and 840 isolated episodes can easily be identified, and again there is correlation with the perspective of the NHS and the patient.

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI100	Symptom score	3	11	4	3	1	8	0	2	4	1	4	1
	Suspect a UTI		■										
	Treated		■	■	■	■	■	■					
	Positive sample	■											
UTI115	Symptom score	15	0	0	0	0	0	0	0	0	0	0	0
	Suspect a UTI	■											
	Treated	■	■	■	■	■	■	■	■	■	■	■	■
	Positive sample												
UTI138	Symptom score	9	12	6	6	4	14	10	10	8	10	8	7
	Suspect a UTI			■	■	■			■	■	■	■	■
	Treated												
	Positive sample												
UTI139	Symptom score	2	1	1	0	1	8	2	2	2	4	0	1
	Suspect a UTI						■						
	Treated					■			■				
	Positive sample				■								
UTI218	Symptom score	0	6	0	0	3	0	0	0	0	0	0	0
	Suspect a UTI		■			■							
	Treated	■	■										
	Positive sample		■										
UTI236	Symptom score	4	2	1	0	0	0	0	0	0	0	0	0
	Suspect a UTI	■	■										
	Treated	■		■									
	Positive sample		■										
UTI266	Symptom score	4	1	3	3	2	2	3	2	3	2	3	2
	Suspect a UTI	■											
	Treated	■											
	Positive sample												
UTI337	Symptom score	6	8	5	2	10	1	7	4	5	4	11	3
	Suspect a UTI		■			■					■	■	
	Treated	■	■	■	■		■	■				■	■
	Positive sample		■				■						
UTI343	Symptom score	7	3	3	6	3	3	2	3	2	7	2	2
	Suspect a UTI	■	■		■					■	■		
	Treated					■				■	■	■	■
	Positive sample	■	■		■	■			■		■		
UTI365	Symptom score	0	1	2	1	0	0	1	0	0	0	0	0
	Suspect a UTI							■					
	Treated	■	■	■	■	■	■						
	Positive sample											■	
UTI376	Symptom score	1	2	1	2	1	1	1	0	0	0	0	0
	Suspect a UTI												
	Treated												
	Positive sample												
UTI383	Symptom score	5	3	3	3	3	3	3	3	2	3	2	1
	Suspect a UTI		■	■	■	■			■				
	Treated						■	■	■	■	■	■	■
	Positive sample												
UTI414	Symptom score	2	10	7	11	6	7	1	0	1	0	0	0
	Suspect a UTI		■	■	■		■						
	Treated		■		■	■	■		■	■	■	■	■
	Positive sample												
UTI468	Symptom score	7	5	8	5	2	4	5	7	3	6	2	4
	Suspect a UTI		■										
	Treated	■	■										
	Positive sample												
UTI524	Symptom score	8	4	6	5	2	4	3	3	3	3	5	3
	Suspect a UTI												
	Treated	■	■	■	■	■	■	■	■	■	■	■	■
	Positive sample												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI531	Symptom score	0	4	0	0	0	0	0	0	0	0	0	0
	Suspect a UTI												
	Treated												
	Positive sample												
UTI536	Symptom score	3	0	0	2	0	0	1	1	1	0	0	1
	Suspect a UTI	■											
	Treated								■				
	Positive sample								■				
UTI562	Symptom score	5	0	0	0	4	0	1	1	2	2	1	1
	Suspect a UTI	■											
	Treated		■						■				
	Positive sample						■						
UTI569	Symptom score	7	6	4	9	10	11	6	8	6	5	9	9
	Suspect a UTI				■	■	■				■		
	Treated			■		■							
	Positive sample				■		■						
UTI675	Symptom score	8	7	10	8	8	6	4	6	4	5	6	6
	Suspect a UTI	■			■								
	Treated			■	■	■	■	■	■	■	■	■	■
	Positive sample	■											
UTI726	Symptom score	9	6	7	8	2	6	4	3	0	1	0	1
	Suspect a UTI	■	■	■	■	■	■						
	Treated	■	■	■					■				
	Positive sample												
UTI755	Symptom score	8	5	6	4	3	4	4	4	4	4	4	4
	Suspect a UTI												
	Treated	■											
	Positive sample												
UTI781	Symptom score	5	5	3	4	3	2	2	2	2	2	2	2
	Suspect a UTI	■	■	■	■		■						
	Treated												
	Positive sample												
UTI834	Symptom score	5	4	5	5	5	5	3	3	6	6	6	6
	Suspect a UTI												
	Treated												
	Positive sample												
UTI840	Symptom score	0	12	1	0	2	1	2	0	1	0	5	0
	Suspect a UTI		■			■	■						
	Treated		■			■							
	Positive sample					■	■						
UTI891	Symptom score	2	4	2	1	2	3	3	5	4	3	4	2
	Suspect a UTI												
	Treated												
	Positive sample		■										
UTI899	Symptom score	5	11	3	6	6	5	6	8	6	13	7	8
	Suspect a UTI		■								■		
	Treated		■										
	Positive sample	■											
UTI924	Symptom score	4	5	7	4	4	2	2	3	4	4	5	2
	Suspect a UTI												
	Treated												
	Positive sample												■
UTI966	Symptom score	2	1	5	2	3	0	4	2	2	0	0	0
	Suspect a UTI												
	Treated				■								
	Positive sample												
UTI974	Symptom score	4	5	2	3	2	2	0	0	0	0	0	0
	Suspect a UTI												
	Treated												
	Positive sample												

Figure 13. Table showing patient symptom scores, self-suspected UTIs, treatment for a UTI and positive urine sample data for all study donations. Patients UTI numbers are given. Numbers prefaced with a 'D' are the study donation numbers. Symptom score taken as a summation of all symptom parameters from the questionnaires. 'Suspect a UTI' shows a filled box if patients answered 'yes' to the question 'Do you feel you are suffering now from symptoms of a urinary tract infection?'. A filled box in the 'treated' row indicates if patients received antibiotic treatment for a UTI within 3 days either side of the study donation. A filled box in the 'positive sample' row indicates if the patients' medical records showed a positive urine sample had been submitted to the NHS laboratories within 3 days either side of the study donation.

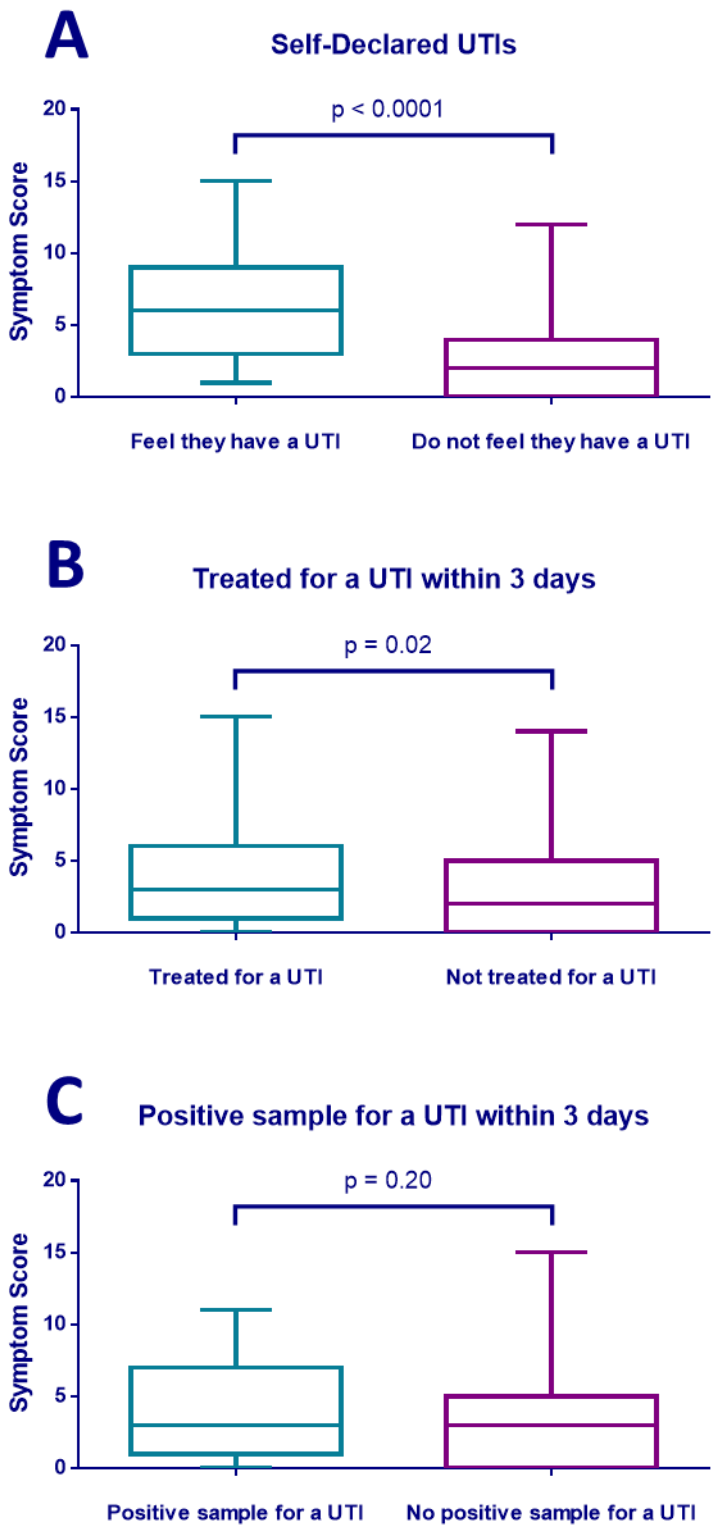


Figure 14. Symptom scores broken down by self-declared UTIs (A) and if patients were treated for a UTI (B) or submitted a positive urine sample (C) within 3 days of the study sample being collected. For all 3 n=360.

5.3. UTI Associated Words

The questionnaires contained a question asking about any changes in their symptoms since the last donation. One of the questions provided a blank space in which the patients could answer in their own words (Appendix 4, p.217). This allowed an insight into the sort of language patients were using to talk about their urinary status. Taking all of these words and creating a word cloud it was possible to see the most frequently used words and phrases patients used to talk about their disease (Figure 15). The language used allowed analysis of the more social aspect of the study.

Text size of the words in word clouds are proportional to how often the word was used in the questionnaires, thus larger words were used most frequently. Some of the commonly used words are medically associated with the disease, such as 'frequency', 'urgency' and 'infection'. However, the majority of commonly used words are often more colloquial, such as 'smell' and 'passage'. This suggests patients are less comfortable with medical terminology and prefer more layman terms to talk about their disease state. Another example of different terminology seen several times in both the questionnaires and during the short hand over meetings when collecting donations, was that several patients felt more comfortable using the word 'water' rather than urine and talked about 'going to the toilet' rather than urinating. Perhaps this lack of use of medical terminology could be due to the age range of the patients, they may be embarrassed to use such terms and favour what they deem to be politer alternatives. This should be kept in mind when HCPs are communicating with patients such as these, as terminology they are not comfortable with may confuse or embarrass them when trying to explain or educate them.

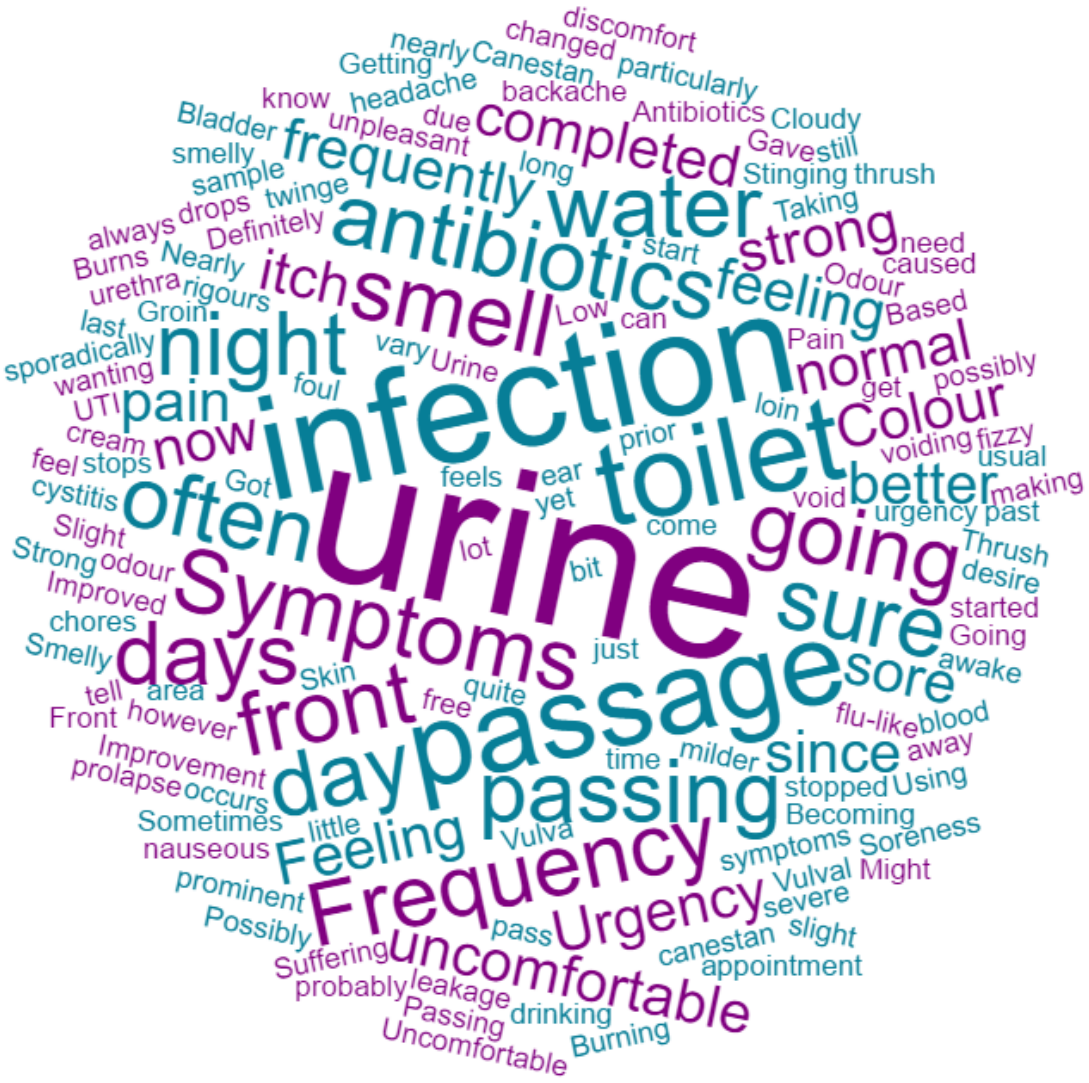


Figure 15. Word cloud of the comments and phrases used in the open answer in the symptom questionnaire. The size of the text is proportional to the frequency of the word being used. Made using www.wordclouds.com.

5.4. UTI Treatments

The data discussed thus far has explored the patients' data and correlations with their perceptions, now attention will be turned to the treatment aspect of the study. From the study questionnaires we can gather basic information about any antibiotics that were taken during the course of the study. The main first line treatments for UTIs are nitrofurantoin and trimethoprim due to their narrow spectrum of action [28,35]. However, elderly patients have been found to have a higher risk of toxicity from nitrofurantoin treatments [28]. Due to the broad-spectrum mode of action of some antibiotics, such as co-amoxiclav as well as some cephalosporins and quinolones, there are increasing numbers of resistant pathogens being observed within the clinics [28,35]. In cases of multi-drug resistance, fosfomycin can be used. However, this is kept for special cases where other drugs can be shown to be ineffective [28,36,37].

Of the 30 patients 22 were prescribed at least one course of antibiotics during the study (73.3%). The most common courses of antibiotic treatment were 7 days or 3 days (Figure 16A). The questionnaire data was also able to reveal the most highly prescribed antibiotics to our patients, for both short course and prophylactic treatment, were nitrofurantoin, cephalexin and trimethoprim (Figure 16B-D).

For short courses of antibiotic treatment Nitrofurantoin was prescribed at 200mg per day for a 7-day course. Trimethoprim was prescribed at 400mg per day for either 3 or 7 days. Cephalexin was always prescribed for 7 days and this was at a dose of 1 to 1.5g per day. The only known course recorded in the questionnaires for pivmecillinam was for 400mg per day for 4 days. Amoxicillin was prescribed twice during the study and both times it was for 7 day courses of either 750mg or 1000mg. There was one course of fosfomycin, this was a short high-dose course of 3g for just 1 day. There was also one short course of co-amoxiclav prescribed during the study, of 500mg twice a day for 8 days.

There were 13 separate prophylactic courses of antibiotics prescribed to 11 different patients during the study. Prophylactic courses are defined from the information given on the study questionnaires, where patients listed their antibiotic treatment as 'on-going'. Prophylactic courses appear on Figure 13 (p.87) as consecutive filled boxes on the treated line of the figure, whereas short courses were usually single filled boxes. 6 separate patients were prescribed nitrofurantoin prophylaxis; this was most commonly 50mg or 100mg once a day. Trimethoprim prophylaxis was prescribed to 2 separate patients and this was always at a dose

of 100mg once a day. Prophylactic courses of Cephalexin were prescribed to 3 different patients and this was most commonly at a dose of either 125, 175 or 250mg once a day. One patient was also prescribed a prophylactic course of methenamine hippurate at a dose of 1g per day. Prophylactic courses could be seen in as little as one study donation, this was counted as 2 weeks and ranges all the way up to 22 weeks of treatment. However, it must be noted that treatment courses could have been longer as they may have been started before the study or continued afterwards, information which is not captured here. The average course of prophylaxis, captured within the remit of this study, is 8.2 weeks of antibiotic treatment.

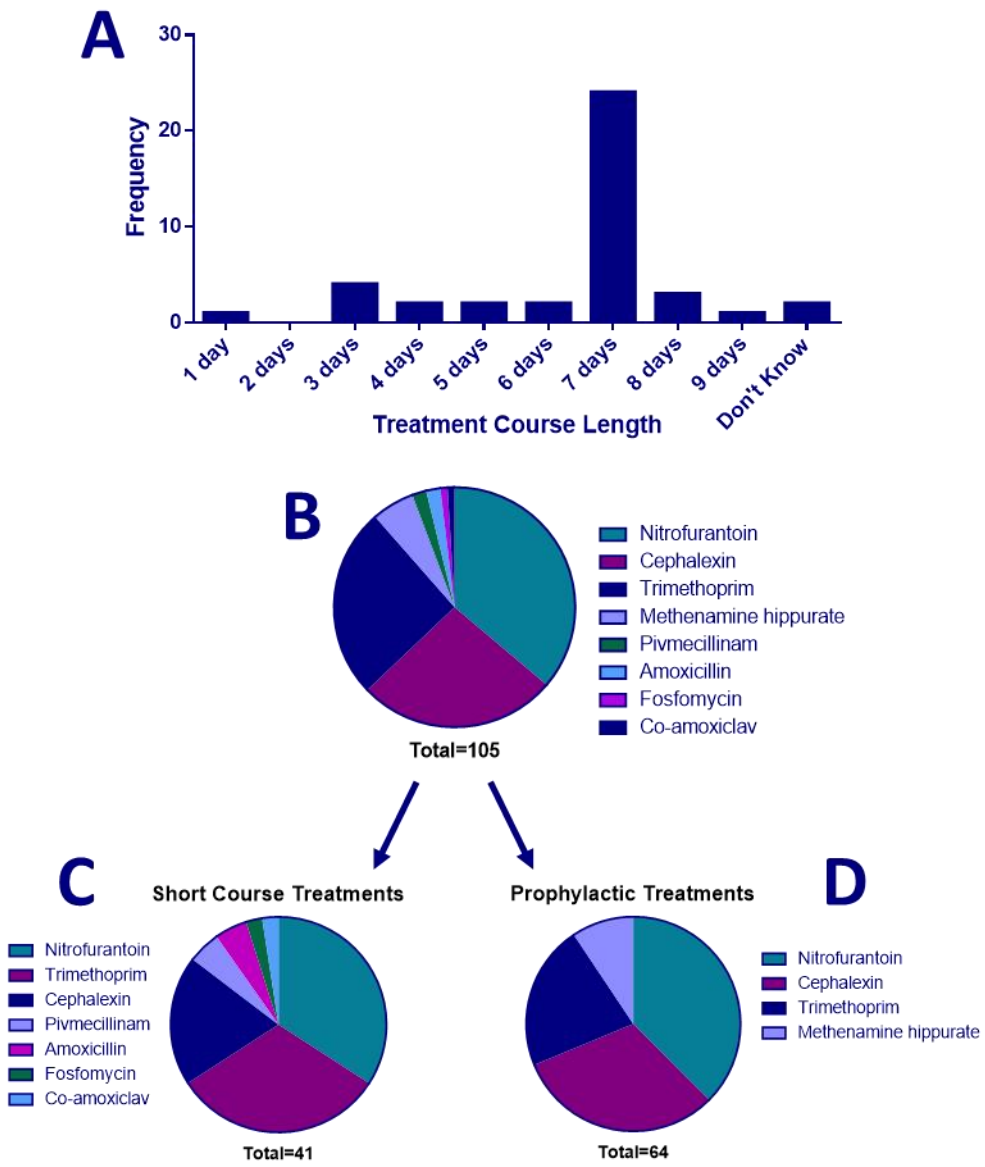


Figure 16. Antibiotic treatment data of all study samples. 'A' shows the frequency of each length of short course antibiotic treatment for a UTI during the study, as collected form the questionnaire data. 'B' is an overview of all UTI antibiotic prescriptions throughout the course of the study. 'C' and 'D' show this broken down by short courses and prophylactic courses of antibiotics for UTI. Prophylactic courses were defined as 'on-going' treatment with antibiotics collected from the study questionnaires.

5.5. Conclusions

By analysing the study patients' clinical and questionnaire data it has been possible to gain a better perspective of what types of patients are suffering from recurrent UTIs, building on what was shown in the previous chapter. There are small proportions of the study patients who are being treated with oestrogen supplementation, who are diagnosed as diabetic and had structural abnormalities in their urinary tracts. Collecting this information will allow for any potential observations in the host and bacterial analysis which may be different in these sub-populations.

The data obtained from the analysis of the questionnaires revealed some areas of possible education needed for both patient and HCP. The questionnaire revealed the type of language patients were using to talk about the disorder was often very polite and not medically orientated. This may be worth noting for professionals talking to patients of this age group that using politer lay alternatives may be a more effective means to communicate with and possibly educate them on the disorder than potentially harsh medical jargon.

Unsurprisingly, most of the study patients identified their usual UTI symptoms to fall under the 'mild' bracket. Symptoms would include smelly/cloudy urine, suffering from increased frequency of needing to urinate and/or feeling pain when passing urine. The most commonly stated symptoms throughout the study were the increased frequency of urination and an increased urgency to urinate. However, these are also the most commonly misinterpreted urinary symptoms in the elderly [29]. This could indicate that patients may feel they are suffering from a UTI, when in fact they are feeling the effect of other age associated urinary problems. This is supported by data showing that patients often overestimated the number of UTIs that they had suffered from in the past 12 months, when comparing to the number of positive urine samples that were actually seen by the NHS. Also there is a large disparity when comparing the times of patient-declared UTI from the study questionnaires with either occasions when a positive sample was submitted or when patients were treated with antibiotics. This may also suggest that, apparent UTI symptoms resolved on their own without treatment or were not severe enough for them to warrant a visit to a HCP. It is interesting to note that long prophylactic courses of antibiotics were not sufficient to keep increases in symptom scores and self-declared UTI episodes away. This may indicate that prophylactic treatments are not the most effective means of treatment in these patient groups and could be simply contributing to antibiotic resistances without actually any symptomatic

or clinical benefits for the patients. In addition, by looking into when a negative result was returned from the lab for UTI testing, it was possible to identify several occasions where antibiotic treatment was given without a positive urine sample result. This could suggest that some HCPs may be starting antibiotic treatment before awaiting a positive result, highlighting a possible disparity between the guidelines and the current clinical practice.

One of the most important conclusions that can be drawn from these data, was the definition of what would be deemed a symptomatic episode during the study. The mismatch of when patients' declared they had a UTI and when positive urine sample was handed in or UTI treatment was given, suggested that self-declared UTIs were not suitable to be used as a definition of symptomatic state. In addition to this, several of the self-declared symptoms were due to patients feeling their urine was odorous, which is not a sufficient reason to be deemed symptomatic. When looking at the periods of treatment as a possible candidate for symptomatic definition, there was also some ambiguity as often patients were given prophylactic courses of antibiotics. This would mean treatment at the time of sampling even where UTI symptoms or positive urine samples were not present. Thus, it was decided that a positive urine sample submitted within 3 days of study sampling to the NHS would be the most reliable definition for the symptomatic state. This would mean the patient, HCP and NHS all deemed the bladder to be suffering a UTI very close to the point of sampling. According to this definition there were 22 periods of symptomatic state throughout the study seen in 15 different patients. Therefore, having clearly defined criteria for a clinically relevant symptomatic state, it provides the possibility to analyse potential changes in the bacterial and host data around changes in these periods of symptoms.

Chapter 6. Microbial Analysis

One of the main aims of the project was to analyse the bacteria isolated from the urine samples. This would give a detailed understanding of the potential changes taking place in the bladder from the microbial perspective. Pathogen changes such as invading species, bacterial load, motility, among other things, could all impact a transition from asymptomatic to symptomatic UTI. Therefore, detailed bacterial analysis would make it possible to ask if the changes in the patient's state could be attributable to the invading organism.

The design of the longitudinal study allowed for more regular sampling of patients than would otherwise usually be done by the NHS. However, apart from the more frequent sampling, all sample analysis was based on NHS hospital lab procedure for testing urines. This way any findings could be directly linked back to current guidelines and protocols.

By analysing any changes in the bladder from the microbial perspective it may be possible to explain what is causing symptomatic episodes in these patients. It will also give a detailed picture of the urinary tract environment over a long period of time, allowing observation into whether it is relatively stable or if change is happening all the time with regards to microbial colonisation. For detailed genotypic and phenotypic analysis, this project has focussed on *E. coli*, since this is widely accepted to be the main pathogen responsible for causing UTIs [100,101]. However, analysis of other potential causative agents will also be performed.

6.1. Bacterial isolation and observation

E. coli was isolated from the urine, as described in section 3.2, using chromogenic agar and indole testing. This protocol produced a bio-bank of 184 *E. coli* isolates collected from the patient cohort. Thus, *E. coli* was found to be present in a total of 51.1% of all urine samples collected from these patients (Figure 17C).

The chromogenic agar plates used in the study enabled the identification of other pathogens within the urine samples. As well as *E. coli* these included *Enterococcus faecalis*, *Streptococcus agalactiae*, *Proteus mirabilis*, *Staphylococcus aureus*, *Candida albicans*. Very few patient samples were clear of any detectable growth on the chromogenic agar plates. As would be expected a larger proportion of healthy urine samples were clear of growth (11.1%) compared to that seen in the patients' cohort (2.8%) (Figure 17A and B).

Polymicrobial growth was defined as the identification of two or more different pathogens on the agar plates. This was sensitive down to a concentration of 100 CFU/ml. The vast majority of study strains, from both patients (65.6%) and healthy participants (64.4%), were polymicrobial (Figure 17A and B). It is therefore very interesting to observe the high level of co-existence between different pathogens within the bladders of these patients. Figure 18 shows a selection of examples of polymicrobial growth from the study urines. The plate of UTI115 D6 shows growth of *E. coli* and *Enterococcus faecalis*. Patient UTI337 D9 has growth of *E. coli*, *Enterococcus faecalis*, *S. aureus* and *C. albicans*. UTI376 D9 shows the growth of 2 different pathogens, *Enterococcus faecalis* and *P. mirabilis*. UTI383 D8 has 3 different pathogens within the urine: *E. coli*, *Enterococcus faecalis* and *S. aureus*. Four different pathogens were identified in the urine sample from patient UTI966 D5: *E. coli*, *Enterococcus faecalis*, *Streptococcus agalactiae* and *P. mirabilis*. An example of single microbial growth is patient UTI138 D12, showing pure growth of *E. coli* (Figure 18).

The majority of study urine samples from patients showed the growth of 1 or 2 pathogens (63.6%). 21.1% of urine samples showed the growth of 3 separate pathogens, and 10.0% showed the growth of 4. The largest number of identifiable pathogens seen in the study was 5, within just 9 of the patient study samples (2.5%) from 7 separate patients (i.e. UTI115, 139, 531, 569, 675, 755, 974) (Figure 19).

Interestingly, the most commonly observed species from patients in this study was *Enterococcus faecalis* (Figure 17C). However, relatively high levels of *Enterococcus faecalis* identification was also seen in the healthy control urine samples (Figure 17D). When looking at the range pathogens within the study urine samples, it is often observed that the same combinations are identified for long periods of time during the study (Figure 19). This would perhaps suggest that this is not contamination but a well-established and reasonably stable microflora within the patients' bladders. The trend of similar pathogen combinations observed long-term is highlighted in patients such as UTI100, 536 and 966. As well as displaying these trends in the bladder colonisation, Figure 19 also give an initial suggestion to the huge variability seen between the different study patients as well as within the patients themselves over time.

There are several patients who present long-term colonisation with *Enterococcus faecalis*, some being colonised for the entire study duration. Three examples are patients UTI115, 266 and 891. To assess the impact these may have on the patient it is important to analyse the

load within the urine. Bacterial load quantification was only done for *E. coli* in this study, however *Enterococcus faecalis* can be seen at diagnostic levels ($>10^5$ CFU/ml) for almost all study samples in patients UTI115, 266 and 891 (Figure 20).

UTI115 saw diagnostic levels of *Enterococcus faecalis* at the start of the study, at donations 6 and 7 there was a shift to *E. coli* (red) before *Enterococcus faecalis* returns to diagnostic levels and appears to drop off again in load towards the end of the study (Figure 20). Patient UTI266 had diagnostic loads of *Enterococcus faecalis* for the entire study duration. Patient UTI891 does not always show diagnostic levels of *Enterococcus faecalis*, but an established colonisation of the pathogen can be seen throughout the study. *Enterococcus faecalis* is widely accepted to be the second most common pathogen for causing UTIs after *E. coli* and the most common Gram-positive urinary pathogen [208]. This study data suggests that *Enterococcus faecalis* may be more common than *E. coli* in certain patient groups.

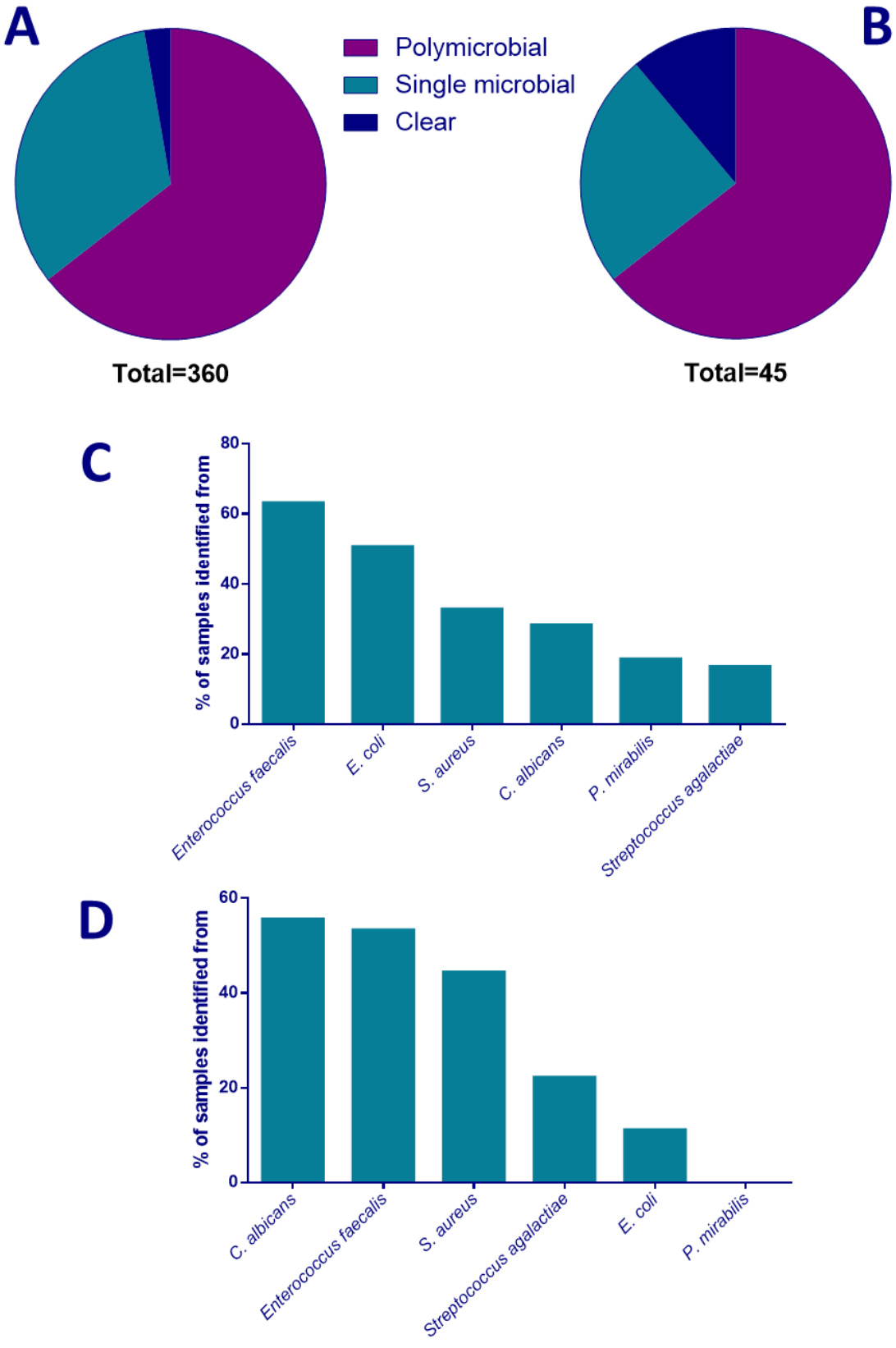


Figure 17. Proportion of all study samples which were polymicrobial, single microbial and clear, from both study patients (A) and healthy samples (B), as well as the breakdown of identified pathogens from patient (C) and healthy control samples (D).

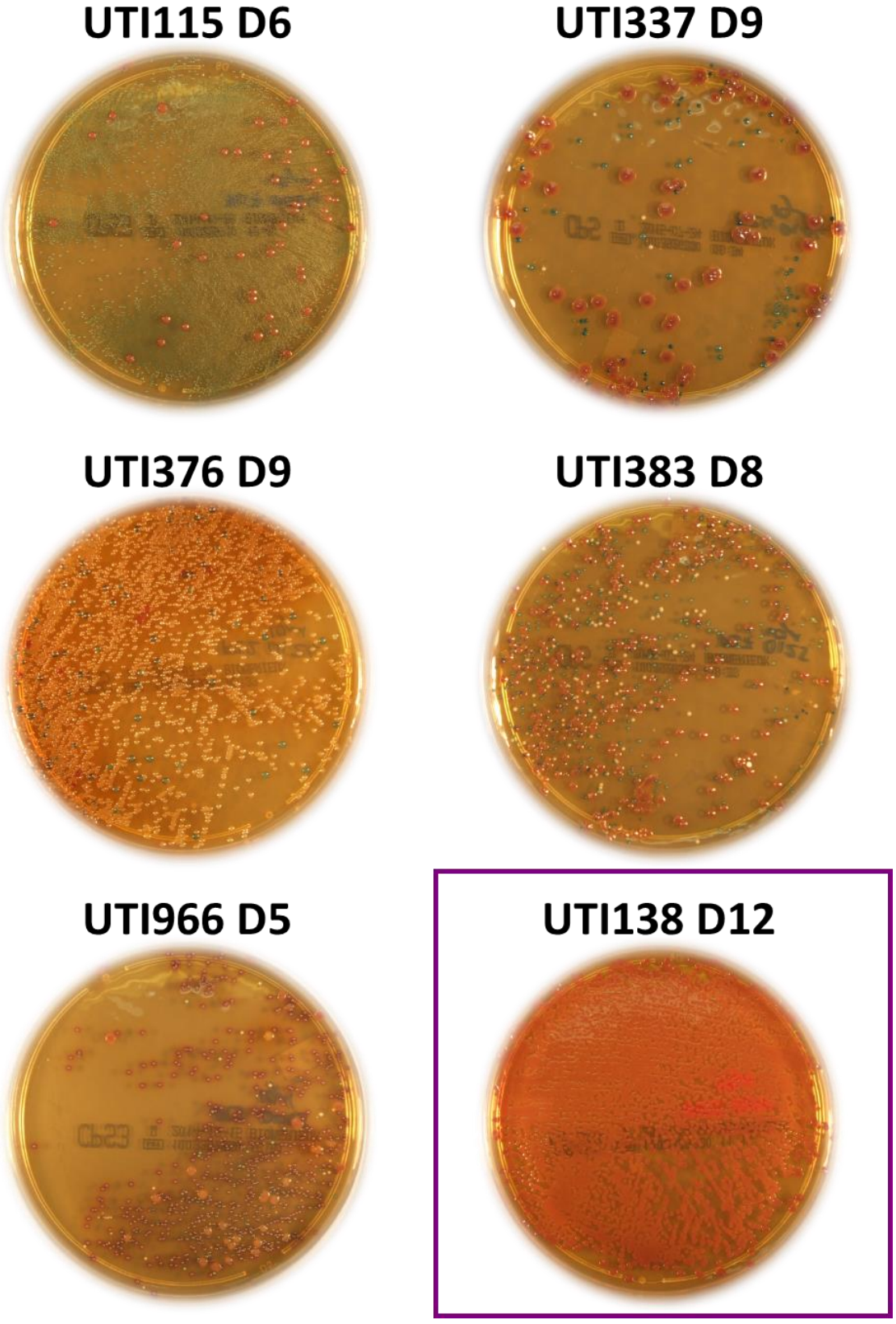


Figure 18. Polymicrobial and single microbial plate picture examples. The purple square highlights the single microbial example. UTI138 D12 is an example of a diagnostic load of *E. coli* in the urine. Red colonies indicate *E. coli*, green/blue colonies are *Enterococcus faecalis*, purple/pink colonies are *Streptococcus agalactiae*, white/brown colonies are *P. mirabilis*, small white are *S. aureus* and tiny white colonies are *C. albicans*.

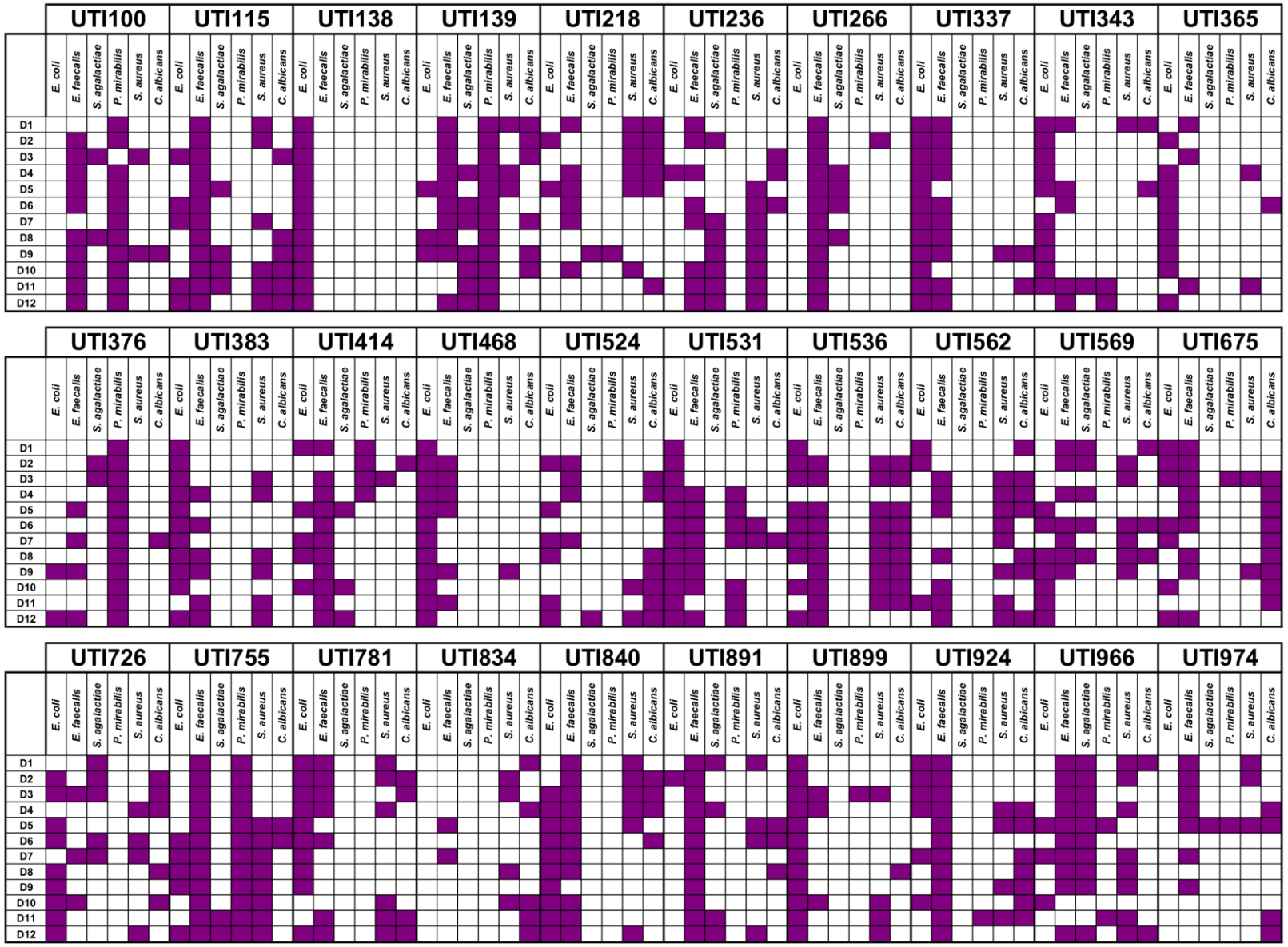


Figure 19. All bacteria observed in the patients' urine samples. A filled box indicates the presence of that organism within the urine sample at a level of 100 CFU/ml or more, taken from the chromogenic agar plates.

E. coli: ***Escherichia coli,***

E. faecalis: ***Enterococcus faecalis,***

S. agalactiae: ***Streptococcus agalactiae,***

P. mirabilis: ***Proteus mirabilis,***

S. aureus: ***Staphylococcus aureus,***

C. albicans: ***Candida albicans***

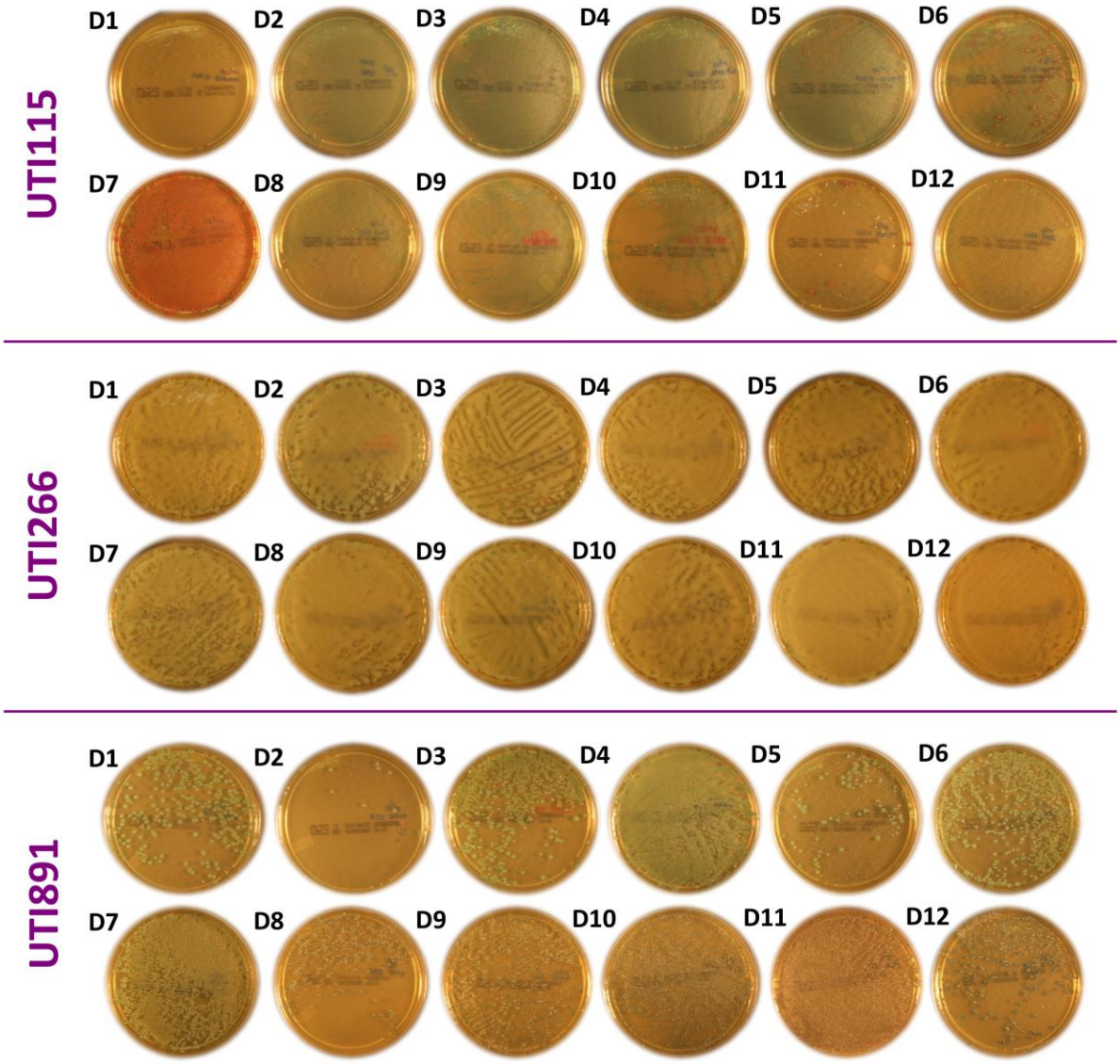


Figure 20. Examples of patients carrying *Enterococcus faecalis* for the entire study duration. *Enterococcus faecalis* is identified by a green colony on the plate. In patient UTI266 the colonies can be hard to see in some of the images due to the mucoid phenotype that this strain exhibited on the plates 'UTI' numbers are the patient's randomised participant ID number and 'D' prefaces the study donation number.

6.2. Bacterial Loads, Symptoms and Treatment

By spreading known volumes of the urine onto agar plates it was possible to make a quantitative estimate of the CFU/ml of any *E. coli* present. Our study design allowed us to isolate *E. coli* at concentrations as low as 100 CFU/ml. Observing the bacterial loads allows distinguishing between urine samples where *E. coli* is established and colonising the bladder from low bacterial loads where *E. coli* presence could just indicate contamination at the time of sampling. In order to see if changes in bacterial load were affecting change in symptomatic state, it was necessary to bring these data together. This begins to address the bacterial aims of the project. As periods of antibiotic treatment could also have an effect on *E. coli* load in the bladder, it was also necessary to include this in the analysis. Figure 21 shows mapping of the changes in *E. coli* load together with the MLST, periods of symptoms and periods of treatment. Therefore, this also allows for identification of the isolated strain and thus observation of any changes in the strain and symptoms with potential spontaneous or induced changes in the bacterial load. The MLST is a strain ID number which allows differentiation of the bacteria, different numbers indicate different strains being isolated in the urine. The MLST data will be discussed in more detail in the next section. In order to aid understanding of these sections it must be noted that a patient profile is available containing all the information discussed for each individual in Appendix 9 (p.223) to Appendix 38 (p.252).

Detailed here are some examples of patients where treatment courses appear to have been unsuccessful in clearing the infection and may even be responsible for making the situation within their bladder worse;

UTI139

The 2nd treatment course patient UTI139 received allowed *E. coli* strain ST131 to colonise the bladder (Figure 21). However, it doesn't cause the patient symptomatic infection. Both treatment courses in this patient appear to clear the *Streptococcus agalactiae* colonisation from the bladder short-term (Figure 19), perhaps suggesting that the *E. coli* is competing with *Streptococcus agalactiae* and the treatment is therefore allowing the *E. coli* to thrive. This patient is a complex case with high levels of polymicrobial colonisation within their bladder throughout the study.

UTI218

In patient UTI218 treatment appears to make matters worse, the antibiotics appear to give rise to symptoms at D2 (Figure 21). This treatment appears to have cleared the *Enterococcus*

faecalis from the bladder at D1 and allowed *E. coli* strain ST73 to thrive, perhaps triggering the symptoms observed (Figure 19).

UTI569

Patient UTI569 suffered from 2 symptomatic episodes directly after 2 separate courses of antibiotic treatment with Nitrofurantoin at D3 and D5 (Figure 21). The first course clears the *Enterococcus faecalis* and *Streptococcus agalactiae* colonisation from the urine, however these both return once treatment is stopped and the patient suffers from UTI symptoms at D4 (Figure 19). The next treatment course clears all pathogens from the urine, except *E. coli* strain ST3640. However, treatment cessation causes polymicrobial colonisation of the urine. This time with *Enterococcus faecalis, Streptococcus agalactiae, S. aureus, C. albicans* and new *E. coli* strain (ST442), suggesting that treatment may be good in the short term but is having no real benefit in the long run.

UTI840

In patient UTI840 it is clear that symptoms occurred only when the bacterial load in the bladder reached that of diagnostic level at D5 (Figure 21). However, this was not cleared by the short course of antibiotics they were given. Earlier on in the study, this patient was given treatment at D2 and it appears to have given rise to *E. coli* strain ST354, which persists for the rest of their study participation.

There are many other examples of where treatment was not successful in clearing the *E. coli* from the bladder, such as patients UTI414, 468 and 726. This could be due to the NHS analysis selecting a different pathogen to run antibiotic susceptibility testing on and the *E. coli* isolates in this study may carry resistance, thus are able persist in the bladder despite treatment.

Prophylactic treatment with antibiotics also appeared to be often ineffective at clearing the bacterial load and as well as symptoms, as has been previously discussed in this chapter. Patients UTI365, 383, 414, 524 and 675 are all examples of prophylactic treatment courses where the *E. coli* load was not consistently kept low.

There were, however, identifiable occasions where treatment appeared to have varying degrees of success in some study patients. Five examples of this are detailed here;

UTI236

Patient UTI236 acquires *Streptococcus agalactiae* in the urine, in addition to the already present *Enterococcus faecalis* at the point where they suffered a symptomatic episode at D2 (Figure 19 and Figure 21). They were treated with pivmecillinam prior to D3 which appears to successfully clear the *Enterococcus faecalis*, which the first treatment course of Nitrofurantoin did not manage to clear.

UTI343

In patient UTI343 treatment appeared to clear the symptoms and the *E. coli* at D5 (Figure 21). However, the later courses of treatment in this patient were not so successful. *E. coli* is replaced by *Enterococcus faecalis* and *S. aureus* at D11, where the patient suffers a symptomatic episode (Figure 19). *Enterococcus faecalis* had replaced *E. coli* twice in this patient, at donations 6 and 12, however no symptoms were seen. This may suggest that it was the colonisation with *S. aureus* was what pushed the immune response into causing symptoms in this patient.

UTI383

Patient UTI383 was given a prophylactic course of antibiotics at D6, this appeared to take around a month or so to clear the bladder of *E. coli* strain ST12 (Figure 21). However, by the end of the study this strain had returned to diagnostic levels along with several other pathogens such as *Enterococcus faecalis* and *S. aureus* (Figure 19). This may be due to developing resistance to the prophylactic anti-infective medicine, methenamine hippurate.

UTI562

Treatment at D2 in patient UTI562 appeared to be sufficient to clear the *E. coli* from the bladder and this strain did not appear to return for the rest of the study duration (Figure 21). However, *Enterococcus faecalis* and *S. aureus* persist within the bladder of this patient almost continuously (Figure 19).

UTI899

Patient UTI899 was treated at D2, which appeared to make an initial impact on the *E. coli* load, it did not clear the bladder from colonisation (Figure 21). However, this patient did not appear to suffer any further symptoms for the study duration, perhaps suggesting the newly colonising strain was less harmful to them. This was strain ST73, the most commonly isolated strain in this group of ABU patients (Figure 22A). Therefore, this strain may be more inherently adapted to avoiding the triggering of symptoms. However, this is the opposite to

what is seen in patient UTI218 (discussed above), suggesting that this strain is not always linked to an asymptomatic state.

Collectively, these data suggest that in some patients, treatment can be effective, however it is very easy to identify patients where it is not. Often seeing that in the long-term treatment is having no real benefit to the patients' bladder colonisation status.

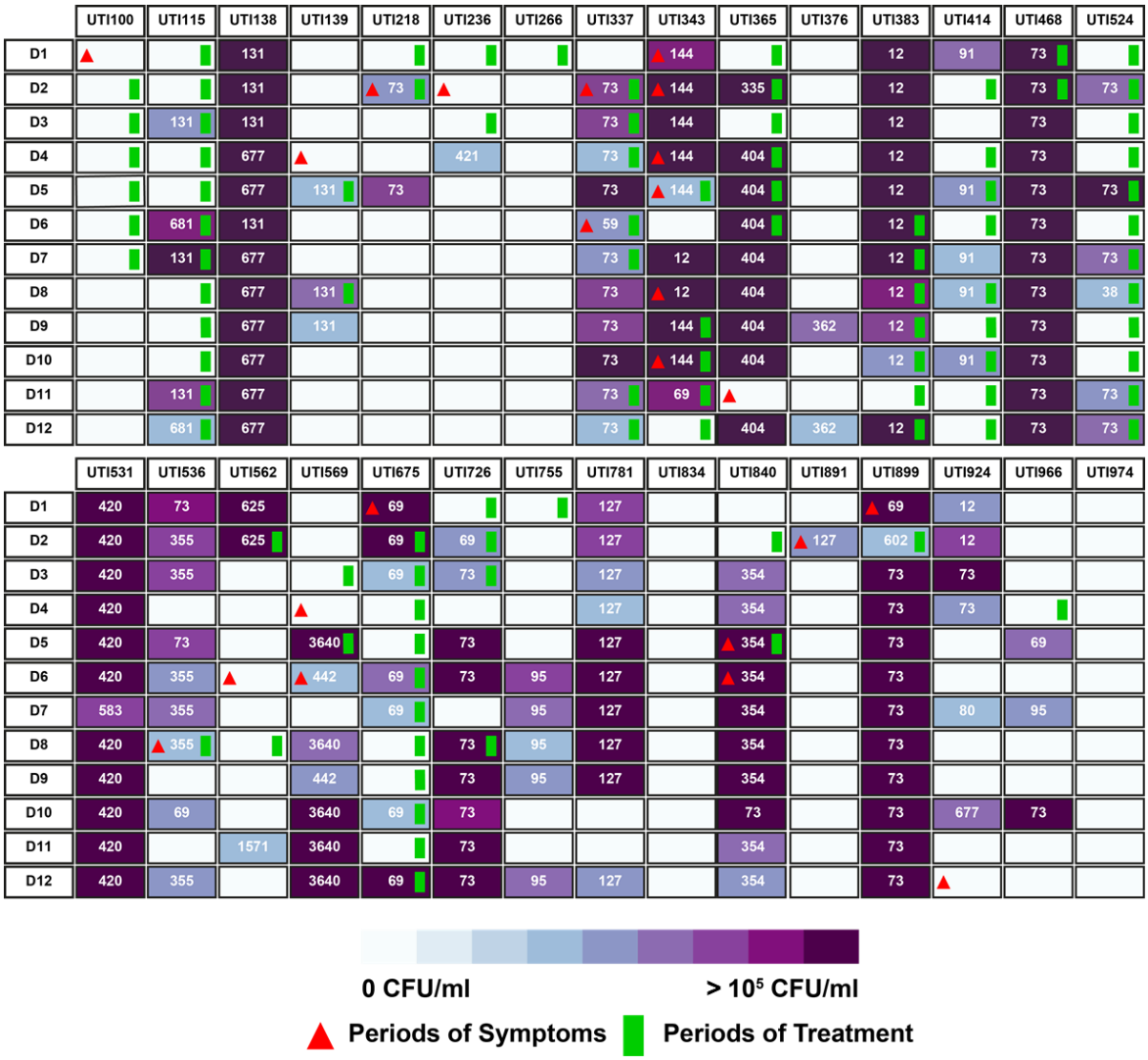


Figure 21. ***E. coli*** **load quantification over the full study duration. Numbers are the MLST strain IDs, these represent samples from which** ***E. coli*** **was isolated. Dark purple represents samples with clinically significant loads of** ***E. coli*** **(>10^5 CFU/ml). Red triangles are episodes defined as symptomatic.**

6.3. Long-term Carriage of E. coli

A number of study patients showed long-term carriage of *E. coli*. Patients UTI138 and 468 had diagnostic loads of *E. coli* in the bladder for the entire study duration (Figure 21). 13 other patients had long-term *E. coli* for at least 3 donations in a row. Interestingly, relatively few patients who had long-term colonisation with diagnostic levels of *E. coli* complained of symptomatic episodes, except UTI343 and 840. The majority appeared to remain symptom free during the periods of *E. coli* colonisation both with and without prophylactic treatment courses. For example, this can be seen in patients UTI 138, 365, 383, 468 and 531. However, the majority of patients showing long-term *E. coli* carriage at diagnostic loads were those not taking prophylactic antibiotics. This long-term colonisation without symptoms could support the argument that certain strains of *E. coli* colonising the bladder could be protective of symptoms. By allowing a less harmful ABU strain to remain in the bladder may stop more harmful potentially symptomatic strains from invading.

In polymicrobial urine samples *E. coli* was most commonly isolated with *Enterococcus faecalis* (55.4%) and *S. aureus* (27.7%). Long-term colonisation of *E. coli* and *Enterococcus faecalis* can be seen in patients UTI337 and 840. *E. coli* and *S. aureus* can be seen regularly isolated together from the bladders of patients UTI383 and 536 (Figure 19, p.101). The least common pathogens to co-exist with *E. coli* in this study were *P. mirabilis* (12.0%) and *Streptococcus agalactiae* (7.6%). The longitudinal study design allowed observation of the fact that when *P. mirabilis* is identified within a sample alongside *E. coli*, it is usually *P. mirabilis* colonising the bladder long-term and *E. coli* is just found sporadically. This can be demonstrated in patients UTI139, 376 and 755 (Figure 19). From Figure 21 it can be seen that these *E. coli* isolates are at very low loads, suggesting that *P. mirabilis* is much more dominant when these 2 strains are competing for the niche the bladder provides. The only example where this is not the case is in patient UTI531 where *E. coli* remains at almost diagnostic levels despite *P. mirabilis* also being isolated (Figure 19). This could be due to a strain advantage conferred by ST420 or perhaps competition from the colonising *Enterococcus faecalis* (Figure 21). *S. aligactiae* colonisation often appears to avoid periods of *E. coli* colonisation. This can be well demonstrated in patients UTI115 and 139, and to a lesser extent patients UTI569 and 726 (Figure 19). In patient UTI966 where *E. coli* was isolated alongside *Streptococcus agalactiae* at donations 5 and 7 it is found at very low concentrations, but when *E. coli* reaches diagnostic levels at D10 the *Streptococcus agalactiae* is no longer found in the urine (Figure 19 and Figure 21). These data suggest that

E. coli and *Streptococcus agalactiae* do not co-exist very successfully. Furthermore, these data may suggest that the different pathogens are not all equally well suited for survival together with *E. coli*.

6.4. E. coli Sequencing Analysis

In order to get an accurate identification of which strain of *E. coli* was present in the urine, the MLST sequencing method was used. This gives a sequence type ID number based on 7 housekeeping genes based around the *E. coli* genome. These ID numbers allow us to differentiate between any potential changes in the colonising pathogen in the bladders of study patients. Another level of identification used during the project was sequencing the *fliC* gene to obtain the H-antigen serotype. This allowed further deduction of any changes in the infecting *E. coli* strain.

E. coli was isolated from 26 of the 30 study patients at some point during the course of the study (86.7%). The most commonly isolated sequence type of *E. coli* during the study was ST73, which was responsible for 29.3% of all the patient isolates, found in 10 different patients (Figure 22A). This is followed by *E. coli* strains ST12, ST60 and ST420 as the next most commonly isolated strains. The most commonly isolated serotypes during the study were H1, H5 and H31 (Figure 22B). A total of 26 different strains of *E. coli* were detected and 15 different *fliC* serotypes.

Figure 23 highlights the large variety of different *E. coli* strains colonising the study patients during the study. Most patients who showed *E. coli* colonisation during the study (80.1%) only had just 1 or 2 different strains of *E. coli* detected during the 6-month study. Patient UTI924, however, saw colonisation of 4 different strains of *E. coli* over the study period, this was the maximum isolated from one study patient. Periods of symptoms, indicated in red on Figure 23, do not align consistently with changes in strain of colonising *E. coli* strain. UTI218, UTI337 and UTI569 have periods of symptoms which coincide with changes in *E. coli* strain, however all of these patients also had another episode of symptoms during the study where this is not the case. This suggests that it is not the change in *E. coli* strain that is responsible for the periods of symptoms. *E. coli* was only isolated from patient UTI891 once during the study, this also coincided with an episode of symptoms the patients experienced. In addition, patient UTI365 suffered from a symptomatic episode at a time where the usually isolated strain ST404 was not detected in their urine, at D11. This may suggest the invasion of a more virulent and disruptive strain. It is possible to see that in this patient at D11 the

colonising bacteria changes from *E. coli* to *Enterococcus faecalis* and *S. aureus* (Figure 19). This could give strength to the argument that ABU *E. coli* strains colonising the bladder may be protective against symptoms. Alternatively, it could be that the body is becoming used to the invading strain and is desensitised to its presence in the bladder. The *E. coli* strain most commonly associated with periods of symptoms throughout the study was ST144, isolated in 5 different symptomatic samples. However, this was all from the same patient, UTI343. *E. coli* strains ST73 and ST69 were each found to be symptomatic twice in separate study patients. *E. coli* strains ST12, ST355 and ST442 were each found to be symptomatic once throughout the study.

6.4.1. Serotype Switching

Interestingly, the flagellin serotype for the same strains within patients is not always the same. In 2 patients, UTI840 and 569, the serotype switches without the strain changing. By looking at the published sequence alignments of the H-antigens it is possible to assess whether these changes in serotype are likely due to full gene (or strain) switches and those that could be caused by point mutations within the *fliC* gene [209]. Unfortunately, H36 was unable to be aligned by Wang *et al*, so it is not possible to see if the switch seen in ST354 in patient UTI840 is due to mutation or full gene switching [209]. However, it seems that the H36 variation of the strain is better suited to survival within this patient's urinary tract as it remains present in almost all further study samples after the switch (Figure 23). In patient UTI569 *E. coli* strain ST442 changes from serotype H21 to H6 at D9 (Figure 23). These serotypes are very different, thus this switch cannot be caused by a single point mutation and suggest a complete change in *fliC* gene [209]. However, these strains were isolated 7 weeks apart with another *E. coli* strain colonising in between (ST3640 H37) (Figure 23). This could suggest that this switch in serotype seen in strain ST442 may be due to a strain invasion event rather than a specific gene switch.

In 5 different patients, changes in serotype within the same strain can be observed, that appear to also switch back to the original serotype during the study. UTI138 was colonised with ST131 with serotype H5, at D2 this changes to H12 and returns to H5 at D3 (Figure 23). The sequences of these serotypes are very different and thus this cannot be due to a single point mutation in the gene [209]. Another example for this is seen in patient UTI365, where strain ST404 switches its serotype from H5 to H37 and back again (Figure 23). These are also not closely related sequences, thus suggest a full gene switch [209]. UTI343 also appears to

have a full gene switch in strain ST144 from H6 to H5 and back again as these genes are also not closely related [209]. After ST144 H6, this patient is colonised with another strain of *E. coli*, ST12 H5 at D7, which is followed by ST144 H5 at D9 (Figure 23). The H-antigen then reverts back to H6, suggesting that this serotype is better suited to survival within this patient's urinary tract. In patient UTI531 *E. coli* strain ST420 changes serotype from H31 to H5 and back again, these are very different genes when analysed by sequence alignment [209] (Figure 23). The strain ST420 changes from serotype H31 to H5 at D6. This appears to be followed by the invasion of a different strain of *E. coli* (ST583) with the same serotype seen previously in ST420, H31, at D7. By D8 the original ST420 strain with serotype H31 has returned and remains in the urine for the remainder of the study. This suggests that the H31 serotype is better suited to survival within the urinary tract of patient UTI531 and that the switch to H5 conferred no survival advantage. Therefore, the H5 strain did not appear to last for long in their bladder before being out-competed. These observations in serotype switching could suggest that some strains are able to change the flagellin, perhaps as a way of evading recognition by the immune system. Finally, patient UTI675 shows a switch from H18 to H6 and back in strain ST69. However, these genes are very closely related upon sequence alignment and could therefore be due to single point mutations in the gene rather than full gene switching [209].

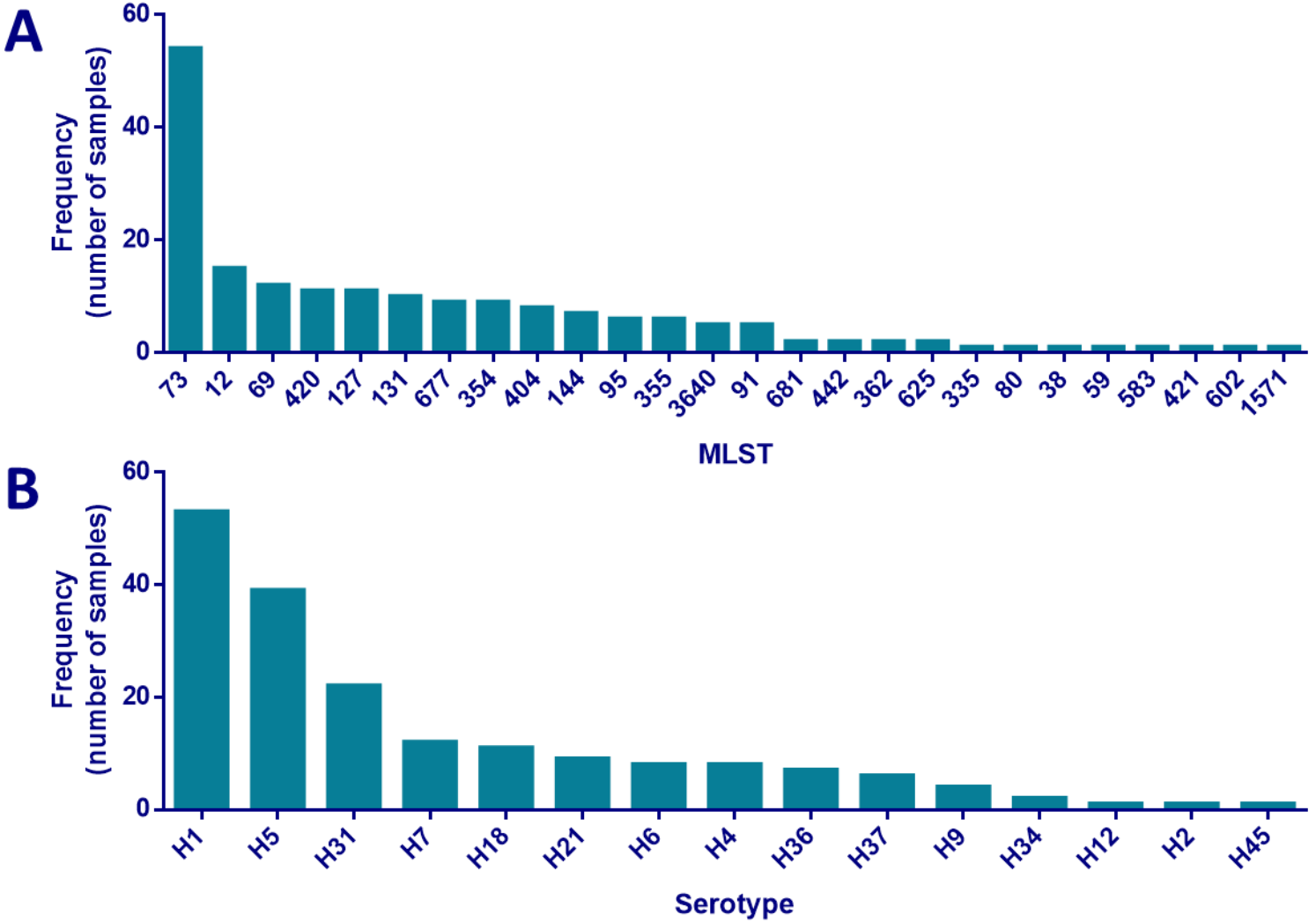


Figure 22 . Frequency of different MLSTs (A) and serotypes (B) of the *E. coli* isolates from patients throughout the study.

		UTI100	UTI115	UTI138	UTI139	UTI218	UTI236	UTI266	UTI337	UTI343	UTI365	UTI376	UTI383	UTI414	UTI468	UTI524
D1	MLST			131					73	144			12	91	73	
	FliC			5					1	6			5	4	1	
D2	MLST			131		73			73	144	335		12		73	73
	FliC			12		1			1	6	7		5		1	1
D3	MLST		131	131					73	144			12		73	
	FliC		5	5					1	6			5		1	
D4	MLST			677			421		73	144	404		12		73	
	FliC			21			7		1	6	5		5		1	
D5	MLST			677	131	73			73	144	404		12	91	73	73
	FliC			21	4	1			1	6	5		5	4	1	1
D6	MLST		681	131					59		404		12		73	
	FliC		5	5					7		5		5		1	
D7	MLST		131	677					73	12	404		12	91	73	73
	FliC		5	21					1	5	5		5	4	1	1
D8	MLST			677	131				73	12	404		12	91	73	38
	FliC			21	4				1	5	37		5	4	1	18
D9	MLST			677	131				73	144	404	362	12		73	
	FliC			21	4				1	5	5	9	5		1	
D10	MLST			677					73	144	404		12	91	73	
	FliC			21					1	6	5		5	4	1	
D11	MLST		131	677					73	69					73	73
	FliC		5	21					1	45					1	1
D12	MLST		681	677					73		404	362	12		73	73
	FliC		5	21					1		5	9	5		1	1

		UTI531	UTI536	UTI562	UTI569	UTI675	UTI726	UTI755	UTI781	UTI834	UTI840	UTI891	UTI899	UTI924	UTI966	UTI974
D1	MLST	420	73	625		69			127				69	12		
	FliC	31	1	7		18			31				18	5		
D2	MLST	420	355	625		69	69		127			127	602	12		
	FliC	31	5	7		18	18		31			31	9	5		
D3	MLST	420	355			69	73		127		354		73	73		
	FliC	31	5			6	1		31		34		1	1		
D4	MLST	420							127		354		73	73		
	FliC	31							31		34		1	1		
D5	MLST	420	73		3640		73		127		354		73		69	
	FliC	31	1		37		1		31		36		1		18	
D6	MLST	420	355		442	69	73	95	127		354		73			
	FliC	5	5		21	18	1	7	31		36		1			
D7	MLST	583	355			69		95	127		354		73	80	95	
	FliC	31	5			18		7	31		36		1	7	7	
D8	MLST	420	355		3640		73	95	127		354		73			
	FliC	31	5		37		1	7	31		36		1			
D9	MLST	420			442		73	95	127		354		73			
	FliC	31			6		1	7	31		36		1			
D10	MLST	420	69		3640	69	73				73		73	677	73	
	FliC	31	18		37	18	1				2		1	5	1	
D11	MLST	420		1571	3640		73				354		73			
	FliC	31		9	37		1				36		1			
D12	MLST	420	355		3640	69	73	95	127		354		73			
	FliC	31	5		37	18	1	7	31		36		1			

Figure 23. All patient *E. coli* isolate sequencing over the full study duration. All donations containing numbers indicate a urine sample where *E. coli* was isolated. Purple text shows the MLST ID number of the strain and blue text gives the H-antigen serotype of the strain's flagellin. Red boxes indicate samples where symptoms were recorded. Grey squares are where no *E. coli* was isolated or symptoms were reported. 'UTI' numbers are the patient's randomised participant ID number and 'D' prefaces the study donation number.

6.4.2. Strain alignment analysis

E. coli is a very highly diverse pathogen, responsible for a wide range of diseases in various locations within the body. As a means of classifying the pathogen in order to better identify them, their phylogenetic groupings are often used. These groups, or clades, are based on whole genome analysis of number of published genetic markers around the genome. These clades also tend to show correlation with the source of the pathogen or the niche in which it most commonly causes pathogenicity [122]. MLST provides an accurate method of assigning different *E. coli* sequences to the different *E. coli* clades based on their sequence alignment [88]. By aligning the MLSTs of the study's *E. coli* isolates to those with known *E. coli* clades, it is possible to better identify the types of strains being observed with respect to the literature [90].

The alignment of the samples isolated in this project shows a reasonable spread of the isolates between the various clades (Figure 24). The most common clade the study isolates were associated with was B2. This was to be expected as this clade is most commonly associated with UPEC [90,125]. The isolates ST677 and ST354 were associated with clades B1 and F respectively. These strains look proportionally well represented, but it must be noted that these were both isolates from single patients; UTI138 and 840 respectively. So when counting the strains from each patient only once, B1 and F only represent 10.9% and 4.3% of the different patient *E. coli* study isolates. To give this some perspective, the B2 clade represents 63.0% of the different patient isolates. ST69, which is from clade D, was isolated from 6 separate patients during the course of the study. This clade represents 17.4% of different patient isolates, which suggests this representation within the D clade is more robust than that of B1, E (4.3%) or F. Clade D is also known to be associated with UPEC strains [125], thus this observation fits with the literature.

Clade A is associated with commensal gut *E. coli* strains [123]. This would suggest very few of the study isolates are present in the urine due to gut contamination. However, this does not rule out the gut as a potential reservoir for UPEC strains before they reach the bladder [59,60]. Thus low numbers of *E. coli* in the urine could still be due to sample contamination.

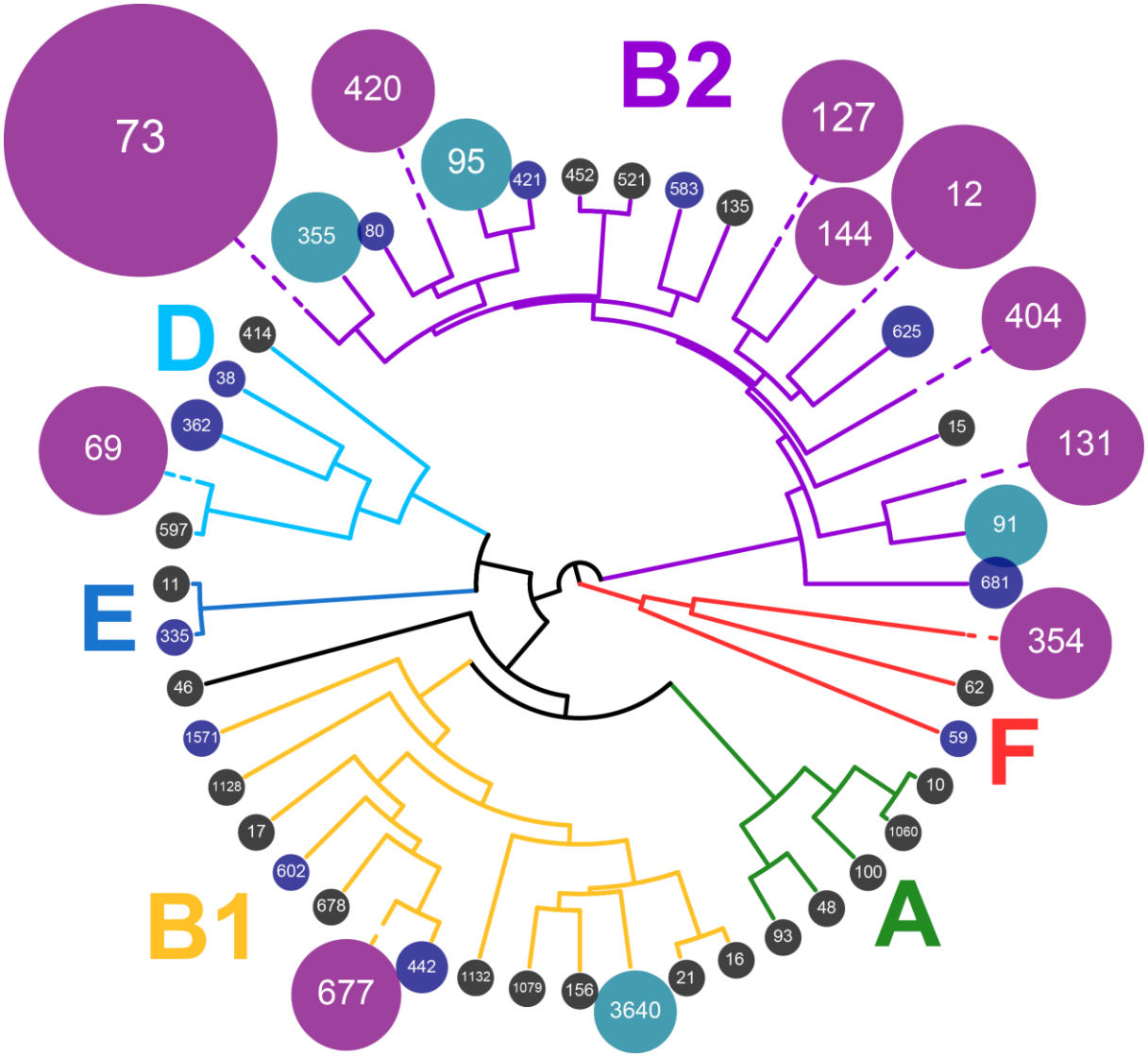


Figure 24. Sequence alignment grouping of all study isolates into the well-recognised *E. coli* clades. Strains identified from this study and in the literature super imposed on the sequence alignment tree. All filled coloured circles containing text (purple, green and blue) are isolates from this study, black circles indicate *E. coli* strains previously grouped by McNally *et al* [90]. The size of the filled circle is proportional to how many times it was isolated during this study (note: black filled circles are a size of 1). Alignment tree produced by the Jalview program use to analyse the multiple sequence alignments of all study MLST strains produced by the ClustalX software [205].

6.5. Motility Testing

The bacterial flagella, which allows the cells to be motile, has been implicated to have a role in causing UTIs [135,141,142]. It has been suggest that they help bacteria ascend the urinary tract during infection [141,142]. It is for this reason it was interesting to assess the study *E. coli* isolates' motility. This may give insight into strains and serotypes that are perhaps better suited to urinary tract colonisation. As well as allowing for observation of whether these more motile strains are more likely to cause the patient symptoms or remain asymptomatic.

The majority (67.4%) of *E. coli* strains isolated throughout the study had a non-motile phenotype after 6 hours on motility agar (Figure 25A). The most commonly observed motile and non-motile serotypes were H1 and H5 for both phenotypes (Figure 25B and C). This suggests that the motility is strain related, rather than H-antigen related. Due to the low level of symptoms seen in these study patients and ABU patients in general, it could be that an evasion mechanism may be being utilised here. ABU strains may have found a way to eradicate or reduce flagella numbers, either per cell or within the population. This would allow them to avoid activating the immune system via the TLR5 pathway [131,140].

It was occasionally observed that some strains would appear motile after being left overnight at room temperature. This could further the argument that these strains may be maintaining low levels of flagellation within the population perhaps to subvert clearance by the immune response. However, this would also allow them to maintain the selective advantage the flagella would give them of ascending the urinary tract and avoiding clearance via micturition. A 6-hour time point was chosen for this project as this is a standard assay time for assessing the motility in *E. coli*. However, this observation could give an argument for measuring motility at different time points in future studies.

E. coli strain ST73 is the most commonly isolated motile and non-motile strain (Figure 25D and E). However, since this is the most commonly isolated strain throughout the study, this is to be expected (Figure 22, p.112). The vast majority (70.6%) of motile *E. coli* strains were only ever seen to be motile, for example ST127, ST677 and ST354. The same was found of most non-motile (70.6%) strains also, for example strains ST355, ST420 and ST95. There were 5 strains which were seen as both motile and non-motile during the study. These were ST73, ST69, ST131, ST144 and ST12. Which may suggest that some strains could be able to switch between a motile and non-motile state. Potentially giving an explanation into the observation that some non-motile isolates were seen to be motile after being left overnight.

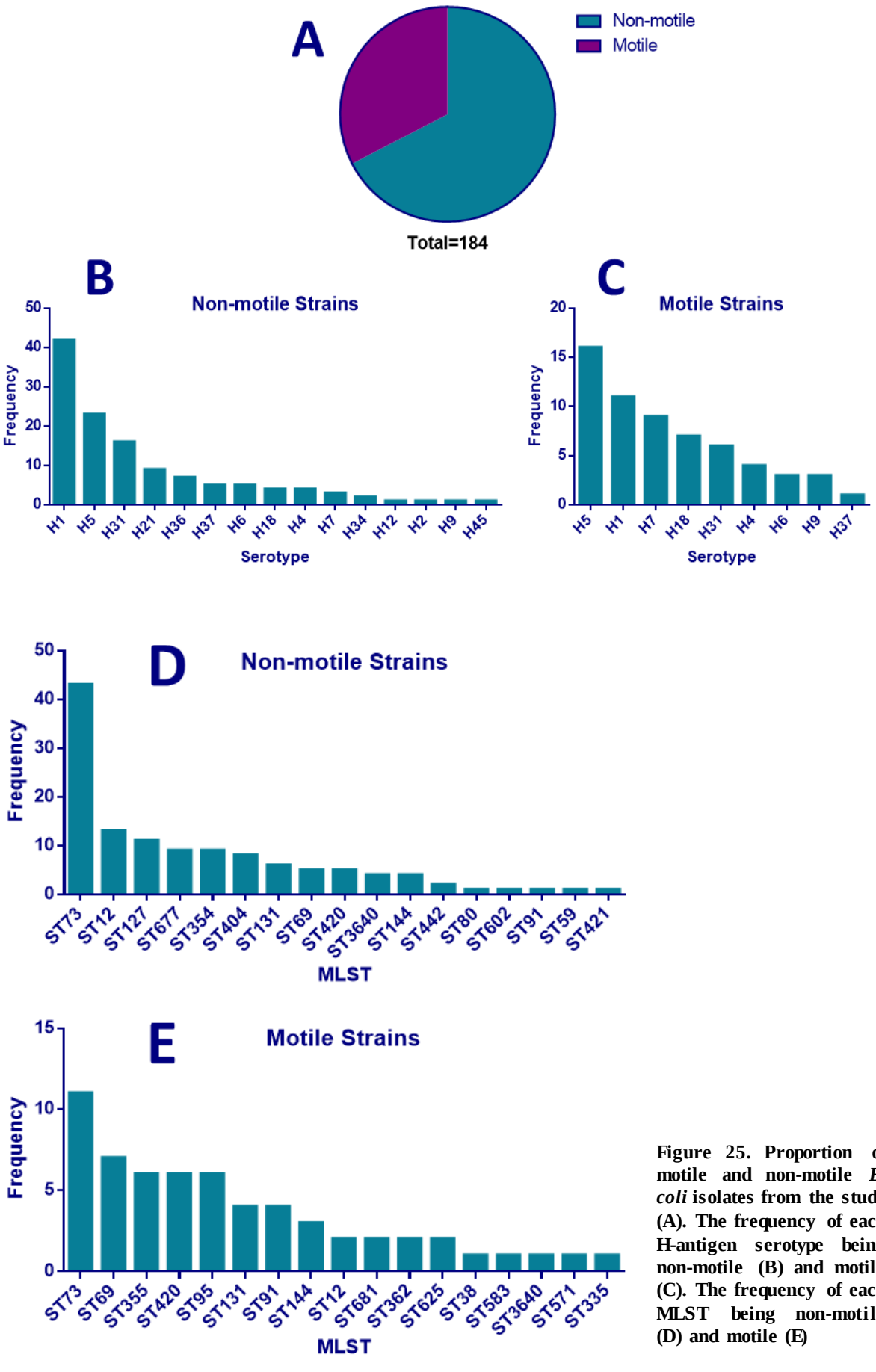


Figure 25. Proportion of motile and non-motile *E. coli* isolates from the study (A). The frequency of each H-antigen serotype being non-motile (B) and motile (C). The frequency of each MLST being non-motile (D) and motile (E)

6.6. Antibiotic Susceptibility Testing

To compliment the treatment and bacterial load analysis, antimicrobial susceptibility testing was also performed on all *E. coli* isolates from the study (Figure 26). All study strains were tested for resistance to the 6 most commonly prescribed first-line antibiotics, as this is what is routinely tested for in NHS laboratories. These were amoxycillin, co-amoxiclav (amoxycillin-clavulanic acid), cephalexin, trimethoprim, nitrofurantoin and ciprofloxacin [28,35].

The majority of *E. coli* isolates were resistant to at least 1 of the antibiotics tested (81.0%), of which 39.1% were resistant to just 1 and 28.8% to 2 of the antibiotics. A total of 41.8% of the study *E. coli* isolates were multidrug resistant. One isolate showed resistance to 5 of the tested antibiotics, this was in patient UTI840 D11 (Figure 26). This was *E. coli* strain ST354, with a serotype of H36 (Figure 23, p.113). The majority of isolates were resistant to amoxicillin (60.3%), followed by trimethoprim (49.5%) and co-amoxiclav (16.8%) (Figure 28).

In several patients it is clear that the antibiotic resistance profile remains constant as the isolated *E. coli* strain remains the same during the study. Such as in patients UTI139, 531, 755 and 781. There are also examples of the antibiotic resistance profile changing as the *E. coli* strain changes. This is clearly outlined in patient UTI924, where 4 different strains are isolated all with distinct resistance profiles. In patient UTI569 the changing *E. coli* strains can also be mapped to the changing antibiotic resistance profiles. To a lesser extent this can also be seen in patient UTI966, however both *E. coli* strain ST69 and ST73 have the same resistance profiles in this patient. In theory the resistance profile could be used as a means of identifying the strain without sequencing. However, there are several patients where different *E. coli* strains can be seen with the same resistance profiles, for example patients UTI337, 365 and 726.

There are some occasions where the *E. coli* strain appears to remain the same across different donations, however the resistance profiles are so different it seems to suggest that the invading strain is completely different. For example, in patients UTI218, 376 and 675, none of which can be aligned to potential pressures caused by any relevant antibiotic treatments.

In patient UTI115 *E. coli* strain ST681 is initially susceptible to all tested antibiotics at D6, but later when this strain is isolated at D12 it appears to have developed the same resistances to amoxicillin and trimethoprim that *E. coli* strain ST131 possessed. Some strains appear to

loose and develop resistances throughout the course of the study, for example, cephalexin resistance is gained at D8 but then lost again after this in patient UTI383. The co-amoxiclav resistance is gained and lost several times throughout the study in patient UTI468 despite the strain appearing to remain constant.

In patient UTI218 the *E. coli* strain ST73 was absent for 2 donations and when it returned at D5 it had developed amoxicillin resistance. This doesn't seem to have been caused by any treatment, as they were only given trimethoprim at D1 and 2 (Appendix 13, p.227). No trimethoprim resistance seems to have appeared in this strain. As seen in UTI218 amoxicillin resistance seems to be readily lost and gained on several occasions by different strains throughout the study. For example, in patient UTI343 both *E. coli* strain ST144 and ST12 both have varying resistances to amoxicillin during the course of the study. Other examples of amoxicillin resistance appearing transiently within the same strain can be seen in patients UTI376, 524 and 675. Amoxicillin is a β-lactam antibiotic and resistance to it is predominantly caused by the bacteria producing β-lactamase encoded on a plasmid [210]. The transient loss and acquisition of this plasmid by the colonising strains could explain the loss and gain of amoxicillin resistance seen here within the study isolates.

Patient UTI524 is an example of where treatment appears to have led to the development of resistance. They are prescribed prophylactic trimethoprim at D5 and this is when trimethoprim resistance is acquired by *E. coli* strain ST72. This resistance remains throughout the rest of the study, despite a change in *E. coli* strain at D8 (Appendix 23, p.237). Patient UTI899 was given trimethoprim treatment at D2, which aligns with a trimethoprim-resistant strain of *E. coli* invading (Appendix 35, p.249). However, this strain is replaced by *E. coli* strain ST73 for the remaining duration of the study. As previously analysed in section 6.4.2 this patient does not experience any further symptoms, suggesting this is a far less harmful strain to this patient. A third example of where prophylactic treatment appears to lead to antibiotic resistances forming is in patient UTI365. They are prescribed trimethoprim for the first 6 donations of the study (Appendix 18, p.232). At D2 They become colonised with a trimethoprim resistant strain of *E. coli* at full diagnostic levels. This could suggest that re-testing urine samples could be useful in prophylactically treated patients. An example of resistances developing due to the pressure of short courses can be seen in patient UTI343. In this patient a short course of cephalexin at D5 appears to clear the bladder of *E. coli* by D6 (Appendix 17, p.231). However, at D7 a cephalexin resistant strain of *E. coli* appears. This is

then treated with trimethoprim, leading to a trimethoprim resistant strain invading. A second course of cephalexin leads to yet another resistant strain colonising the bladder of this patient.

It is also worth noting that none of the *E. coli* strains isolated during the study were resistant to nitrofurantoin (Figure 28), despite this being the most commonly prescribed antibiotic for both short course and prophylactic treatment (Figure 16, p.93). Patient UTI675 was being treated with Nitrofurantoin and appear to have developed no resistance to this antibiotic throughout the study (Figure 26). It is known that Nitrofurantoin does not reach effective concentrations in the blood stream to clear kidney infections [28,35]. So it is possible that *E. coli* strain ST69 in patient UTI675 had ascended the urinary tract and established itself in the kidneys, thus avoiding this prophylactic clearance by the antibiotic.

6.6.1. Susceptibilities of E. coli From Healthy Controls

E. coli was only isolated from 4 of the 45 healthy urine samples, from participants H16 and H18 (Figure 27). Participant H18 had the *E. coli* strain ST73 isolated from their first urine sample, however this was at just 100 CFU/ml, so it is likely to be gut contamination during sample collection. *E. coli* strain ST404 was isolated from H16 in all 3 study samples, these all shared the same serotype and antibiotic resistance profile. This participant was carrying a diagnostic load of *E. coli* for the first 2 study samples, this then dropped to just 1100 CFU/ml in the final sample, despite them receiving no antibiotic treatment. This participant declared that they had never suffered from a UTI in their life. All *E. coli* isolates from the healthy participants were non-motile. It is interesting to note than even in the otherwise healthy population that diagnostic loads of *E. coli* can still be found.

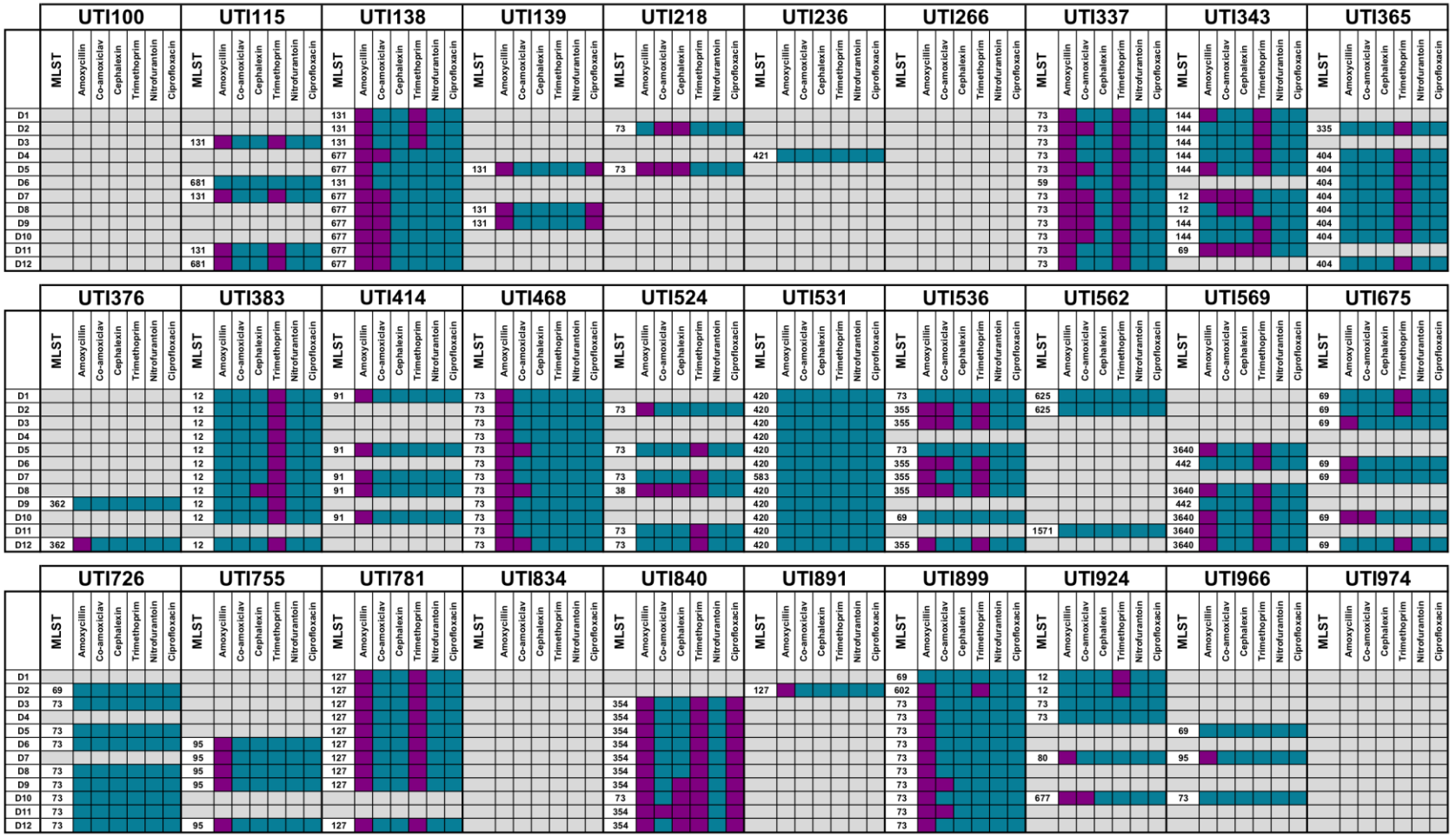


Figure 26. Antibiotic resistance of all *E. coli* isolates over the full study duration. Numbers shows the MLST ID number of the strain. 'UTI' numbers are the patient's randomised participant ID number and 'D' prefaces the study donation number.

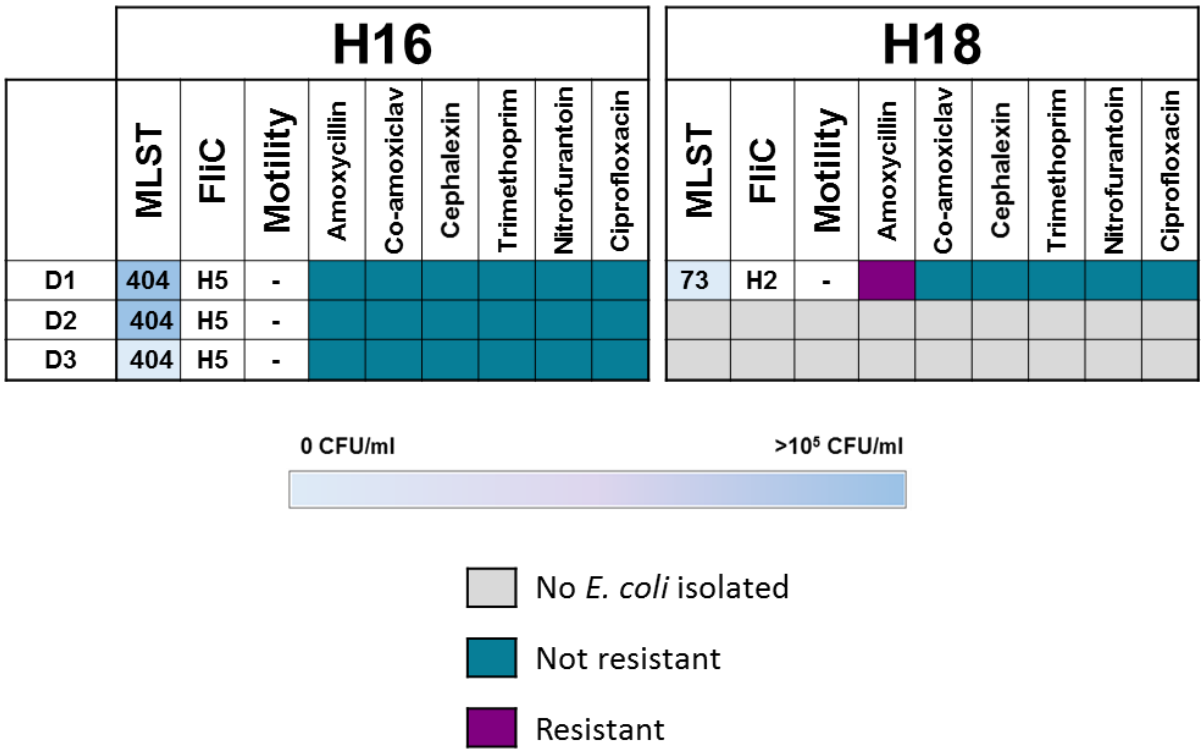


Figure 27. ***E. coli*** **load quantification, MLST and** ***fliC*** **sequencing, motility analysis and antibiotic resistance testing of all** ***E. coli*** **isolates isolated from healthy participants. Numbers shows the MLST ID number of the strain. 'H' numbers are the healthy participant's randomised participant ID number and 'D' prefaces the study donation number.**

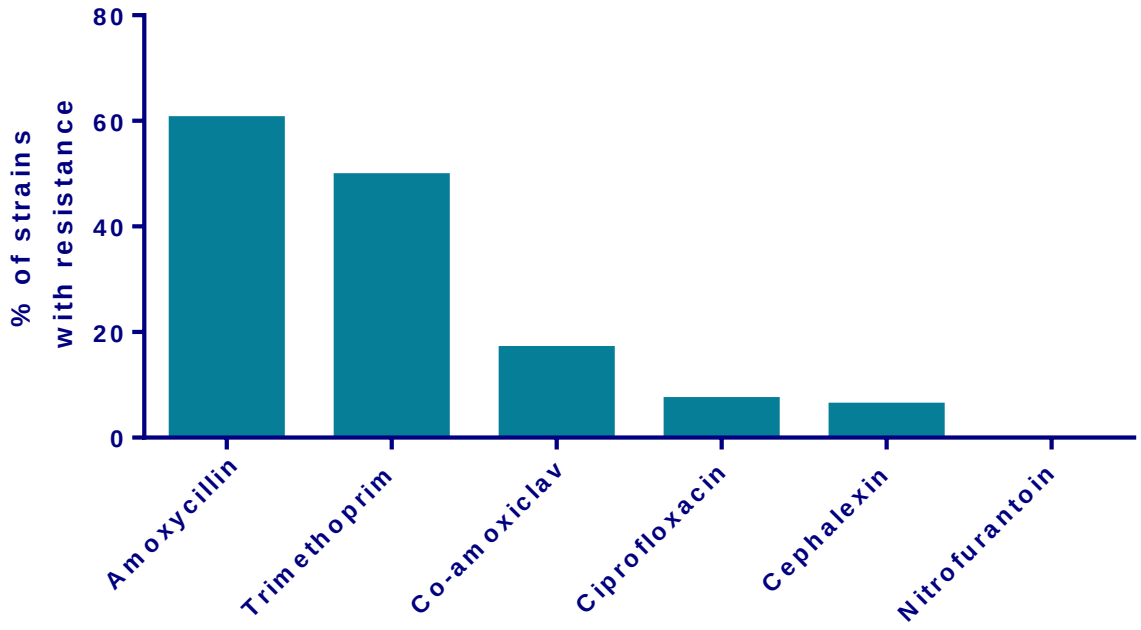


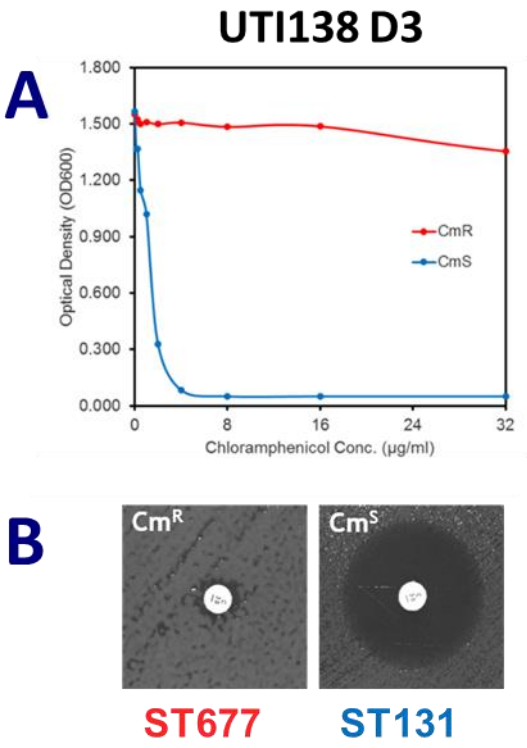
Figure 28. Proportion of all *E. coli* isolates with resistances to the different antibiotics tested.

6.7. Patient UTI138: A Case Study

Due to the vast amount of data presented here it is difficult to focus on individual patients. Thus patient UTI138 has been chosen as a case study, to look into more detail about the bacterial changes taking place in their bladder over time. In addition, it was in this patient that an extra piece of analysis was completed which could be applied to other patients but was not possible to complete within the remit of this project. Patient UTI138 was unique in this study as they had pure *E. coli* growth throughout the full study duration (Figure 19, p.101). This colonisation was found at diagnostic levels in every study sample (Figure 21, p.107). However, they did not show any periods of symptoms and were not treated with antibiotics throughout (Figure 21). Two different *E. coli* strains were isolated from the patient's urines using the single colony selection method undertaken throughout this study, these were ST131 and ST677 (Figure 21). The *fliC* serotypes for strain ST677 in this patient was H21. Strain ST131 was isolated with 2 different serotypes H5 and H12, which has been previously discussed in section 6.4.1 (Figure 23, p.113). *E. coli* strain ST131 is found in the B2 clade (Figure 24, p.115), which is the most commonly associated clade with UTIs [90,125]. However, ST677 is found in the B1 clade, which is a sister group of clade A, commonly associated with commensal gut strains [123,211].

It was upon antibiotic resistance testing that something unusual was observed. It must first be noted that the co-amoxyclav zones of inhibition were just below the threshold for conferring resistance. Thus, the resistance profiles for donations 4 onwards all looked very similar. This seemed unusual since the resistance profile for ST131 at donations 1, 2 and 3 were very different (Figure 26, p.121). It was for this reason the plate washes taken during the study for D3 (among others, data not shown) were analysed for multiple strains of *E. coli* (Roca-Bayerri and Aldridge, unpublished data). For this 100 separate *E. coli* colonies were picked from the plate wash re-activations and sequenced. This analysis revealed that both ST131 and ST677 were present in the urine at the same time. This data reveals that multiple strains of *E. coli* can co-exist within the urine at the same time. Figure 29 (p.126) shows phenotypic and genotypic evidence of the 2 distinct strains of *E. coli* within the same urine sample of this patient (all data shown is courtesy of Roca-Bayerri and Aldridge, unpublished data). This could suggest that future studies should select more than one *E. coli* colony from the urine in order to attempt to capture this information. However, this is a large undertaking and would not have been feasible to perform on a study of this size within the remit of a PhD project. This was one reason for the decision to only isolate one colony was made, the other was that

this protocol would mirror that of the NHS laboratories. Thus these data may give an argument for the NHS to potentially isolate more than one bacterial strain for antibiotic resistance testing, perhaps in hard to treat patients. Or perhaps look into alternative ways of analysing microbial populations rather than single isolates.



C

	MLST Allele ID	
	adk	*icd*
Cm^R	6	18
Cm^S	53	13

Figure 29. Isolation of two different strains of *E. coli* from the same urine sample (D3 from patient UTI138). 'A' shows the differing killing concentrations of the 2 different strains isolated from the same urine sample. 'B' shows representative images of the antibiotic resistance zones of inhibition from the 2 different strains and their corresponding MLST. 'C' gives the different allele ID numbers from sequencing the MLST genes *adk* and *icd* for the 2 separate isolates. All data shown is courtesy of Roca-Bayerri and Aldridge (unpublished data).

6.8. Conclusions

The vast biobank created by the study allowed for a wealth of sequencing to be done to further explore the isolated *E. coli* strains. The biobank will allow for in depth analysis of the specific strains that are colonising this patient group. These analyses will provide insight into the next project aim of exploring potential changes in the bacterial phenotype and genotype during periods of change in the symptoms and treatment of these patients.

The data shows that several study patients are able to carry *E. coli* for long periods of time. *E. coli* was most commonly isolated with *Enterococcus faecalis* during the study, this occurred in 55.4% of all samples containing *E. coli*. Alternatively, the least common pathogens to be isolated together with *E. coli* in this study were *P. mirabilis* and *Streptococcus agalactiae*, which were found in just 12.0% and 7.6% of samples containing *E. coli* respectively. This could be due to the organisms utilising the same nutrient source and therefore having to out-compete each other in order to survive within the urinary tract.

A total of 26 different strains of *E. coli* were isolated during the study from both patients and healthy participants. The most commonly isolated strain was ST73. A finding that fits with other work that has been recently done on UTI *E. coli* isolates, which has also identified ST73 as one of the most prevalent clinical UPEC strains [212]. This has been a recent shift in prevalence, as ST131 was previously reported to be the most common UTI strain of *E. coli* [124,198,212]. ST73 is associated with the B2 clade of *E. coli* which is most commonly associated with UPEC. As would be expected with a UTI clinical study, the vast majority of strains isolated fell into this B2 clade (63.0%) and to a lesser extend the D clade (17.4%). These are the clades most commonly associated with UPEC [90,125].

There were several times that the *fliC* serotype changed and the MLST appeared to remain the same. This could be the invasion of a new strain or a mechanism by which the strain is switching the expressed flagellin, perhaps in order to subvert the adaptive immune response. Feng *et al* have shown that *E. coli* is able to undergo unilateral flagellar phase variation, whereby it switches its *fliC* gene entirely, thus giving the strain an entirely different serotype [213]. This may be the mechanism we see taking place in this study. Another potential mechanism the cells may be using to subvert immune detection is by keeping motility at a lower than threshold level. This would allow the infection to survive within and ascend the urinary tract whilst perhaps also remaining undetected and thus not cleared by the innate

immune system. This is supported by the fact that very few of the strains isolated in our study were motile on motility agar after 6 hours.

One of the most interesting observations during the analysis of these data are the various downfalls of the antibiotics treatments that patients received throughout. For example, there are several cases during the study where treatment courses appeared to clear the way for the invasion of new pathogens, sometimes much more harmful than before. This can be seen in patients UTI236 and 569 during the course of the study (Figure 21, p.107). It was also interesting to note the lack of correlation between symptomatic episodes and commencement of treatments. It is clear from these data that many HCPs are not awaiting the return of a positive urine sample before beginning antibiotic treatment, such as in patients UTI414 and 562. In addition to this it also seems that the treatments themselves are often ineffective at clearing the bacterial load and in some cases also the symptoms. For example, this can be seen in patients UTI218 and 468. This may be due to the selection of the wrong antibiotic by the HCP or poor course compliance by the patients themselves. This lack of efficacy can be highlighted in several study patients who received prophylactic courses of antibiotics. Many of whom still saw varying bacterial loads and still suffered symptomatic episodes during the treatment, such as patients UTI365 and 383.

By analysing the antibiotic resistances of the study strains it was possible to add another dimension to the bacterial analysis, as well as align this to some of the treatments given during the study. On several occasion it was possible to see the isolated *E. coli* acquiring and losing antibiotic resistances without any changes in antibiotic treatment. This may be due to horizontal gene transfer between different *E. coli* strains or even different bacteria colonising the bladder together [214]. This is supported by the vast majority of study samples being returned as polymicrobial. This may be a useful mechanism the bacteria might use to avoid clearance from the urinary tract and ensure survival. Notably, no isolated *E. coli* showed resistance to Nitrofurantoin despite being one of the most prescribed antibiotics during the study, including for prophylactic courses. This could therefore be the best option for long courses of treatment, however it is not effective at clearing infections from the kidney and is associated with a higher risk of toxicity in older people [28,35].

On several occasions it can be seen that treatment courses are causing the acquisition of antibiotic resistances or leading to new more resistant strains colonising the bladders of these patients. Whilst it must be noted that not all of these occasions lead to the patients

experiencing symptoms, it is increasing the reservoir of more resistant pathogens. This could therefore easily lead to the spread of the pathogens between hosts or the resistance genes within the bacterial community. There are also some cases during the study where treatment courses appeared to clear the way for the invasion of new pathogens, sometimes much more harmful than before. Thus further suggesting the treatments being given could even be having harmful effects to these patients, as well as potentially increasing antibiotic resistances.

Many patients had colonisation of several different strains of *E. coli* throughout the study. By looking at the strain changes compared to periods of defined symptoms, it was possible to see that the strain changes alone were not correlated with symptomatic episodes. There were even some cases where the *E. coli* colonisation of the bladder appeared to prevent the patient from suffering further symptomatic episodes. This fits with some of the research being done by Sundén *et al,* in which they have shown inoculating the bladders of certain patients who suffer from UTIs with a harmless ABU strain (83972) can actually prevent symptomatic UTIs [53].

There also appeared to be no distinct link between these symptomatic episodes and sudden changes in the bacterial load within the bladder. This was perhaps more unexpected as changes in load would be expected to see changes in the immune response and thus perhaps symptoms. However, it could be that these patients' bodies are used to the presence of bacteria in the urine and these fluctuations no longer cause sudden disturbances in the inflammatory response. The patients' host immune responses to these bacterial colonisations will be the focus of the next chapter. [50,215]

Chapter 7. Urine Analysis and Host Response

Is has been possible to analyse the clinical notes and questionnaire data to delineate the study population as well as define episodes of symptoms within the study duration. The *E. coli* isolates from the study provided a detailed perspective of the bacterial colonisation taking place within the bladders of the study participants. This also allowed correlation of potential changes around defined symptomatic episodes. The clinical study also provided a large database of 360 patient urine samples on which a host analysis could be run. This analysis would address the study aim of analysing changes in potential host immune urinary biomarkers around periods of symptoms. Potentially identifying markers within the urine which could predict such symptomatic episodes. As well as this 45 urine samples were also collected from healthy controls as a means of comparison and baseline threshold deduction.

7.1. Dipstick Outcomes

Dipstick analysis is a tool used by HCPs in order to non-invasively test various parameters of the patents health, including kidney and liver functioning. Upon collection all urine samples were analysed using the standard dip-stick according to the NHS protocols. In order to maximise data acquisition from this analysis all dipstick parameters were captured and not just those that indicate potential urine infections. These included glucose, bilirubin, ketones, specific gravity, protein, pH, urobilinogen, nitrites, leukocytes and both haemolysed and non-haemolysed blood within the urine. Any potential findings could therefore easily be translated into working practice within an NHS clinical setting.

7.1.1. Patient and Healthy Control Dipsticks

In order to define the healthy urine baselines for the dipstick scores, the healthy dipstick outcomes were required. The most frequently returned outcome for each of the 10 parameters of the dipstick in the healthy control urines was chosen as the 0 score on the dipstick scoring system, as outlined in Table 3 (p.49). Frequency analysis shows clearly which levels were chosen as healthy. For glucose, bilirubin, ketone, protein, nitrites, leukocytes and blood were all set at ‘Negative’. The healthy level of specific gravity was set at 1.010, for pH it was set at 6 and for urobilinogen at 0.2mg/dL. These were all backed up by previously reported normal

ranges [65]. This analysis provided the baselines to determine dipstick scores for the patient cohort, which is discussed later in this chapter (Table 3).

Frequency histograms of the study patients and healthy controls allow for an overview of the type of results being returned in the two cohorts (Figure 30). The majority of patient samples (99.2%) returned a negative result for glucose. Only 3 samples produced a non-negative result for glucose, these were UTI100 D10 (2000+mg/dL of glucose), UTI139 D12 (100mg/dL) and UTI414 D1 (250mg/dL). These patients all stated that they had diabetes upon recruitment to the study (Appendix 9; p.223, Appendix 12; p.226 and Appendix 21; p.235). All 3 donations positive for glucose exhibit growth of *Proteus mirabilis* (Figure 31), which may suggest that this organism thrives especially well in the presence of glucose. Therefore, this pathogen may be a key threat to patients with poorly controlled diabetes [64].

Levels of bilrubin, ketones, specific gravity and protein were all seen at slightly higher levels in patient urines compared to the healthy controls. 13.3% of patients had a 'small' bilirubin result or higher, whereas 100% of healthy controls had a negative result. 10.3% of patient samples had 5ml/dL or higher ketone levels in the urine, whereas all healthy controls again were negative. 28.9% of patient urines had a protein level of 5mg/dL and above, compared to just 10.0% of healthy urine samples. 80.1% of patient urine samples had a specific gravity over 1.010 compared to 49.0% of healthy urines seen above this designated 'normal' level. Higher levels of ketones, specific gravity and protein all suggest a slightly poorer overall kidney heath and potential bacterial presence in the study patients [65]. Which may be to be expected, considering the long-term carriage of pathogens within the urinary tract of most study patients (Figure 19, p.101). However, the vast majority of patients showed the same result as was seen in the healthy controls.

As would be expected, the urine of patients showed proportionally much higher levels of nitrites and leukocytes, as well as a higher pH. These are all indicators of bacterial colonisation [65], which was seen in almost all study patient urine samples (Figure 19). 22.2% and 61.1% of the patient samples had a positive dipstick outcome for nitrites and leukocytes respectively, compared to 0.0% and 8.0% of healthy urines. The average pH in patient samples was pH6.3 and in the healthy urines was pH6.2.

Finally, the frequency of detecting blood, both haemolysed and non-haemolysed, in the patients' urines was much higher (37.2%) than was seen in the healthy control urines (8.0%). One possible explanation for this, is that patients may have damaged uroepithelial linings,

which could lead to the presence of blood in the urine [22]. This could be being caused by the combination of direct action of the colonising bacteria as well as the subsequent inflammation induced by the host immune response in reaction to such colonisation is causing [22].

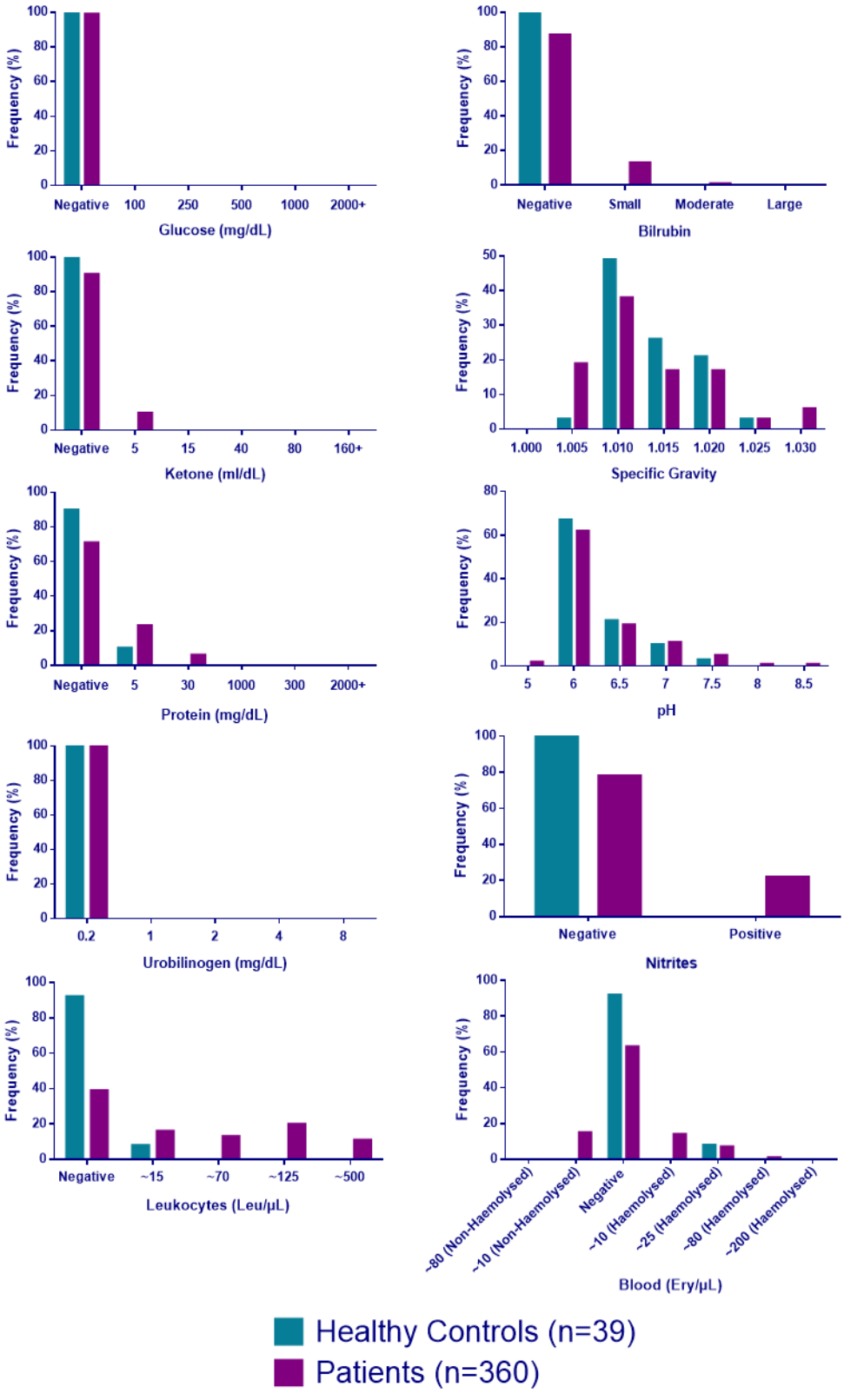


Figure 30. Dipstick outcome frequency analysis of healthy and patient urines. Samples positive for nitrites and leukocytes are what a HCP would deem a positive urine sample for a potential UTI [28,65].

Figure 31. Plate pictures of urine samples returning a positive glucose result from the dipstick analysis. All plates show the growth from 10µl of study urine. 'UTI' is anonymised patient ID number and 'D' indicates the donation number (of 12). CFU of each plate: UTI100 D10 $>10^5$ CFU/ml, UTI139 D12 = $1.55*10^4$ CFU/ml, UTI414 D1 = $3.6*10^3$ CFU/ml.

7.2. Dipstick Scores

By quantifying the outcomes of the dipstick analysis it was possible to assign each of the study urines a dipstick score (Table 3, p.49). This enabled a holistic overview of the general sample health, as a higher dipstick score should correlate to a poorer overall sample health. It was possible to therefore align such scores with defined periods of symptoms over the duration of the study. If correlation was seen this could provide a very simple and cheap method of predicting symptomatic episodes in these patients.

By analysing the dipstick scores, the HCP outcomes and symptoms all together over time, it is possible to see occasions where the symptom scores correlated with symptomatic episodes (Figure 32). For example, in patients UTI236 and 343. However, there are many more where this is not the case, such as in patients UTI138, 891 and 824. In patient UTI569 a high dipstick score can be seen at their first symptomatic episode, at D4, but not at their second episode, at D6. Alternatively, in patient UTI675 a high dipstick score correlates to their symptomatic episode, at D1, but the same high score at D12 where no symptomatic episode was recorded. Thus, the dipstick score does not appear to be consistently reliable at predicting periods of symptomatic UTI, when using a positive urine sample to define symptoms.

Another interesting finding from this analysis was the lack of apparent correlation between positive HCP outcomes and symptomatic episodes. The symptomatic episodes in this study are being defined as a positive urine sample being handed in within 3 days of a study samples. Thus, it would be expected to see a positive urine dipstick score for nitrites and leukocytes at these times. However, only 34.1% of symptomatic urine samples gave a positive HCP outcome. Negative HCP outcomes and positive urine samples can be seen in patients such as UTI139, 218, 536, 562 and 569.

A positive HCP outcome is stated to predict an ~80% chance of a UTI, when a patient takes a urine sample to their HCP for testing [216]. However, this does not discriminate between symptomatic episodes. The data shown here suggests that the dipstick scores and HCP outcomes this gives, may not always be completely reliable at predicting or perhaps even diagnosing periods of symptomatic episodes in these patients.

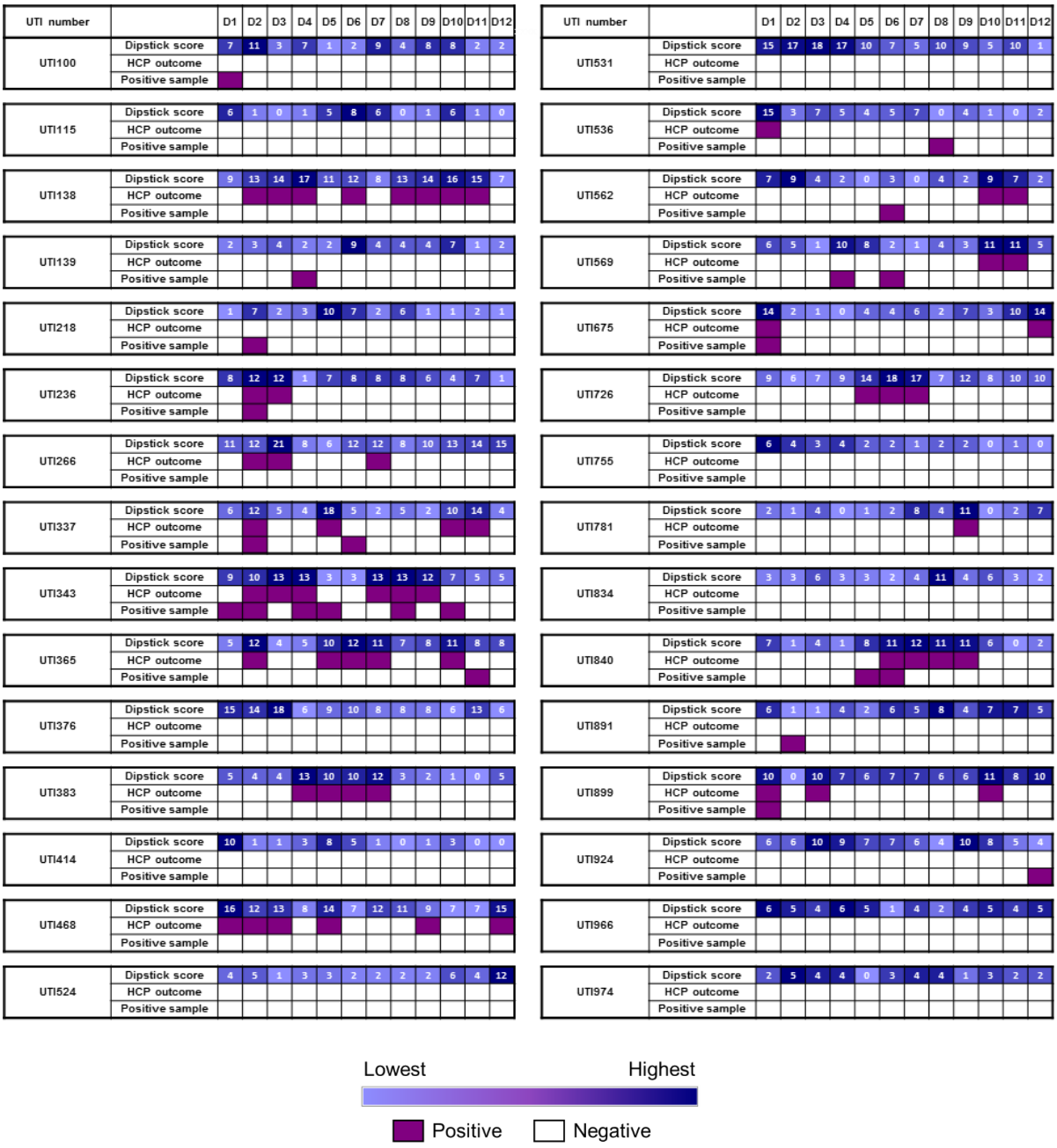


Figure 32. Table showing patient dipstick scores, HCP outcomes and positive urine sample data for all study donations. Patients UTI numbers are given. Numbers prefaced with a 'D' are the study donation numbers. Dipstick score taken as a summation of all measured dipstick parameters. 'HCP outcome' shows a filled box if the dipstick returned a positive result for both nitrites and leukocytes. A filled box in the 'positive sample' row indicates if the patients' medical records showed a positive urine sample had been submitted to the NHS laboratories within 3 days either side of the study donation.

7.3. Blood Analysis

In order to get an idea of the study patients' general health, certain parameters were measured in the blood. This is a common investigation requested by urologists in order to better understand the patients' kidney and urinary functions which cannot be deduced from a simple urine sample. Serum creatinine was measured as a marker of kidney filtration function. An elevated serum creatinine concentration is usually an indicator of impaired kidney function, as it suggests it is not being properly filtered out of the blood [75]. The vast majority of the patients had serum creatinine levels within the normal range (Figure 33A). Only one patient, UTI755, showed elevated levels of serum creatinine in the blood, this may suggest that this patient had decreased kidney function. A low serum creatinine, as seen in UTI100, is not usually such a large cause for concern and is usually associated with a low body muscle mass, which is commonly seen in older people [217].

Vitamin D was measured from the bloods of study patients as there has been evidence to suggest that vitamin D deficiency can put people at higher risk of recurrent UTIs (Figure 33B) [79–81]. Only 40.0% of the study patients showed sufficient concentrations of vitamin D in their blood. A large proportion of the recruited patients had insufficient vitamin D in their blood, which fits with what has been previously shown in the literature. This could suggest one of the reasons for their UTI history could be linked with their low levels of circulating vitamin D. Low levels of vitamin D have also been shown to be associated with malnutrition [218]. This link could explain the low levels of vitamin D and serum creatinine seen in patients UTI100, as malnutrition could be causing the patient to have a low body muscle mass both leading to lower levels of these blood parameters.

Sodium, potassium and urea are all other blood based parameters routinely tested in order to give an overview of general kidney function. Sodium concentrations in the blood were only seen to be marginally too low in 3 study patients, UTI414, 536 and 966 (Figure 33C). Low sodium can be associated with reduced kidney function, but is usually explained by a larger than normal consumption of fluids or the use of diuretics, which is not unlikely in this patient cohort [76]. Potassium was only seen to be marginally too high in 3 study patients, UTI266, 524 and 726 (Figure 33D). Though there were no patterns here with any of the other blood results. None of the patients had levels over 5.5mmol/L, this is the level where clinician concern would be raised and levels above 6.5mmol/L would be handled as an emergency, as this is

associated with high levels of morbidity and mortality [77]. So all the study patients were within a safe range of potassium concentration.

Elevated urea levels are also indicators of poor kidney filtration function [78]. Three study patients had levels of urea in the blood higher than the normal range, these were UTI755, 834 and 899 (Figure 33E). UTI755 showed the highest concentration of urea at 14.7mmol/L, the other elevated patients 834 and 899 had levels of 10.5mmol/L and 9.7mmol/L respectively. UTI755 was the patient with elevated serum creatinine, this could indicate that this patient's kidney status could be some cause for concern, as there has been some links between raised serum creatinine and urea with a poorer prognosis in kidney disease [219]. However, their other blood parameters were within the normal ranges.

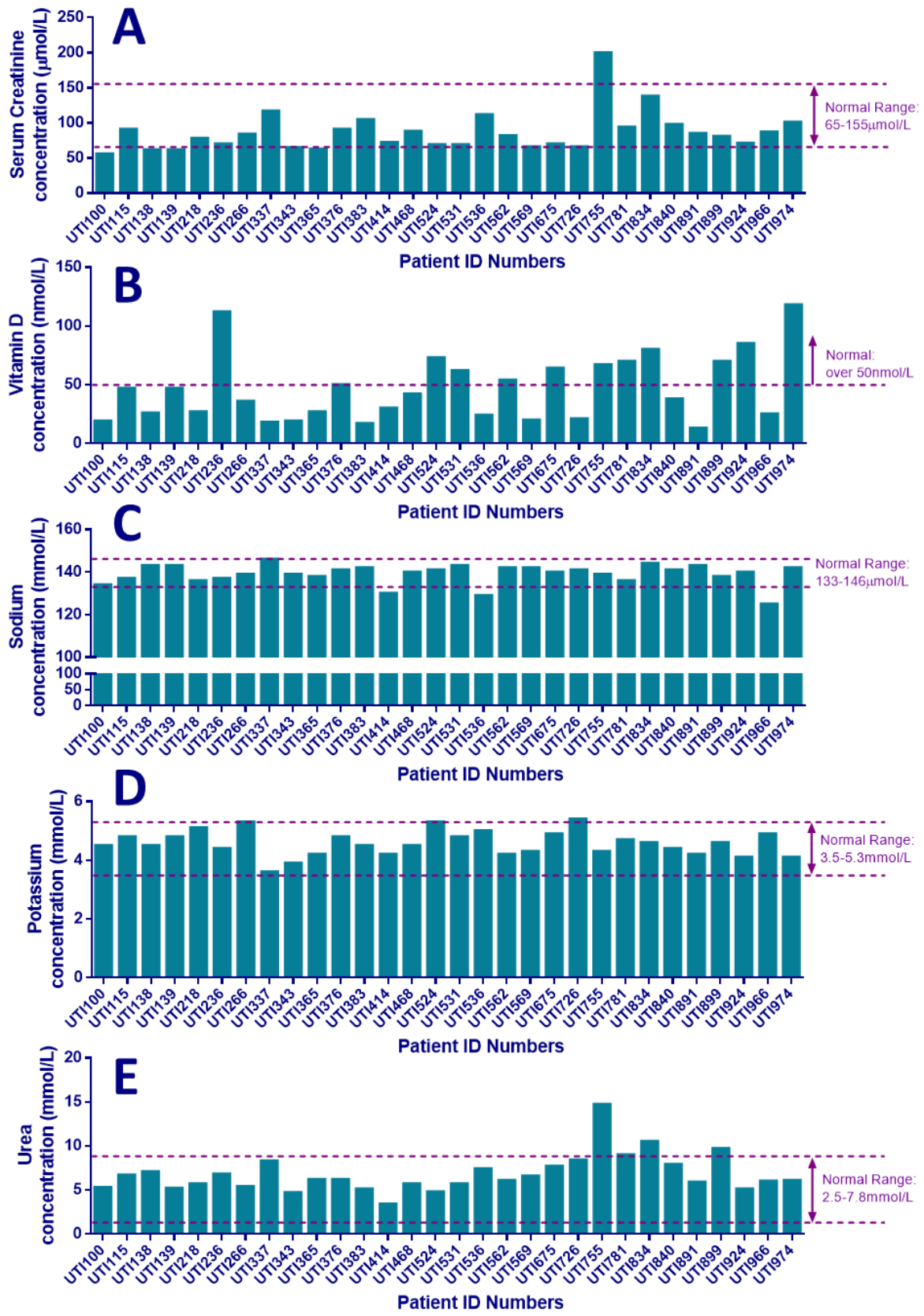


Figure 33. Blood results for all study patients. 'A-E' show blood concentrations of serum creatinine, vitamin D, sodium, potassium and urea in patients. Normal ranges defined by hospital laboratory reports.

7.4. Cytokine Analysis

A key aim of the study was to analyse changes in immune markers that could be measured within the urine as a potential method of predicting symptomatic episodes of UTI within the ABU patient population. A wide selection of cytokines were chosen to be measured in the urine based on their reported involvement in UTIs. Several pro-inflammatory (IL-1β, IL-6, IL-8, IL-12 p70, IL-17A, TNFα and IFNγ) and anti-inflammatory (IL-4, IL-5 and IL-10) cytokines were chosen for analysis as both have been shown to be involved with the immune response in UTIs. Each of the cytokines chosen were suggested to show some involvement in UTIs, details of these are given in section 1.4.2 [54,94,147,150–155,159,160,162,165,173–181,220,221]. The chosen cytokines also needed to be easily measured by commercially available ELISA kits, this would mean a large screen could be done and that any potential findings or markers could be viably tested in a reproducible way in the future.

7.4.1. Urinary Creatinine and Healthy Adjustments

Urinary creatinine was measure within the study urine samples as a potential reference marker to adjust the immune protein levels to. As urinary creatinine is a marker of urine solute dilution levels which is governed by kidney function [222]. Thus theoretically, adjustments of the protein concentrations would allow normalisation to that of the urine sample concentration. When plotting the raw data with the data adjusted for the urinary creatinine concentration it is possible to see a large variation in all the measured cytokines (Appendix 5, p.219).

Based on the advice of the project supervisor, Professor Robert Pickard and some experts in this field, Dr Björn Wullt and Dr Christina Svanborg, it was decided that the raw values would be most informative for the analysis within this study. This was based on several reasons regarding their previous experience with this issue, published literature and the nature of urinary creatinine production. Firstly, experience with measuring urinary cytokines gave very similar overall findings when looking at the raw data and normalising to urinary creatinine compared in parallel (Wullt and Svanborg, unpublished data). As a result, such an adjustment would not impact the analysis and potentially make data reproduction overly complicated. Therefore, the best thing to use was the raw concentrations found in the urine. This is in agreement with Rodhe *et al* (2009) where a similar observation during their analysis was noted and suggested that testing without correction to urinary creatinine would be possible and not change the overall impact of the data significantly [155]. Finally, urinary

creatinine is produced by the kidney and is thus a marker of kidney function. Thus, normalisation to urinary creatinine would be more appropriate for proteins being produced in the kidney, rather than the urinary tract and bladder as these chosen cytokines are. For these reasons it was decided that creatinine normalisation was inappropriate for these analyses. However, the data has been captured during the study, so this analysis can always be completed if different questions were to be asked of the data in the future.

By using the cytokine concentration raw data from the healthy control urine samples, it was possible to produce an average healthy concentration for each of the measured cytokines (Figure 34). This could then be deducted from the individual raw values from the patient samples. This would now give a more realistic overview of the changes within the patients that are based on the illness and not normal healthy concentrations. Several of the averages obtained within this study fit with other reported concentrations from the bladders of ABU patients [155,223] (Table 2, p.39).

The average cytokine data reveals the concentrations of each protein being seen in the patient group compared to that of healthy bladders (Figure 34). IL-1β, IL-5, IL-8 and IL-10 all show higher concentrations in the bladders of patients than healthy controls. IL-8 stands out as a protein which could separate the two cohorts due to its clear elevation elevated in patients compared to healthy controls. Interestingly, IFNγ, TNFα, IL-17, IL-12 and IL-6 all show reduced concentrations in patients compared to the healthy controls. This could suggest that the patients' immune systems are somehow supressing the production of these proteins or perhaps that constant immune activation has caused a desensitisation in the pathways which lead to their production. Due to their presence in the bladders of healthy age-similar individuals at relatively high concentrations, these proteins would probably not make good predictive biomarkers in the urine of older people. As peaks may be due to normal fluctuations, which could give a high rate of false positive readings.

The purpose of measuring the cytokine levels in the bladders of healthy controls was to normalise the patient data. Values obtained by deducting the healthy average concentration from the individual patient values will be the concentrations used for the remaining analysis of this project (Appendix 6, p.220).

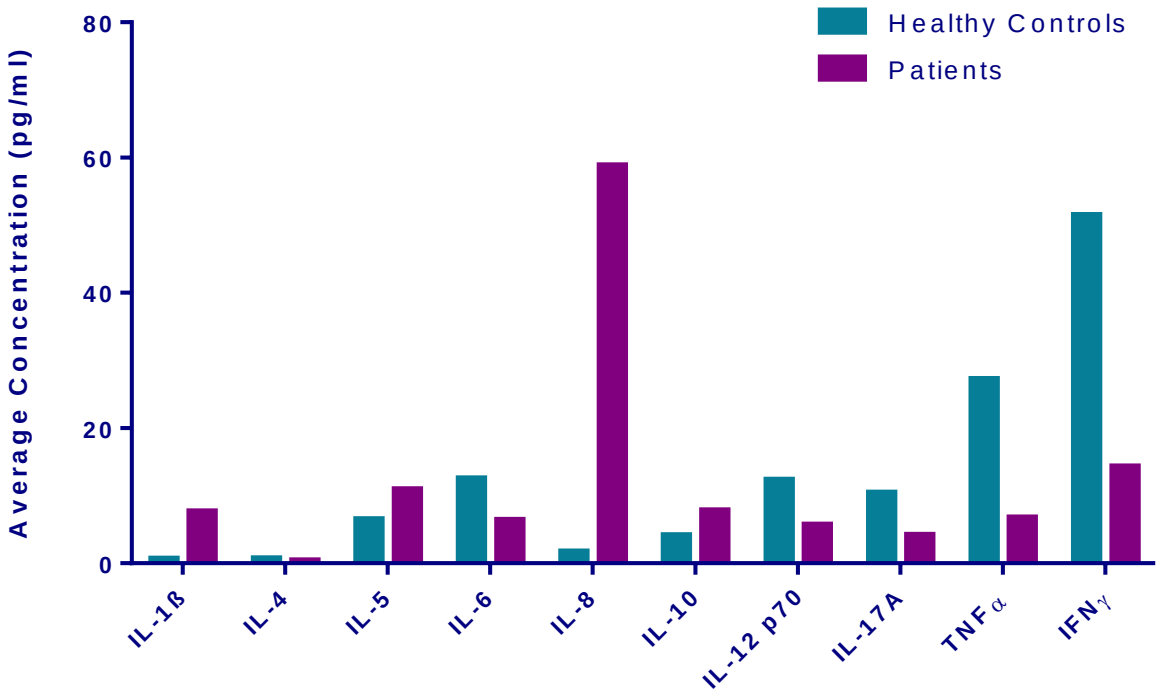


Figure 34. Average cytokine concentrations from all healthy and patient urine samples.

7.4.2. Cytokine Fold Change Analysis

In order to visualise elevations in the urinary protein concentrations together, it was necessary to use fold change data. By calculating all the fold changes of the patient to healthy control data for all the analysed cytokines, a general fold change of 11.2 was observed (Figure 34). Thus, as a cut off, it was decided that concentrations over 10-fold higher than the lowest detectable concentration in each patient would be reported as a positive. Analysing different options for fold change cut-offs 10-fold would allow inclusion of and average of 10.2% of all study samples (Figure 35). The 2-fold and 5-fold cut offs would have included averages of 18.8% and 14.1% respectively. Therefore 10-fold was a tight cut-off but this is necessary when trying to identify potential biomarkers of disease. By using the tighter control of 20- or 50-fold cut-offs would have left on average just 6.8% and 3.5% of the samples above this level respectively. As these were so low they were discarded because they may begin to prevent any observations being made within the data. Using the 10-fold change data would allow for a comparable overall visualisation of concentration peaks for all of the measured proteins together (Figure 36). Fold-increase allows for the detection of fluctuations above normal which is a standard diagnostic method in clinical practice. However, it would also be possible to calculate and analyse the fold decrease in proteins which showed suppression in patients compared to healthy controls. As a single standardised method was required for these analyses, a fold increase method was chosen for the cytokine data produced.

Visualising the data in this way shows the huge variability of the immune response over time within patients. It also shows the variation between different patients with the same disease. It is not consistently one or a combination of cytokines that are being produced in response to ABU. This suggests a huge level of variability of the immune response to pathogenic colonisation of the bladder in different patients.

Interestingly, patient UTI343 who showed 6 separate symptomatic episodes during the course of the study had no immune protein concentrations over 10-fold that of their lowest. This is surprising as it would be expected that a high frequency of symptoms would show a highly active immune response. Looking at the raw values the only cytokine found above that of the healthy control was IFNγ at UTI343 D3, which is not even a sample which was defined as symptomatic. However, the concentration was much lower than the assays detectable range (0.47pg/ml). This patient may give rise to the argument that patients with long-term

pathogenic presence in the bladder, have an overall desensitised immune response to colonisation due to the constant activation.

The fold change data suggests that there are no clear or consistent patterns in immune protein concentration around the periods of symptoms. By visualising all the data for the chosen cytokines together, it is possible to see if any of the measured proteins show significant peaks in concentration around times of symptoms. These peaks will be captured within the 10-fold change cut-off. From this analysis, there appears to be no distinct protein or protein combination that can be identified to predict periods of symptomatic infection (Figure 36). However, it is important to also look at the raw values of the cytokines in patients over time in case potential observations are excluded by the 10-fold concentration cut off.

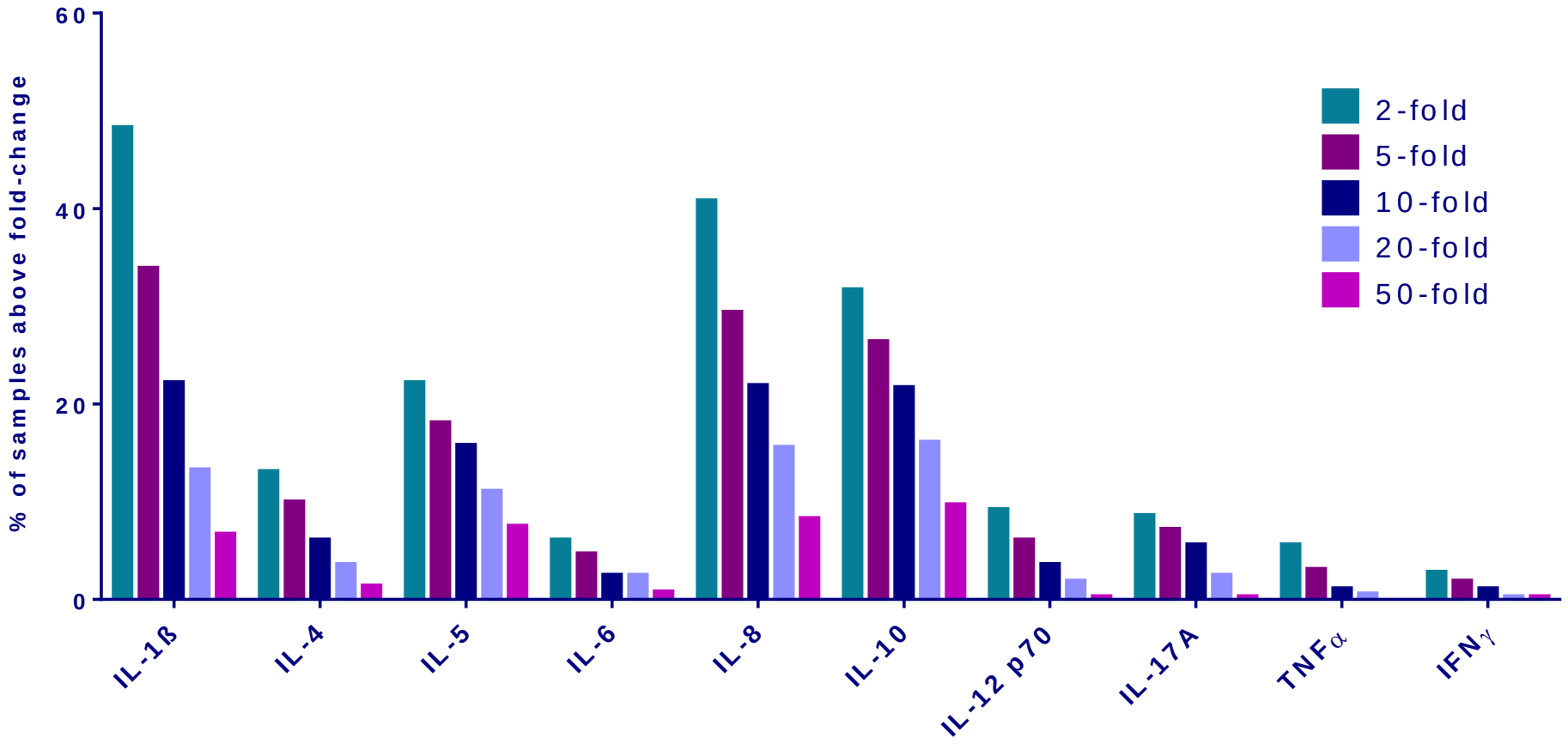


Figure 35. Proportion of samples above varying thresholds of fold change in protein concentration for each cytokine.

Figure 36. 10-fold change cytokine data alongside symptomatic episodes. A filled box indicates the protein concentration was 10-fold higher than the lowest detectable concentration seen in that patient. Pro- and anti-inflammatory cytokines are shown in separate colours.

7.4.3. Host Response and Symptomatic State

It was previously outlined in Chapter 5 how a symptomatic episode would be defined. This stated that the best definition for a period of symptoms in our study would be based on the patients handing a positive urine sample to a healthcare professional for a suspected UTI within 3 days of the study urine sample being collected. This allowed the identification of samples could be classed as 'symptomatic' at the time of collection, these episodes occurred in 15 patients during the study. These symptomatic episodes could then be plotted against the various markers being analysed in the urines. Figure 37 to Figure 46 show the 10 analysed immune protein concentrations in all 15 patients who suffered from a symptomatic episode during the study. This provided a comparison of the cytokine concentrations while comparisons to other potential definitions of a symptomatic state, such as self-declared symptoms from patients and positive HCP outcomes, will follow in the next chapter.

These timeline-based representations allow the data to be analysed for any trends in protein levels around the time of symptoms. It must be noted that the scales are based on the levels seen in that patients so direct comparisons of levels cannot be made across patients within the figures. In addition, it is important that not all donations were evenly spaced, most were ~2 weeks apart however some had longer gaps, this must be kept in mind when interpreting these figures. However, the definition of symptomatic state is based on a positive sample being submitted within 3 days either side of the donation that is marked (green bars).

IL-1β

Of the 23 individual identifiable symptomatic episodes defined in the study, only 2 occurred at times of peak IL-1β concentrations in that patient, these were in patients UTI236 and 569 (Figure 37). However, UTI569 suffered another symptomatic episode and no such increase in IL-1β was seen. UTI236 showed one other raise in IL-1β levels of about half the magnitude however this did not correspond to another symptomatic episode. Other patients saw some IL-1β concentration rises around symptoms, such as in patients UTI337, 365, 562 and 840. However, all of these patients saw larger peaks in IL-1β at other points in the study which did not correlate to a symptomatic episode.

IL-4

The highest levels of IL-4 within patients corresponded to just 2 of the 23 symptomatic episodes, in UTI675 and 924 (Figure 38). However, the peak in IL-4 concentration seen in UTI675 was very low and in UTI924 was below the detectable range for the assay so no

conclusions can reliably be drawn from these variations. The only detectable concentration of IL-4 in UTI675 was at a period of symptoms. Very few detectible concentrations of IL4 were found in any of the 15 symptomatic patients' urine samples. This fits with the concentrations Davidoff et al (1997) saw when measuring urinary IL-4 in patients with bacterial cystitis [153].

IL-5

IL-5 concentrations were at maximum concentrations for just 3 of the 23 symptomatic episodes, in UTI236, 337 and 891 (Figure 39). All 3 of these patients showed clear increases in IL-5 protein concentrations only at periods of symptoms, indicating a possible correlation. IL-5 patterns in patient UTI236 and to a lesser extent UTI337 show some correlation with IL-1β around periods of symptoms. UTI569 also showed a correlating symptom and peak in IL-5, but another symptomatic episode did not.

IL-6

Only 3 of the 23 symptomatic episodes corresponded to peak concentrations of IL-6 in patients UTI236, 569 and 891 (Figure 40). UTI236 and 891 seemed to show a correlation with the protein concentration and symptom state. However, UTI569 displayed another episode of symptoms but without an increase in IL-6 concentration, which was similar to their concentration profile of IL-1β. The IL-6 peak seen in patient 891 showed similarity to the clear peak in IL-5 at the patient's symptomatic episode.

IL-8

IL-8 was shown to have the highest average concentration in patients compared to any other measured cytokine (Figure 34, p.142). IL-8 peaked at just 2 of the 23 periods of symptoms in UTI337 and 569 (Figure 41). The peak profile in UTI337 was similar to that of IL-1β. However, both of these patients had other episodes of symptoms without another corresponding peak in IL-8 protein concentration. The IL-8 concentrations mirrored those of the IL-1β and IL-6 profiles in patient UTI569. It should also be noted that the levels of protein seen in UTI337 were much higher than could reliably be detected using the assays. So whilst the peaks seen in this patient will likely indicate an increase in the protein level, the exact values are likely to be inaccurate. On 5 separate occasions the IL-8 concentrations peaked within 1 or 2 donations after a symptomatic episode, suggesting the immune system activating to clear the infection. This would not therefore be a good choice of predictive marker for UTI symptoms.

IL-10

In several cases the concentration of IL-10 seemed to increase on or shortly after symptomatic episodes (e.g. UTI100, 536, 562, 675, 891) (Figure 42). This could be due to a delayed immune response or perhaps a result of subsequent treatment. However, this trend was not consistent for all symptomatic episodes. Also this would also suggest it would not be a suitable predictive marker for symptoms.

IL-12 p70

IL-12 concentration peaks correlated with symptomatic episodes in 3 of the 23 identified cases, these were seen in patients UTI337, 569 and 891 (Figure 43). However, both UTI337 and 569 had other periods of symptoms which did not show corresponding increases in IL-12 concentrations. As was seen in the concentrations of IL-10 for UTI891, despite a peak in IL-12 at the period of symptoms, the patient had other increases in the protein where symptoms were not present.

IL-17

IL-17 increase only corresponded to 1 symptomatic episode in UTI569 (Figure 44). However, this patient also presented with another period of symptoms but did not show another increase in IL-17 concentration. This was similar to what was seen in IL-1β, IL-6 and IL-8 in this patient.

TNFα

Maximum levels of TNFα corresponded to symptomatic episodes in just 2 patients; UTI337 and 569 (Figure 45). However, both patients had other periods of symptoms but did not show elevated TNFα levels in the urines. The TNFα concentration profile for patient UTI337 was similar to that of the IL-12 profile. Very low levels of this protein are seen across the symptomatic patients. This is likely due to the healthy average adjustment, as this was one of the proteins which showed a marked lower average concentration in the patients' urines compared to the healthy controls (Figure 34, p.142).

IFNγ

Finally, the peak levels of IFNγ did not appear to correlate with any symptomatic episodes (Figure 46). IFNγ did have a tendency to increase shortly after a symptomatic episode in a few cases. However, the concentrations were generally too low to have any significance. As with TNFα, this protein shows consistently low concentrations due to the healthy adjustment,

as this protein was also seen at higher levels in healthy urine than in patients (Figure 34, p.142).

These data taken together show that there is huge variability in the immune response between patients and even within patients as individuals. However, it is clear that of the proteins measured in this study, there is no single immune marker that consistently shows marked elevation before symptomatic episodes. It is possible that there may be a combination of protein patterns which may be able to predict episodes of symptoms. For this it is therefore necessary to observe periods of symptomatic episodes alongside elevations in all the urinary proteins together.

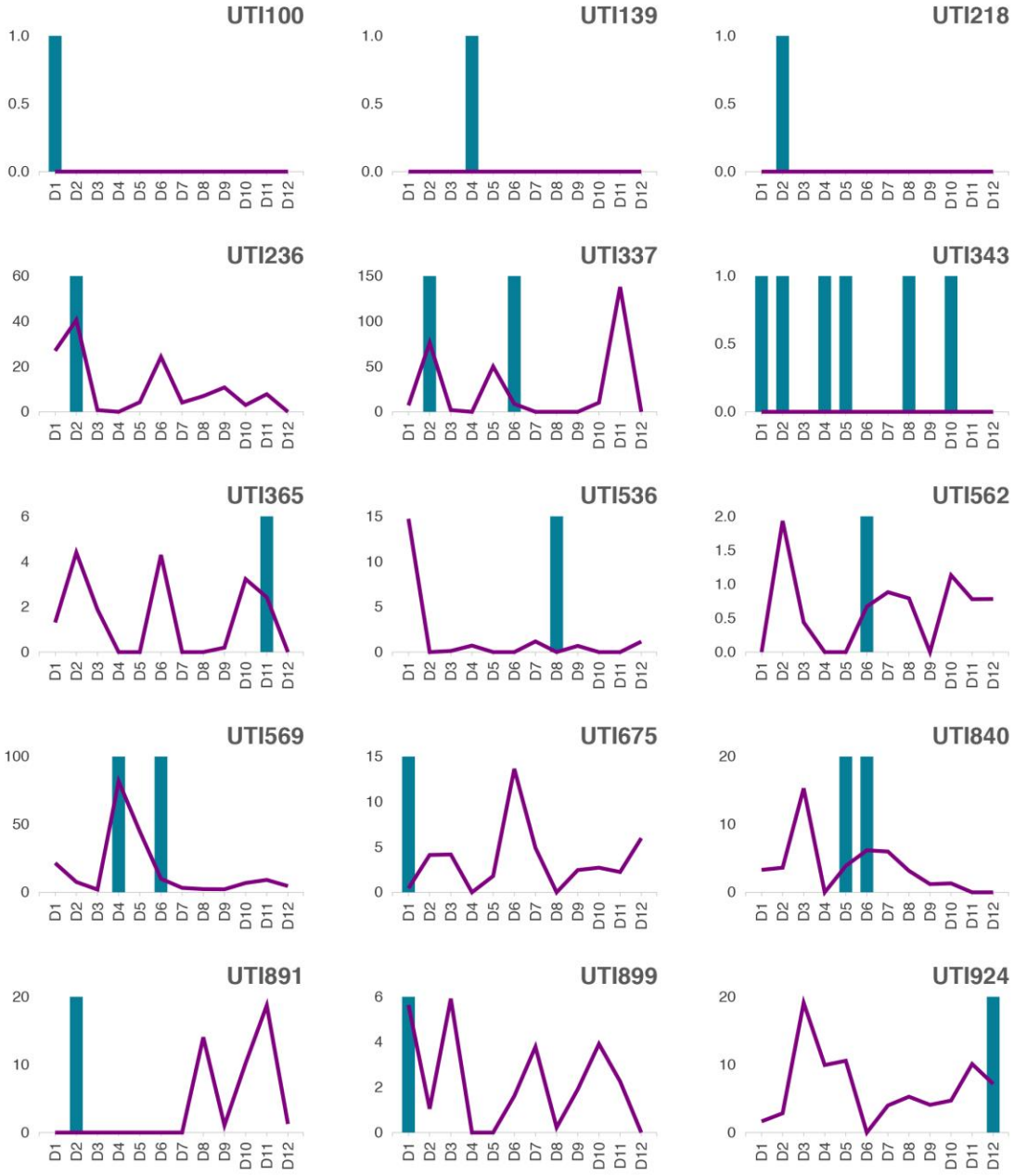


Figure 37. IL-1β concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

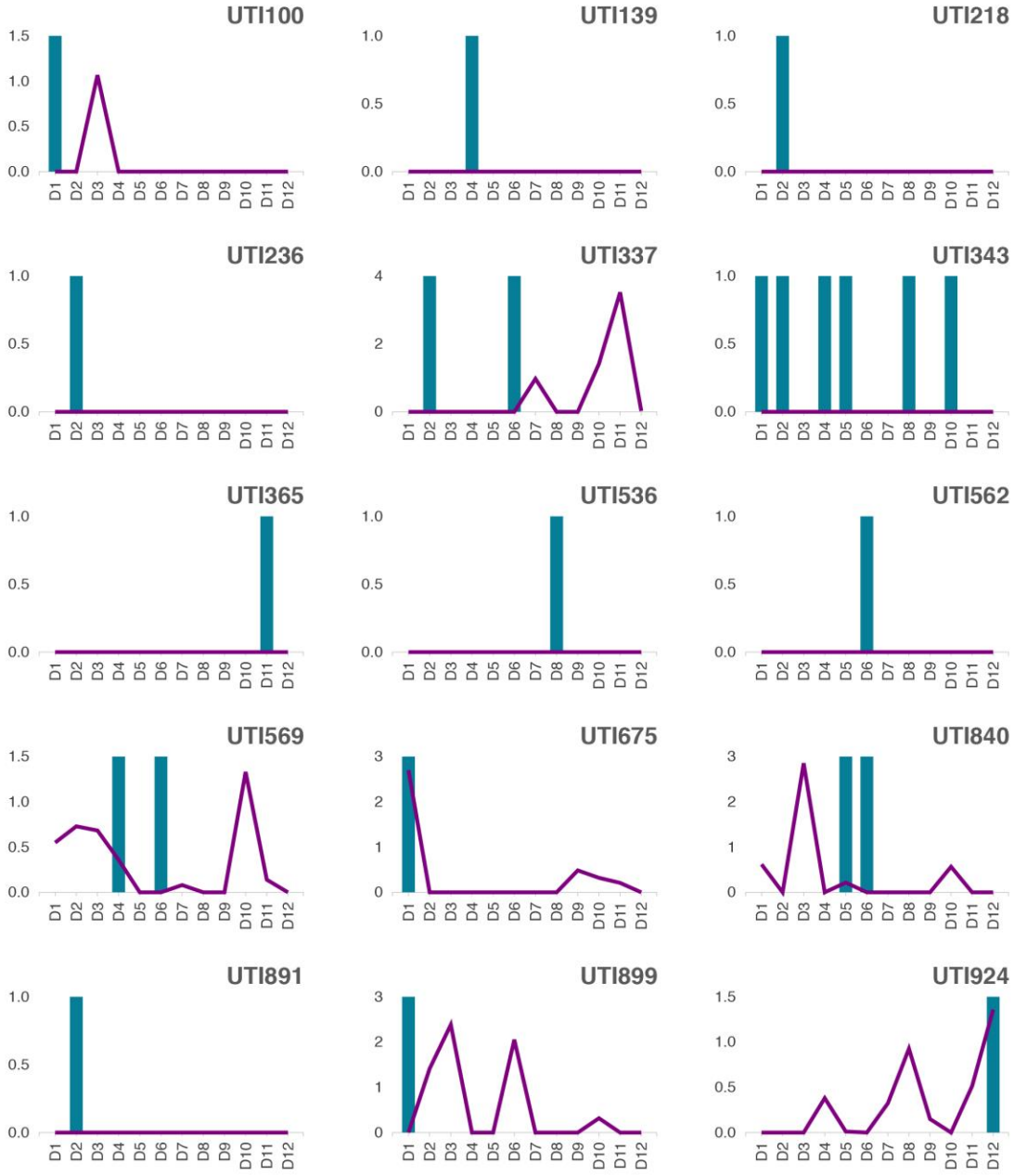


Figure 38. IL-4 concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

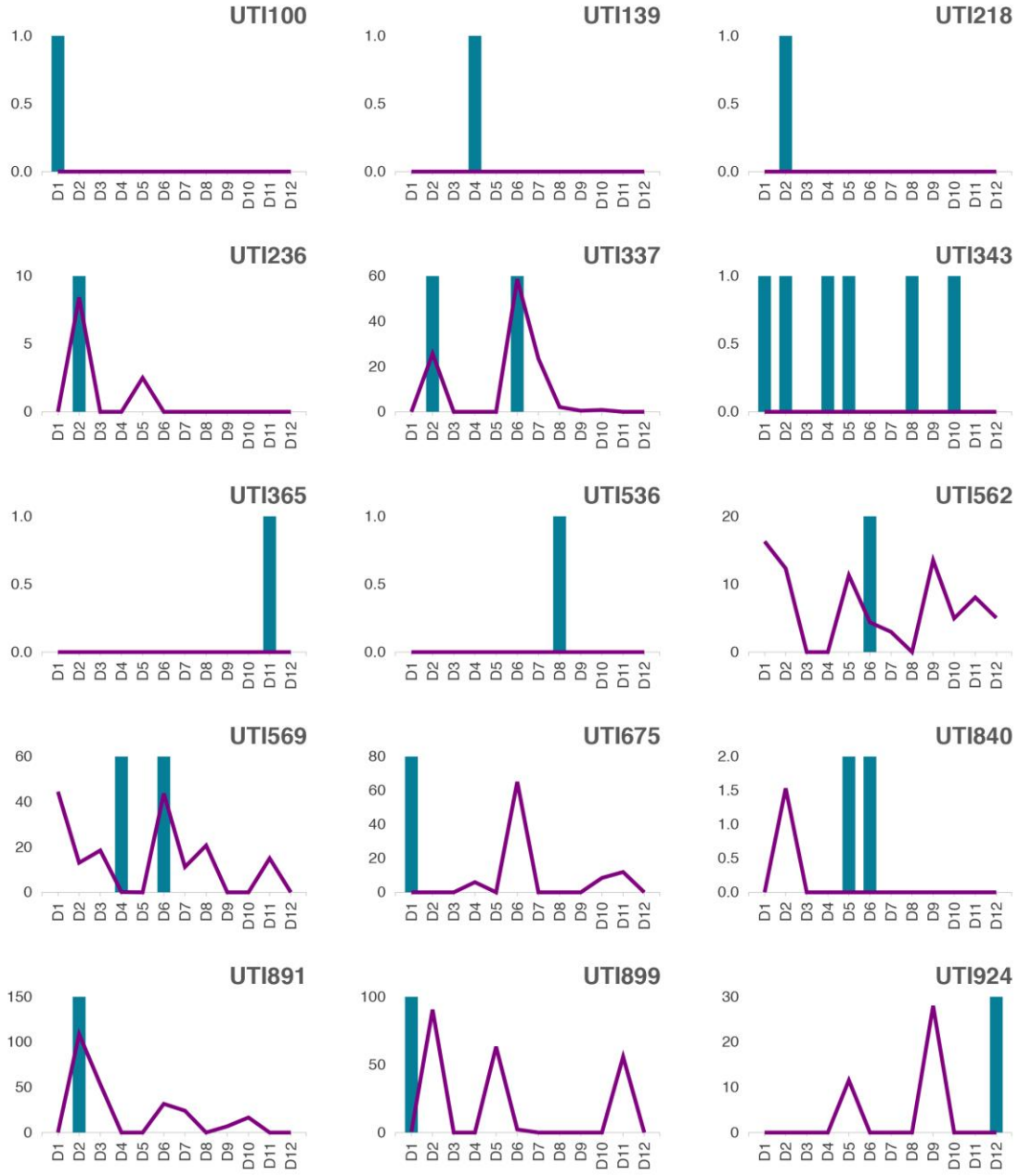


Figure 39. IL-5 concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

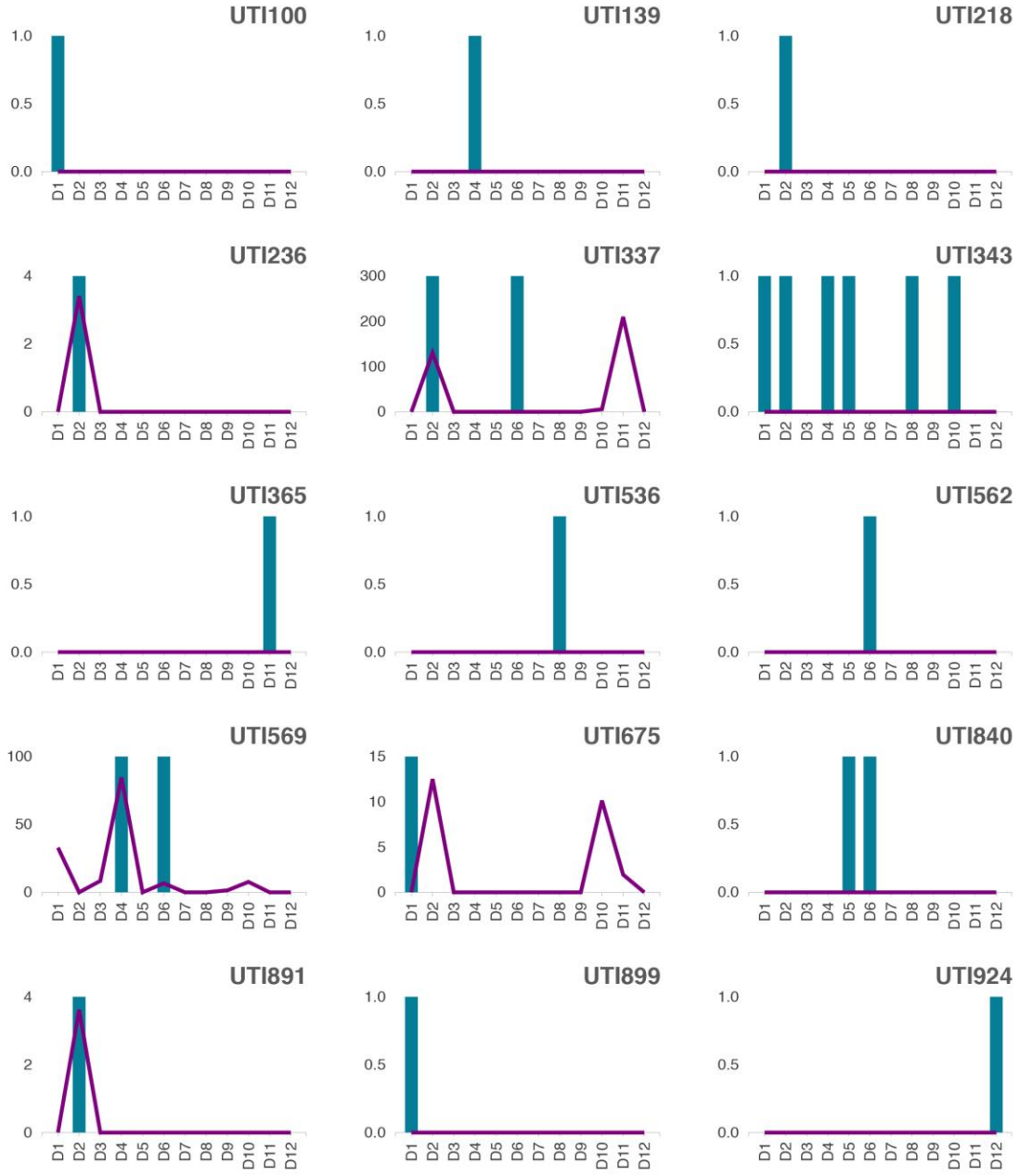


Figure 40. IL-6 concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

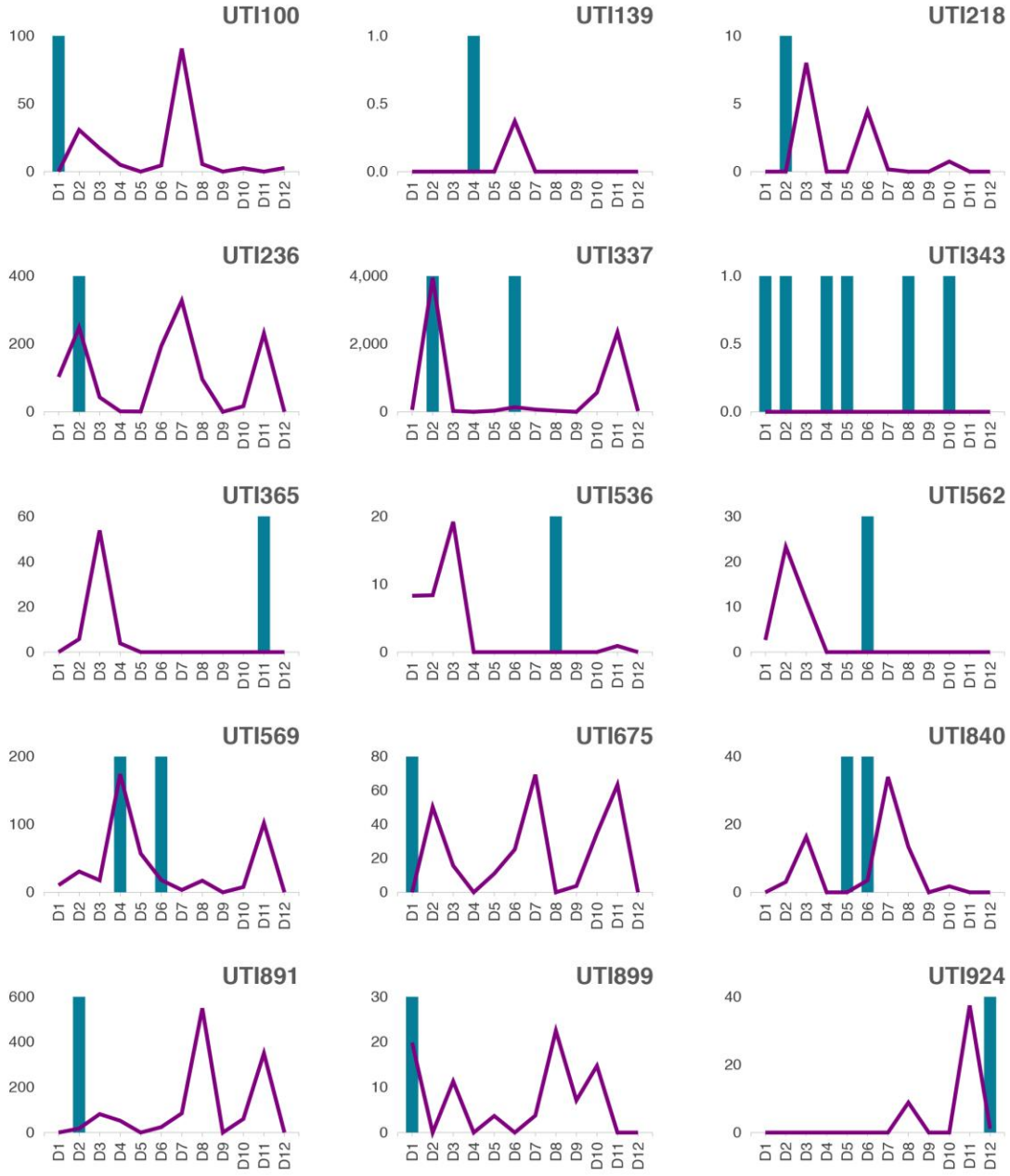


Figure 41. IL-8 concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

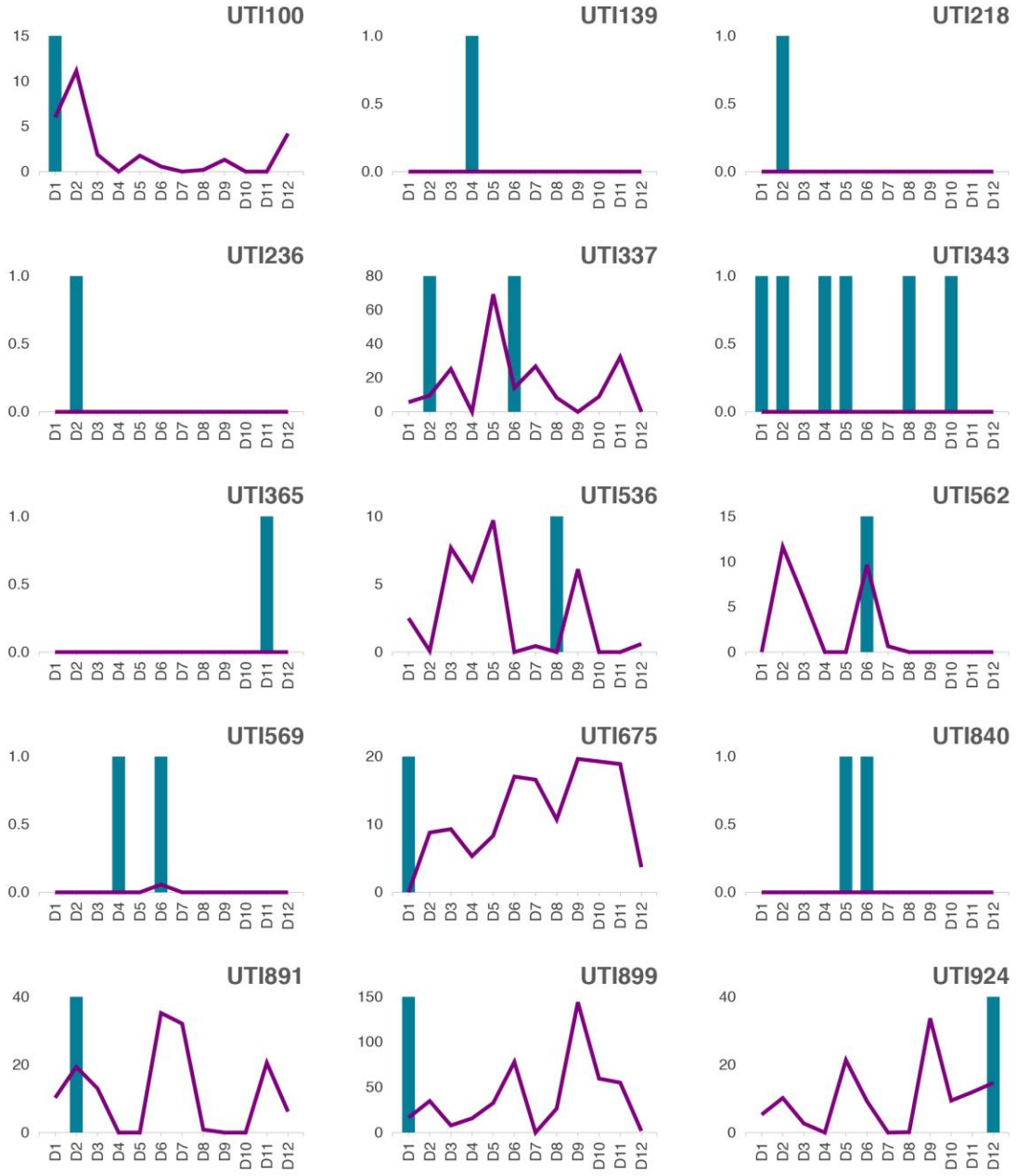


Figure 42. IL-10 concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

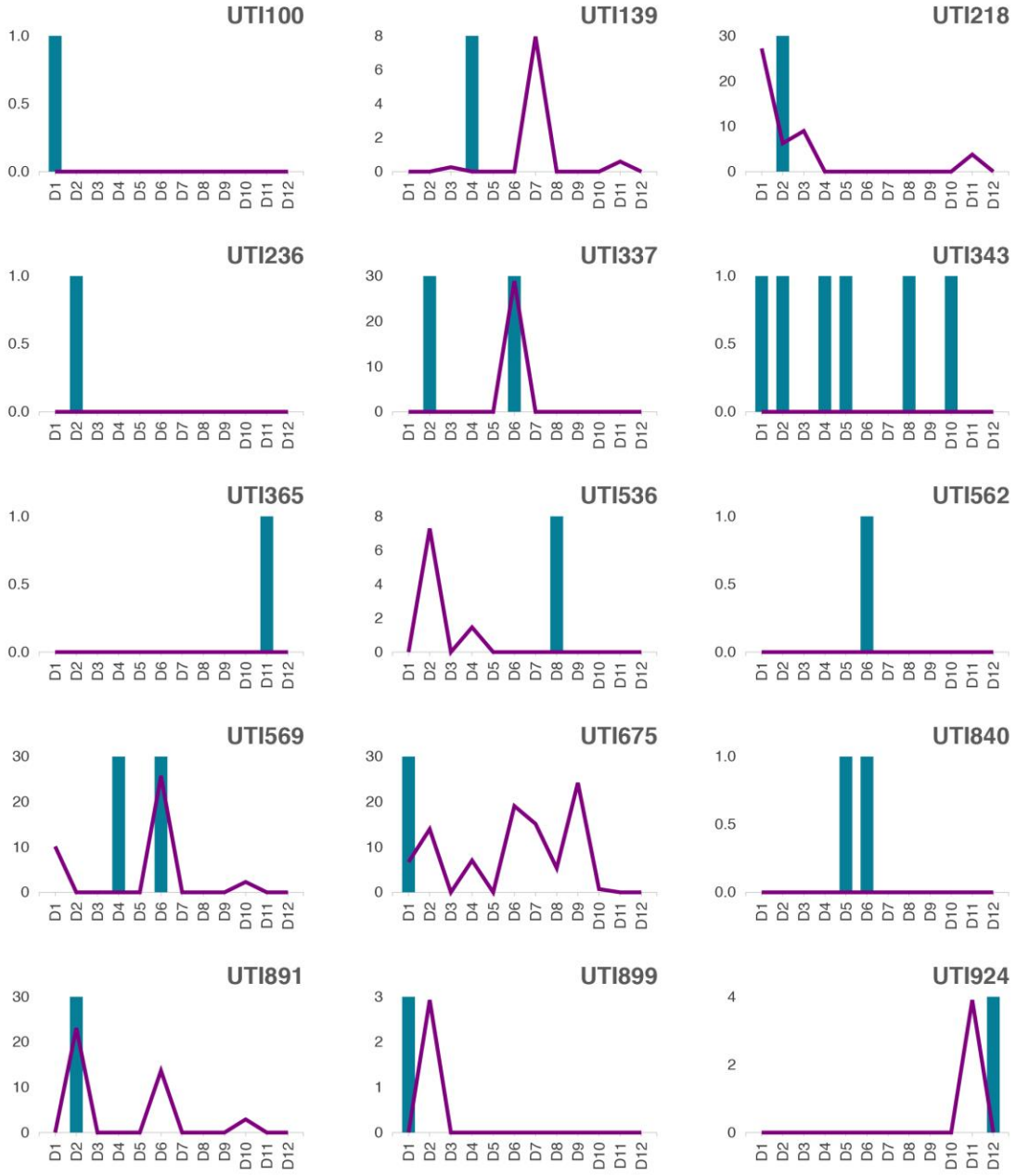


Figure 43. IL-12 p70 concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

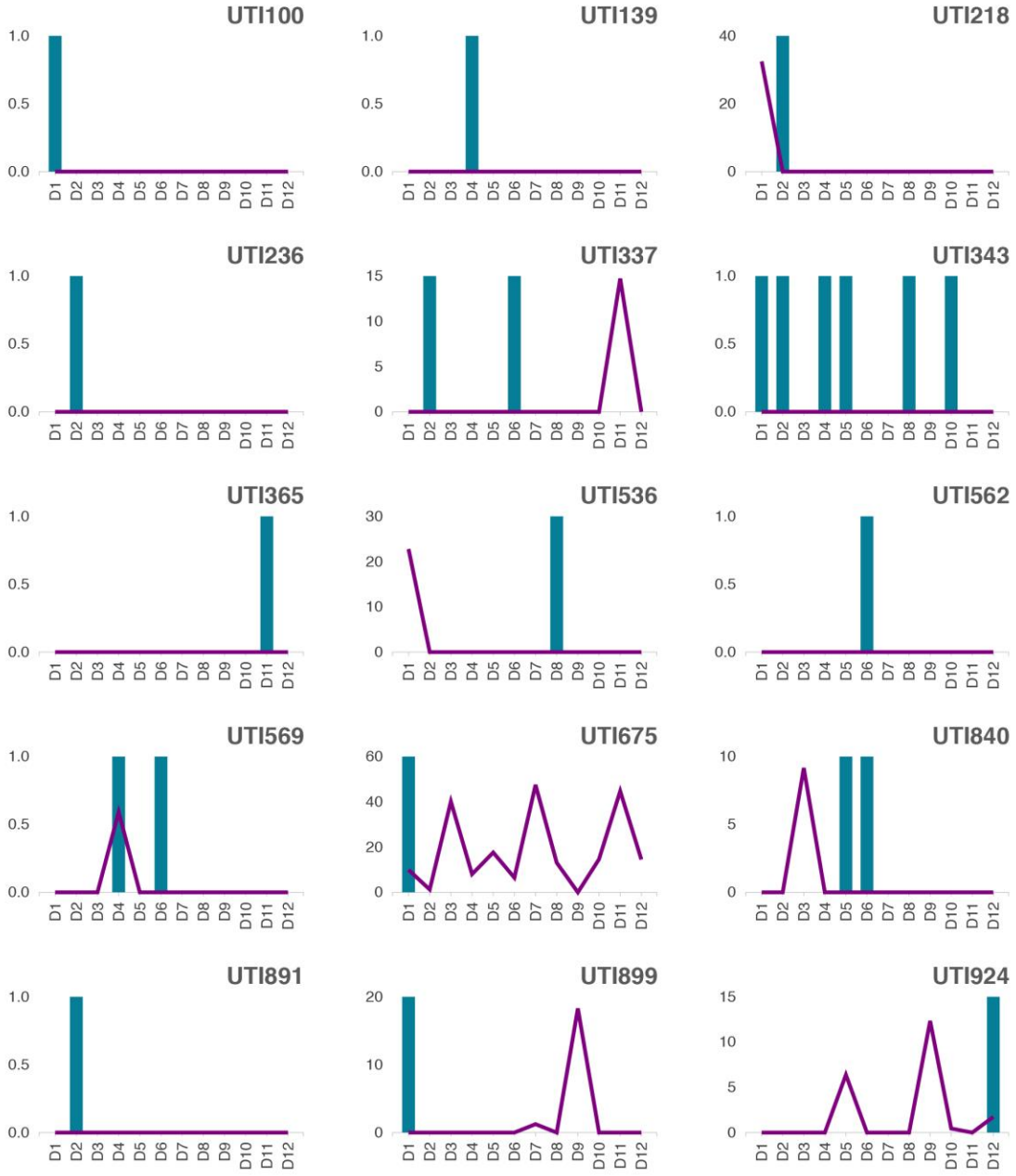


Figure 44. IL-17A concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

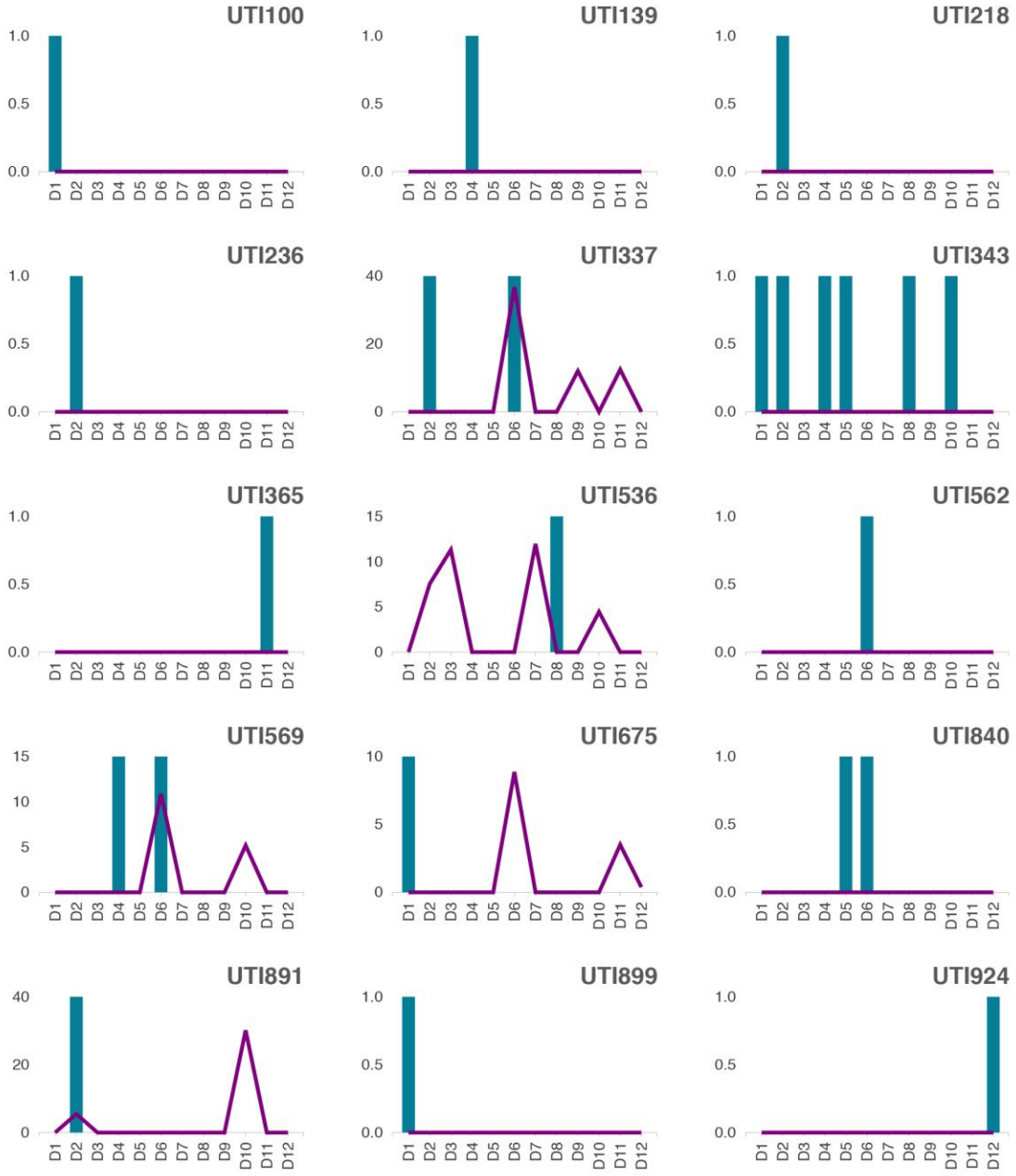


Figure 45. TNFα concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

Figure 46. IFNγ concentrations in all patients who experienced symptomatic episodes during the study. Purple line indicates the concentration (pg/ml) of protein in the urine. Green bars indicate symptomatic episodes, defined as a positive urine sample being submitted within 3 days of the study sample being collected. 'D' is the study donation number, 'UTI' is the patient's anonymised study ID.

7.5. Conclusions

The urine and blood analysis outlined in this chapter gives a better look at the hosts reaction to the long-term colonisation of the bladder and how their body is coping with this. The blood analysis provided insight into the study patients' general well-being, with special regard to their urinary health. An interesting finding from the blood results is that a large proportion of patients were vitamin D deficient. It has been shown previously that low levels of circulating vitamin D can be associated with an increased risk of urinary disease [79–81]. Thus it may be worth HCPs strongly recommending vitamin D supplementations to any patients with low levels of vitamin D in the blood, as this could be an explanation for why some of this patient cohort are suffering from such a disorder to begin with. Whether this would have an impact on improved UTI outcome would need further investigation. Early evidence suggests it may have some benefit to patients who suffer from UTIs [224]. One element of data that was not collected in this study was the vitamin D status of the healthy controls. This could be an important explanation for some of the observations made regarding their urinary cytokine concentrations as well as the bacterial presence previously mentioned. Bloods were not collected from healthy participants due to ethical and time restrictions. However, future studies should certainly consider collecting this information in order to check this potential explanation for any variabilities observed.

The blood analysis also revealed that the only 3 study samples with detectable levels of glucose reported on the dipstick shows growth of *P. mirabilis*. There seems to be no obvious link between diabetic patients and *P. mirabilis* infection in the literature. However, in this study all samples which returned a positive reading of glucose on the dipstick analysis contained *P. mirabilis*, suggesting some kind of link. Perhaps the pathogen survives especially well in the presence of glucose, which could make it a particular threat to patients with poorly controlled diabetes. This is just 3 samples of course, so would need further investigation in other glucose-positive urine samples to analyse the significance.

Basic dipstick analysis of the study urine samples reveals that these patients appear to have slightly poorer overall kidney health compared to age-similar controls. They also had higher levels of blood in the urine than was seen in the healthy controls. These results are to be expected as they are likely to be caused by both the direct action of the invading pathogens as well as the subsequent inflammation and damage this will cause within the urinary tract. It is this inflammatory response that was further analysed in this chapter. By quantifying the

outcomes of the dipstick analysis, it was possible to assign each study urine sample a dipstick score, this gave an idea of overall sample health. By analysing these dipstick scores along with the HCP outcomes of the dipstick (positive for nitrites and leukocytes), it was possible to see that neither of these are able to reliably predict or even necessarily diagnose periods of symptomatic UTI. This lack of reliability is also beginning to be seen within the literature and care guidelines [28,68,216].

Extensive cytokine analysis was performed on all study urine samples from both patients and healthy controls. This provided a vast database of information of the immune responses within the bladders of people of this age group who both did and did not suffer from long-term bladder colonisation. There was enormous variation in the immune protein concentrations within the patients themselves over time and between the patients as a whole. This variation was also seen when all proteins were analysed together alongside periods of symptoms. This suggests that different symptomatic infections can produce very different immune responses within different patients. This could be down to the quantity and diversity of pathogens being carried within the bladder and the length of time such colonisation has been present. As this long-term carriage may desensitise certain aspects of the immune response due to constant activation.

Analysing the data of protein concentrations over time together with the symptomatic episodes provides an interesting overview of the immune protein profiles over time in patients with defined symptomatic episodes, but it does not produce any consistent correlations in the data. Whilst there are some correlations of protein concentration increases with symptomatic episodes, there are far more examples of where these correlations do not occur. There are no clear patterns in the cytokine data to suggest they would be useful for predicting symptomatic episodes. There are many more immune proteins that could be further analysed in urines which may indeed increase or drop in concentration prior to symptoms, but none of the proteins analysed in this project have provided robust evidence for taking forward as a potential predictive biomarker.

Chapter 8. Cumulative Study Analysis

By analysing the clinical, bacterial and host aspects collected during the study it has been possible to get an overview of the patients and their urinary health status. The previous chapters have explored each aspect individually. Analysis has been done within patients over time by analysing potential changes around periods of symptoms. In this chapter all these parameters will be brought together to analyse the whole study database in a cross-cutting manner. This will allow visualisation of potential correlations from different sides of the study analysis.

To perform this analysis, by taking all urine samples as individuals, the study database as a whole was subjected to statistical analysis to explore potential trends in the data. By doing this, samples can be segmented without losing the sample size (n=360) to power such statistical analysis. Appendix 9 (p.223) to Appendix 38 (p.252) give full profiles of all study analysis for each of the individual patients, which can be referred to for further detail throughout this chapter.

The chapter will then go on to explore any observations which can be made when compiling study data. This will involve bringing all aspects of the analyses together from previous chapters. This will also look into any trends which can be seen around the alternative definitions of symptoms.

8.1. Total Scores of All Study Samples

In order to analyse the study database for statistical differences it was essential to normalise all the samples in a way that made them comparable to each other. This would allow analysis of all 360 samples as individuals, giving the sample size to power statistical comparison. In order to achieve this a 'total score' was calculated for each study sample based on all aspects of the study. The total score, therefore, reflected basic, clinical, host and microbial information (Table 6, p.58). This total score provides a summary of sample health, with a higher score indicating more of a divergence from an expected healthy outcome. A score of 0 would suggest a healthy outcome from all study factors, which is highlighted when comparing the total scores from patients' samples and healthy control samples (Figure 47), giving a statistically significant difference in scores ($p<0.0001$).

Upon calculation of the total scores for each study sample it is then possible to break down the database and run statistical analysis on the various sub-categories and ask specific questions with respect to sample health. This allows for comparisons to be made upon the many different aspects of the study analysis.

8.1.1. Basic Patient Data

Gender

A statistically significant difference can be seen in the total scores from female and male patients from the study (p=0.0012) (Figure 48A). UTIs are much more common in women than men [26,207]. A potential explanation for the difference is that it is possible when bacteria are able to successfully colonise the male bladder the resulting interactions are more stable and less harmful than those seen in the female bladder. This could suggest that bacteria found in male urinary tracts are more capable at avoiding activation of the patient's immune response and avoiding symptoms. Bacteria from the gut travelling periurethrally to the urethra have much further to travel in order to reach the male bladder than the female bladder [59,60] (Figure 1B and C, p.17). Thus, perhaps the ones that are successful are far better adapted to surviving undisturbed within the urinary tract once they are there. However, when splitting the average cytokine concentrations by sex, no significant differences were seen between the female and male averages for any of the measured proteins (Appendix 8, p.222).

Age

When total scores are broken down by different age groups of patients in the study it can be seen that all age groups are statistically significant to those from patients aged 75-79 years (p-values given in Appendix 7, p.221) (Figure 48B). This suggests patients of this age range are more likely to have poorer sample health compared to those older and younger than them. It might have been expected that sample health would degenerate the older the patient. However, it is known that the average life expectancy in the UK is 81.2 years [225]. Thus, one potential explanation for this observation is that people older than this are intrinsically better suited to survival and are therefore better at coping with disturbances within the body such as UTIs. Thus it could be that internal coping mechanisms within older patients that are preventing the scores from being higher, perhaps by modulation of more efficient action of their immune responses.

Impact of Diabetes and UTI Severity

There were no significant differences seen between diabetic patients (p=0.366) (Figure 48C). This suggests that this disease does not impact sample health. However, the lack of a difference could be due to the diabetic patients within the study successfully managing their disease, therefore leading to no marked differences within the urine of diabetic patients and non-diabetic patients. Patients with poorly controlled diabetes may indeed have a very different sample health and with the correct ethics in place this is a testable hypothesis. The 3 samples within the study with detectable glucose within the urine, suggesting unsuccessful diabetes management at that time [64], from patients UTI100 at D10, UTI138 at D12 and UTI414 at D1 gave total scores of 14, 21 and 18 respectively. These are all relatively close to, if perhaps a little higher than, the average total scores for both diabetic (15.9) and non-diabetic (15.2) patient samples. All 3 of these patients stated that they were diabetic at recruitment.

There were also no statistical differences between patients who claimed to suffer from different types of disease severity (p-values given in Appendix 7, p.221) (Figure 48D). This suggests there is variable sample health for all different types for UTI severity and that it is not just specific to patients with one sub group of symptom types. It may also suggest that patients are not always consistent at reporting their most common UTI symptoms, which could be a psychological difference of how different patients perceive symptom severity.

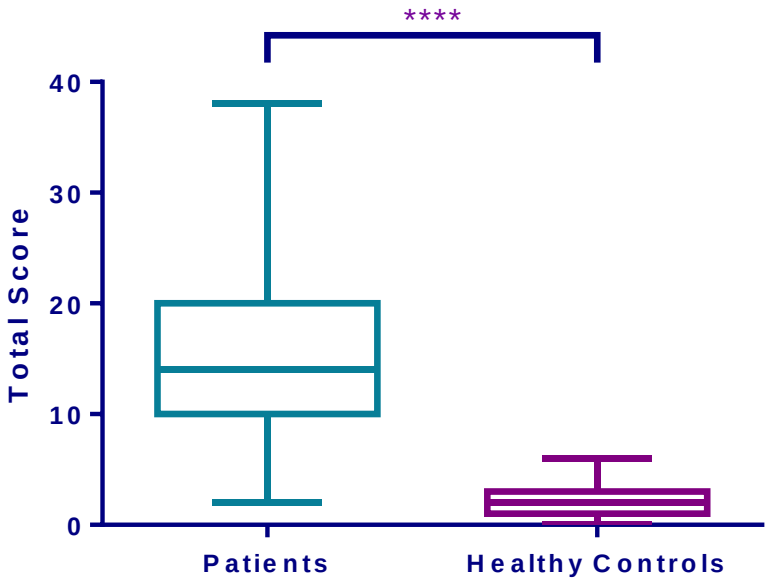


Figure 47. Total scores from all patient and healthy control samples.

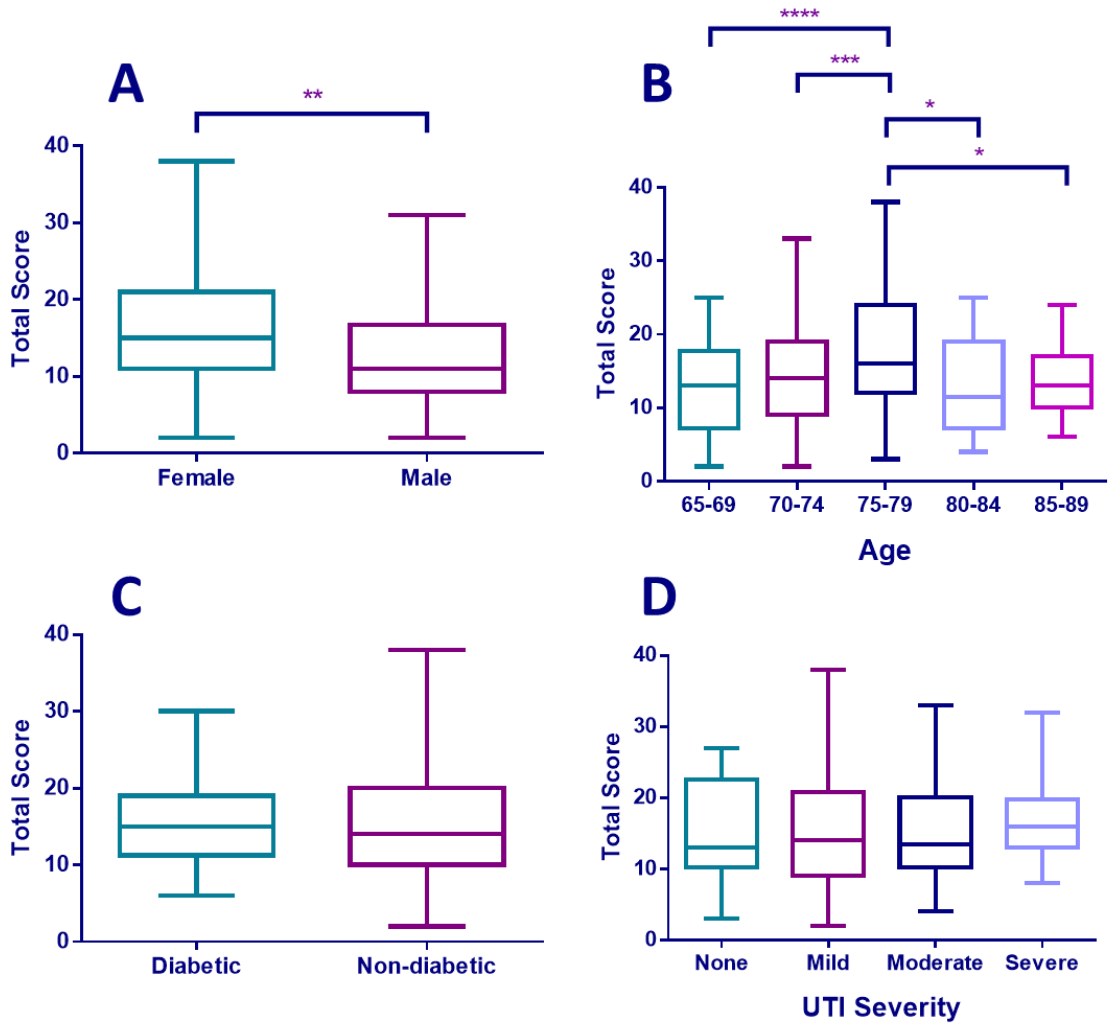


Figure 48. Total scores broken down by aspects of the basic patient data. 'A' Shows the total score of all samples from female and male patients (n=360). 'B' shows the scores segmented by the age of patients at recruitment (n=360). 'C' shows scores from diabetic and non-diabetic patients (n=360). 'D' breaks down the total scores from patients based on the perceived level of UTI symptom severity they usually suffer from (discussed in section 5.1) (n=360).

8.1.2. Clinical Patient Data

As would perhaps be expected samples where the patients felt they were suffering from a UTI had significantly higher total scores to those from samples where they did not ($p<0.0001$) (Figure 49A). This significant difference potentially supports using the self-declared UTI as a potential definition for symptomatic episodes. A large proportion of the total score is based on the symptoms scores that patients gave on a questionnaire. Therefore, it is logical that patients who felt they were suffering from a UTI would give higher scores to each of the symptom questions, which could be responsible for weighting their scores.

A significant difference ($p<0.0001$) was also seen between samples with a positive and negative HCP outcome (Figure 49B). This indicates that a positive dipstick outcome is not only an indicator of a possible urinary tract infection, but also potentially a good indicator of patient and sample health [28,216]. This also suggests that a positive HCP outcome could be used as a potential definition of a symptomatic episode. However, it has been shown previously that a positive HCP outcome does not always align with urinary symptoms (Figure 32, p.136). Despite Figure 49A and Figure 49B looking very similar, of the samples with self-declared UTIs (n=62) and the positive HCP outcomes (n=64), only around half of these matched (n=27). Thus, demonstrating a lack of agreement between when a patient may feel they have a UTI and when a HCP would define a UTI. This may be due to a potential lack of reliability in patients accurately reporting symptoms and self-diagnosing based on diffuse urinary symptoms.

Samples from patients who had handed in a positive urine sample to their doctor within 3 days were statistically more likely to have a higher total score ($p=0.002$) (Figure 49C). This is a breakdown which is not influenced by any other factor in the study, making this difference very important. This therefore further supports the decision for choosing this to be the criteria used to define periods of symptoms in earlier chapters.

Interestingly, no significant difference was seen between samples in which the patient received antibiotics to treat a UTI within 3 days of the study sample being collected and those that were not being treated ($p=0.305$) (Figure 49D). This might suggest one of two possible scenarios that the treatments being given to these patients are not benefitting them and causing an improvement to their sample health. Alternatively, it could suggest that the treatments do in fact improve the sample and therefore patient health. Further work would be needed in order to make this distinction.

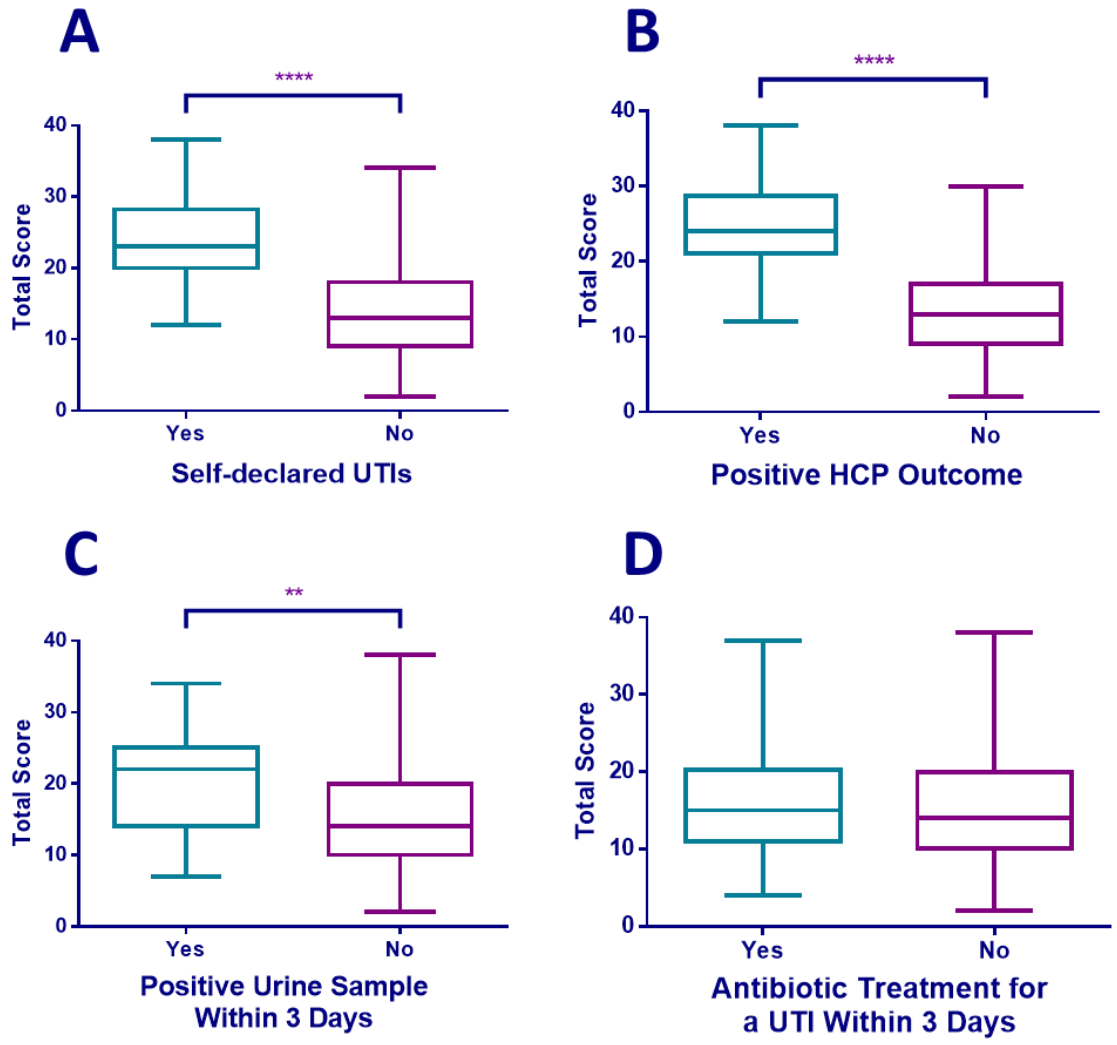


Figure 49. Total scores broken down by patients' clinical data. 'A' Shows the total score from samples where patients declared on the study questionnaire that they felt they had a UTI at the time of sampling and those that did not (n=360). 'B' shows the scores segmented by whether the patient received antibiotic treatment for a UTI within 3 days of the study sample (n=360). 'C' shows the scores segmented by whether a positive urine sample was received by the NHS within 3 days of the study sample (n=360). 'D' shows scores from samples which returned a positive and negative HCP outcome on the dipstick (n=360).

8.1.3. Bacterial Data

Samples which contained *E. coli* showed a statistically higher total score to those that did not contain *E. coli* ($p<0.0001$) (Figure 50A). This difference was also seen in samples with diagnostic loads of *E. coli*, where the box-plots show even less interquartile range overlap ($p<0.0001$) (Figure 50B). These data suggest that *E. coli* in the urine of patients indicated a poorer sample health. However, it is important to keep in mind that *E. coli* analysis contributed to the bacterial aspect of the total score. Thus, other bacteria were not quantified here and samples clear of *E. coli* would almost always give a lower score. Thus, to test this significance the total scores from samples with varying *E. coli* load were broken down (Figure 50C). Interestingly, there is still a statistically significant difference between all 3 cohorts (p-values given in Appendix 7, p.221). This further suggests that *E. coli* presence in the urine, especially at diagnostic quantities, does cause a deterioration in sample health.

A significant difference was also seen between motile and non-motile *E. coli* strains, samples containing non-motile *E. coli* gave significantly higher total scores ($p=0.001$) (Figure 50D). For this analysis only scores from samples containing *E. coli* were used ($n=184$) as including scores from samples clear of *E. coli* would skew the data and prevent a reliable comparison to be made. This suggests that non-motile strains are responsible for a reduction in sample health. This could potentially be due to motile strains of *E. coli* somehow being better able to subvert detection by the host and avoid immune activation, which could in turn potentially prevent urinary symptoms.

By asking the database if there was any variation in the total score of samples with high levels of antibiotic resistance, it was possible to see no significant differences between samples with 1 or no resistances and those with 2 or more ($p=0.073$) (Figure 50E). This may suggest that, despite some bacteria having a survival advantage of antibiotic resistances, they are not necessarily responsible for a more aggressive disease phenotype which could lead negative effects on the patient's health indicated by a higher total score. In agreement with this observation, studies have shown that more virulent strains of *E. coli* are more often associated with antibiotic susceptibility [226,227].

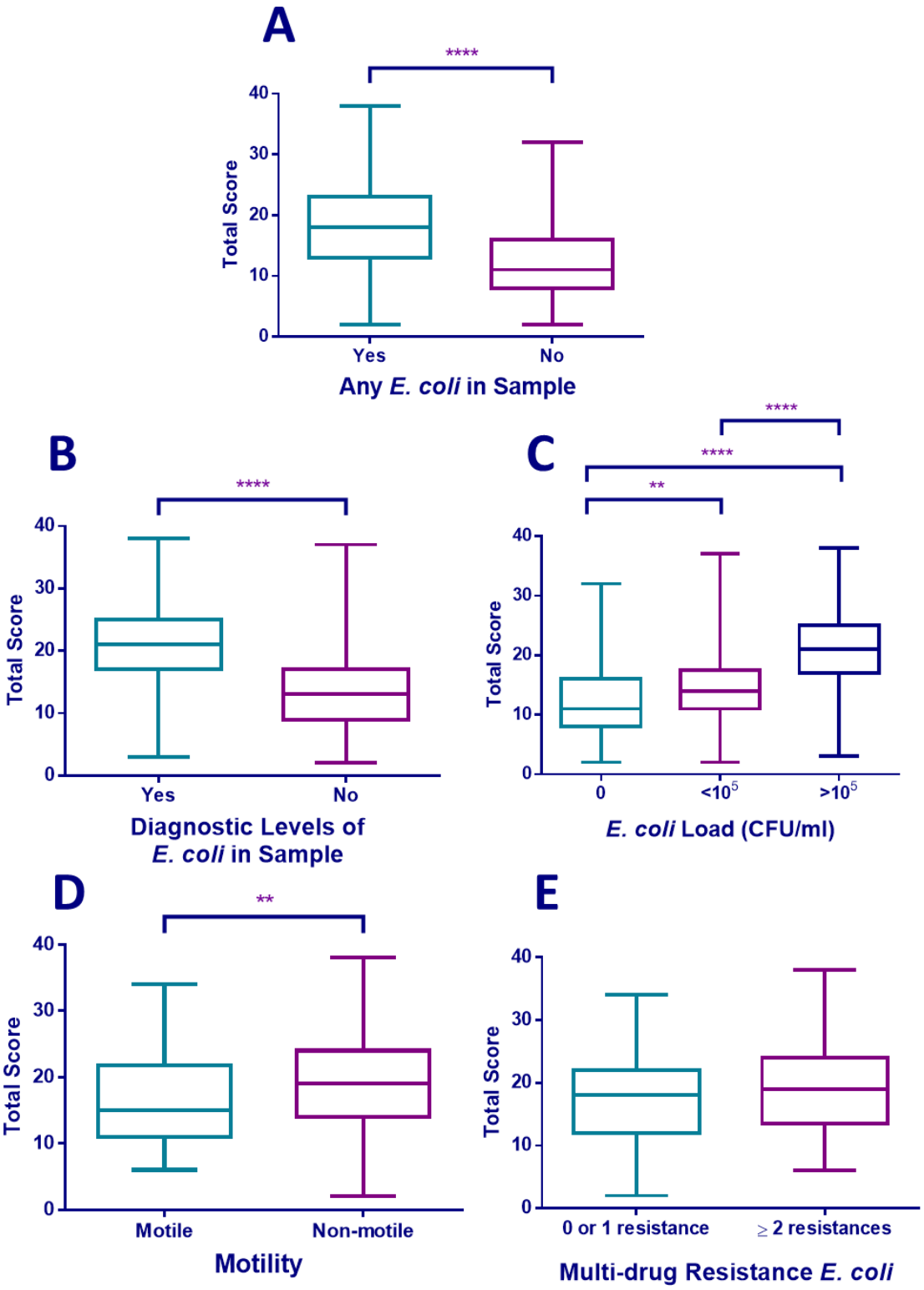


Figure 50. Total scores broken down by *E. coli* data. 'A' Shows the total score from samples which did and did not contain any amount of *E. coli* (n=360). 'B' shows the scores from samples which contained diagnostic loads of *E. coli* (>10^5 CFU/ml) (n=360). 'C' shows the scores segmented by the load of *E. coli* in the sample (n=360). 'D' shows scores from samples which contained motile and non-motile *E. coli* (n=184). 'E' shows scores from samples which contained *E. coli* that was resistant to 2 or more of the antibiotics tested and those that were only resistant to 1 or less (n=184).

8.2. Cytokines, Alternative Symptom Definitions and E. coli Load

Previously, in section 7.4.3 the symptom definition of a positive urine sample being received by the NHS within 3 days of the study sample being collected was analysed against the measured cytokine concentration over time. In this section the cytokine data will be analysed alongside all the alternative definitions for a symptomatic episode as have been discussed in section 5.2. These definitions are;

a) A study urine with a positive HCP outcome
b) Self-declared UTIs by the patients at the time of sampling
c) Antibiotic treatment being taken for a UTI within 3 days of the study sample

In addition to this the changes in cytokine concentrations will also be analysed with respect to the presence of diagnostic loads of *E. coli* in the urine. Due to the large volume of data generated, each cytokine was initially analysed individually in order to begin to visualise the data.

By visualising changes in IL-1β alongside all possible definitions of symptomatic episodes, small observations can be made. However, no trends appear to be consistent or reliable across patients and even within individuals. This appeared to be similar for all measured cytokines in this study Appendix 39 (p.253) to Appendix 47 (p.261). For this reason, IL-1β only will be discussed in relative detail here as an example of the types of observations being made across all the proteins measured.

Colonisation of the bladder with a diagnostic load of *E. coli* appears to match with the peak in IL-1β concentration in patient UTI115 (Figure 51). UTI337 shows the best correlation with HCP positive outcomes and peaks in IL-1β concentrations, which also align with the self-declared symptomatic UTIs (Figure 51). Apart from the first IL-1β peak in patient UTI337, the later trends to not align with a positive urine sample received by the NHS, the previously used definition for symptoms. A similar trend to this was also seen in patient UTI536. Patients UTI365, 531 and 781 also show some correlation with a positive HCP outcome and increases in IL-1β concentrations. Patient UTI383 shows alignment of IL-1β concentration and HCP outcome at D4. However, the positive dipstick outcome continues in donations 5, 6 and 7 but the IL-1β concentrations are lower here. At these later donations patient UTI383 appeared to lose *S. aureus* from the urine (Figure 19, p.101), suggesting this pathogen may have been causing the IL-1β increases. However, *S. aureus* returns later on in this patient and

does not correlate to elevations in IL-1β. In patient UTI 468 a positive HCP outcome aligns well with peaks in IL-1β at donations 1, 2 and 12 (Figure 51). However, there are also peaks in the protein concentration at donations 8 and 10 which do not show a positive HCP outcome and these do not appear to align with any marked changes in the presence of other pathogens in the urine (Figure 19). There are also several patients in which the HCP outcome does not align with changes in the IL-1β concentration, such as patients UTI115, 266, 562, 675 and 924.

Patients' self-declaration of a symptomatic UTI showed some alignment with increases in IL-1β concentrations, for example in patient UTI899. The first peak aligns with the only NHS-perspective symptomatic episode. Patients UTI138, 236, 383, 414 and 569 believe they have a UTI on most occasions when the IL-1β concentration in their urine increases. However, all of these patients declare that they feel they have a UTI on other occasions where their IL-1β concentrations do not peak. There are, however, many patients where the self-declared UTIs did not align with fluctuations in IL-1β, for example in patients UTI365, 562, 675, 726 and 781.

In patient UTI236 a gap in their antibiotic treatment between donations 1 and 3, appeared to align with an increase in IL-1β concentration in their urine. This peak in IL-1β also correlates with the NHS-positive symptomatic episode seen in patient UTI236. There are other examples of patients whereby IL-1β is found at lower concentrations throughout treatment duration. For example, this can be seen in patients UTI562, 755 and 899. It can also be seen on several occasions where prophylactic antibiotic treatment was not sufficient to prevent peaks in IL-1β concentration. For example, this can be seen in patients UTI115, 365, 524 and 675.

In addition to trends in the cytokine changes, visualising the data in this way also allowed comparison between of the NHS-positive urine samples, bacterial load, HCP outcome, self-declared UTI and treatment data. For example, patient UTI337 had 4 occasions during the study in which the dipstick was positive for both nitrites and leukocytes, these all matched with the 4 self-declared UTI episodes the patient suffered. Two of these also aligned with full diagnostic loads of *E. coli* and one aligned with an NHS-positive urine sample. However, this correlation was not seen in other patients. In patients 236 and 569 an NHS-positive urine sample appeared to align with gaps in antibiotic treatment. Positive urine samples were submitted to the NHS after diagnostic loads of *E. coli* appeared to be cleared from the urine

in patients UTI337, 365 and 569, not necessarily due to treatment. These observations could suggest that these patients' bladders are reacting to a more aggressive pathogen. All periods where NHS-positive urine samples were submitted align with *Enterococcus faecalis* colonisation in these patients, which could suggest this pathogen is responsible for causing the ill-effect (Figure 19, p.101).

Interestingly, it can also be observed that a full diagnostic load of *E. coli* in the urine did not always correlate with a positive HCP outcome on the dipstick. For example, in patients UTI468, 726, 781 and 899, which calls into question the reliability of the dipstick to identify samples containing diagnostic bacterial loads. Visualisation of the different definitions for symptomatic episodes shows the lack of agreement between them. This highlights one of the biggest issues with such a study, is the difficulty of defining a symptomatic episode as none appear to be consistent or reliable.

In order to best display the observations being made with regards to the cytokine concentrations and different symptom definitions, patient case examples will be used for the next section of analysis. This is due to the large amount of data being displayed in Figure 51 and Appendix 39 (p.253) to Appendix 47 (p.261) making it hard to observe trends. This will also allow for multiple cytokine concentrations to be visualised together.

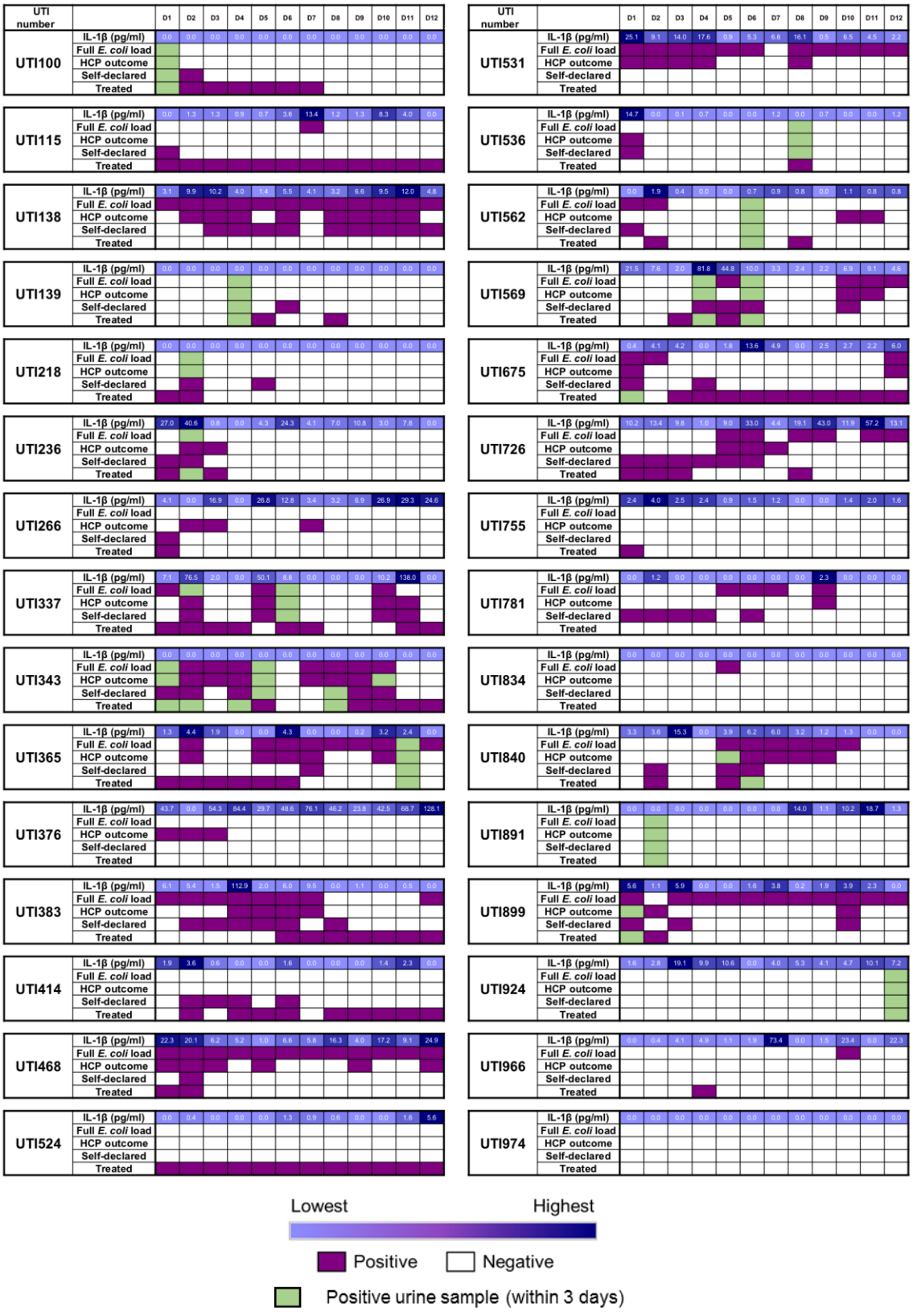


Figure 51. IL-1β concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation. Green columns indicated samples which were collected within 3 days of a positive urine sample being received by the NHS.

8.3. Patient Case Examples

As discussed in section 8.2 some trends could be seen within the cytokine data when compared to the different symptomatic definition. For these analyses each definition will be analysed in detail and patient case examples will be given to display the points of observation. For ease of reading, therefore, some data will need to be repeated across Figure 52 to Figure 57. Case examples will also be used to display observations made about the timing of cytokine concentration fluctuations.

8.3.1. Diagnostic E. coli Load

In order to demonstrate some of the trends between the cytokine concentrations and the *E. coli* load data, patients UTI531, 562, 726 and 781 will be used as case examples. Patient UTI531 saw peaks in IL-4, IL-5, IL-10, IL-12 and IL-17 at D6 towards the end of a long-term colonisation with *E. coli* strain ST420 (Figure 23; p.113 and Figure 52). After a short gap at D7 where diagnostic loads were not isolated, ST420 returns at full loads at D8, however this time a different cytokine profile is seen, with only peaks in IL-5 and IL-8 concentrations. Of the cytokines shown here, with the exception of IL-5, most proteins were found at lower levels during the gap in diagnostic *E. coli* load at D7. This patient did not receive antibiotic treatment for a UTI for the study duration. This could therefore suggest that the combination of cytokines seen peaking at D6 were sufficient to clear the full load of *E. coli* strain ST420 from the bladder of this patient. However, the subsequent dips in the immune proteins appears to allow the strain to re-establish for the remainder of the study. Further rises in these cytokines at D10 do not appear to be sufficient to clear the bladder again.

Patient UTI562 also sees peaks in cytokine concentration before a full load of *E. coli* is cleared from the bladder. The combination of proteins seen in this patient are IL-1β, IL-5 and IL-10 (Figure 52). Alongside a course of trimethoprim this appeared to be successful in clearing the colonising *E. coli* from the bladder for the rest of the study duration (Appendix 24, p.238). Which could suggest that the combination of treatment and increased immune response was effective at clearing the bladder. Perhaps explaining why this was not sufficient in keeping the *E. coli* from returning in patient UTI531.

A peak in the concentration of IL-1β and IL-10 in the bladder of patient UTI726 at D6 appeared to clear the diagnostic load of *E. coli* from the bladder without the aid of antibiotics (Figure 52 and Appendix 29, p.242). However, the same *E. coli* strain appears to return at D8,

ST73 (Figure 23, p.113). The patient is given a short course of trimethoprim which appears to be unsuccessful at clearing this colonisation (Appendix 29, p.243). Increases in the concentrations of IL-1β, IL-4, IL-5 and IL-6 at D9 do appear to clear this diagnostic load of ST73 (Figure 23; p.113 and Figure 52). As was seen in patient UTI531, however, the strain appears to return to diagnostic loads shortly afterwards. This could suggest that if the treatment was given when the immune response was also being activated it could have been more successful at clearing the long-term infection, as was seen in patient UTI562.

At D6 and D7 in patient UTI781 increases in the concentrations of IL-5, IL-10, IL-12, IL-17 and IFNγ appear to help clear the diagnostic load of *E. coli* from the bladder by D8 (Figure 52). This patient does not receive antibiotic treatment for the duration of the study (Appendix 31, p.245). However, the same strain returns at D9, ST73 (Figure 23, p.113). Ongoing elevations in the listed cytokines plus IL-1β and IL-4, appear to prevent the strain from re-establishing for several weeks. However, the strain does appear again at low levels by D12 (Figure 27, p.122).

These data taken together may suggest that treating a patient when their immune response is also being activated, is a more effective method of clearing long-term colonisation from the bladder. However, the protein profiles of activation are very different between different patients.

IL8 Concentrations

One of the observations made when looking through the cytokine data with respect to the pathogens identified was that the majority of proteins showed some correlations with *E. coli* load. One exception to this was IL-8, which appeared to show relatively few correlations with the *E. coli* load and appeared to be more influenced by the changing presence of other pathogens within the urine, especially *Enterococcus faecalis*.

For example, in patient UTI100 *Enterococcus faecalis* disappears entirely at D7, leaving just *P. mirabilis* in the urine (Figure 19, p.101). This aligns with a large spike in IL-8 concentrations (Appendix 9, p.223). This pathogen loss may have caused the immune system to suddenly increase. At D8 the *Enterococcus faecalis* returns and the IL-8 concentrations returns to a low level. This may suggest that *Enterococcus faecalis* may be supressing the IL-8 immune response in some way in this patient. Other examples of where the loss of *Enterococcus faecalis* appears to cause a peak in IL-8, can be seen in patients UTI218 D3 and UTI376 D8 (Appendix 13; p.227 and Appendix 19; p.233).

There are also examples of patients where *Enterococcus faecalis* appears at the time of an IL-8 peak too, such as patients UTI337 D11 and UTI726 D3 (Appendix 16; p.230 and Appendix 29; p.243). There are other large peaks in IL-8 associated with the appearance of new pathogens in the urine. For example, *Streptococcus agalactiae* appears at D7 in patient UTI236 (Appendix 14; p.228), *S. aureus* appears at D2 in patient UTI266 (Appendix 15; p.229) and *C. albicans* and *Streptococcus agalactiae* appear at D8 and D11 respectively in patient UTI891 (Appendix 34; p.248).

In patient UTI236, the treatment courses at D1 and D3 appear to lower the IL-8 concentration of the urine, which is outlined by a peak in the protein seen at D2 (Appendix 14, p.228). This is also highlighted by treatment cessation after D1 in patient UTI266, which appears to lead to a gradual increase in IL-8 concentrations in their urine (Appendix 15, p.229). Perhaps suggesting that the immune activating pathogen has been able to return. This could be being caused by the appearance of either *S. aureus* at D2 or *Streptococcus agalactiae* at D3 in patient UTI266 (Figure 19, p.101). In patient UTI562, *E. coli* disappears from the urine at D3 and appears to be replaced by *Enterococcus faecalis*, *S. aureus* and *C. albicans*, which causes an increase in IL-8 (Figure 19 and Appendix 26, p.240). The IL-8 peak at D3 in patient UTI726 correlates with a new colonisation with *Enterococcus faecalis* (Appendix 29, p.243). However, the opposite can be seen in this patient at D8, where *Enterococcus faecalis*, *Streptococcus agalactiae* and *S. aureus*, are lost from the urine leaving just *E. coli* and *C. albicans* at the time where IL-8 concentrations increase.

These observations suggest that IL-8 concentrations within the urine may be more influenced by the changing colonisation of pathogens other than *E. coli* within the bladder. This was observed for IL-8 far more than for the other measured cytokines. Pathogens such as *Enterococcus faecalis* appear to be largely responsible for IL-8 fluctuations. This may suggest that *Enterococcus faecalis* could be an important focus of future analysis performed on the study biobank.

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI531	IL-4 (pg/ml)	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	1.2	1.5	0.2	0.0
	IL-5 (pg/ml)	12.9	0.0	30.1	15.7	2.1	25.9	23.8	28.7	0.0	22.3	0.0	15.6
	IL-8 (pg/ml)	33.3	45.4	32.1	19.2	0.0	1.3	0.0	100.1	0.0	5.4	11.5	0.0
	IL-10 (pg/ml)	0.0	0.0	0.0	0.0	2.7	20.6	1.4	0.0	0.0	14.3	0.0	0.0
	IL-12 (pg/ml)	0.0	0.0	0.0	0.0	0.0	13.4	0.0	0.0	0.0	0.0	0.0	0.0
	IL-17A (pg/ml)	0.0	0.2	0.0	0.0	0.0	2.8	0.0	0.0	0.0	2.7	0.0	0.0
	Full *E. coli* load												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI562	IL-1β (pg/ml)	0.0	1.9	0.4	0.0	0.0	0.7	0.9	0.8	0.0	1.1	0.8	0.8
	IL-5 (pg/ml)	16.3	12.3	0.0	0.0	11.3	4.4	3.0	0.0	13.5	5.0	8.1	5.0
	IL-10 (pg/ml)	0.0	11.6	6.0	0.0	0.0	9.6	0.7	0.0	0.0	0.0	0.0	0.0
	Full *E. coli* load												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI726	IL-1β (pg/ml)	10.2	13.4	9.8	1.0	9.0	33.0	4.4	19.1	43.0	11.9	57.2	13.1
	IL-4 (pg/ml)	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0
	IL-5 (pg/ml)	3.7	7.6	0.0	0.0	0.0	0.0	5.1	0.0	68.4	0.0	20.5	8.8
	IL-6 (pg/ml)	8.4	3.5	12.7	106.6	0.0	5.2	0.0	22.3	87.6	22.9	9.2	0.0
	IL-10 (pg/ml)	0.0	0.3	0.5	0.0	0.0	7.1	0.0	0.0	0.0	0.2	0.0	0.0
	Full *E. coli* load												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI781	IL-1β (pg/ml)	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0
	IL-4 (pg/ml)	0.3	0.0	0.0	0.0	0.2	0.2	0.0	0.0	1.5	1.7	0.5	0.0
	IL-5 (pg/ml)	25.4	3.2	68.1	0.0	0.0	187.5	36.1	22.0	1.6	87.2	68.9	0.0
	IL-10 (pg/ml)	0.0	3.3	5.0	17.0	0.0	0.5	39.9	4.8	0.0	35.9	27.3	15.0
	IL-12 (pg/ml)	0.0	0.0	0.0	0.0	0.0	5.4	14.8	0.0	0.0	17.3	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	13.1	0.0	0.0	0.0	0.0	0.0
	IFNγ (pg/ml)	0.0	31.9	0.0	0.0	0.0	53.9	0.0	0.0	0.0	0.0	0.0	0.0
	Full *E. coli* load												

Figure 52. Patient case examples for trends in cytokine concentrations and diagnostic loads of *E. coli*.

8.3.2. Positive HCP outcome

A positive HCP outcome is when a sample gave a positive result of both nitrites and leukocytes from the urinary dipstick. This result would generally cause a HCP to send a sample to an HNS lab for urinalysis to investigate whether the patient had a UTI. To observe trends in the cytokine concentrations collected in this study and positive HCP outcomes, patients UTI138, 236, 337, 376 and 468 will be used as case examples.

Peaks in IL-1β, IL-10 and IL-17 in patient UTI138 appear to correlate throughout the study relatively well with positive HCP outcomes of the samples (Figure 53). IL-10 appears to begin to rise slightly before a positive HCP outcome compared to the other cytokines listed. Despite this varying level of immune activation as well as detectible nitrite and leukocyte levels within the urine, at no point in the study did the patient submit a urine sample to their GP. This suggests that they did not experience UTI symptoms throughout.

Patient UTI236 shows marked increases in the concentration of IL-1β, IL-5 and IL-6 at D2 which is accompanied by a positive HCP outcome (Figure 53). However, the same outcome at D3 shows no such increases in the listed cytokines. This demonstrates the enormous variability and inconsistency of immune activation seen in the vast majority of study patients during the study. This suggests that, despite an apparently identical dipstick outcome, the overall situation within the bladder can be very different, even within the same patient. This variation in cytokine profile, with a continued positive HCP outcome can be further demonstrated in D1-3 in patient 376 (Figure 53).

Patients UTI337 and 468 show a degree of correlation in the concentrations of certain cytokines with positive HCP outcome from the urinary dipstick (Figure 53). However, the combination and concentrations of the proteins being released appears to vary each time within both patients. Both patients experienced long-term stable colonisation with *E. coli* strain ST73 (Figure 23, p.113). This suggests that patients do not always exhibit the same response to colonisation and even stable colonisation with the same bacteria can cause sporadic fluctuations in immune activation.

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI138	IL-1β (pg/ml)	3.1	9.9	10.2	4.0	1.4	5.5	4.1	3.2	6.6	9.5	12.0	4.8
	IL-10 (pg/ml)	25.8	27.4	17.7	4.8	19.9	32.4	14.5	0.0	7.0	15.9	4.1	0.0
	IL-17A (pg/ml)	1.7	19.4	6.5	0.0	3.6	13.6	2.2	0.0	3.9	0.0	4.7	0.0
	HCP outcome												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI236	IL-1β (pg/ml)	27.0	40.6	0.8	0.0	4.3	24.3	4.1	7.0	10.8	3.0	7.8	0.0
	IL-5 (pg/ml)	0.0	8.4	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-6 (pg/ml)	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	HCP outcome												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI337	IL-1β (pg/ml)	7.1	76.5	2.0	0.0	50.1	8.8	0.0	0.0	0.0	10.2	138.0	0.0
	IL-6 (pg/ml)	0.0	130.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	209.8	0.0
	IL-8 (pg/ml)	58.6	3664.1	13.9	0.0	43.9	120.3	48.8	31.6	0.0	369.0	2381.2	15.1
	IL-10 (pg/ml)	5.7	9.7	25.3	0.0	69.3	14.1	26.8	8.3	0.0	9.0	32.3	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.7	0.0
	HCP outcome												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI376	IL-5 (pg/ml)	51.5	89.9	208.6	0.0	22.1	11.4	27.9	6.7	0.0	3.2	9.0	0.8
	IL-8 (pg/ml)	51.2	80.2	556.0	218.1	54.1	14.2	114.1	554.4	15.0	39.2	127.3	339.1
	IL-10 (pg/ml)	0.0	0.0	5.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	HCP outcome												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI468	IL-1β (pg/ml)	22.3	20.1	6.2	5.2	1.0	6.6	5.8	16.3	4.0	17.2	9.1	24.9
	IL-6 (pg/ml)	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	2.1
	IL-8 (pg/ml)	0.8	564.9	222.4	7.0	0.7	29.0	98.5	128.6	0.0	12.2	68.2	816.6
	IL-10 (pg/ml)	4.0	0.1	3.9	0.0	25.5	14.4	3.9	1.6	12.0	11.8	0.0	0.0
	IL-12 (pg/ml)	0.0	9.8	0.0	0.0	27.8	2.2	9.2	0.0	0.0	7.6	1.5	0.0
	HCP outcome												

Figure 53. Patient case examples for trends in cytokine concentrations and positive HCP outcomes on the urinary dipstick.

8.3.3. Self-declared UTIs

Within the study questionnaire, patients were given the opportunity to state if they felt they were suffering from a UTI at the time the study sample was taken. Comparing these self-declared UTIs with fluctuations in the measured cytokines, allows a more patient-focussed method of analysing changes within their bladders. In order to demonstrate some of these changes, patients UTI236, 337, 414, 569 and 899 will be used as case examples.

Concentrations of IL-1β, IL-5 and IL-6 peak at D2 in patient UTI236, which is also a time when they state they feel they are suffering from a UTI (Figure 54). However, they also state this at D1 and only IL-1β appears to be increased of these 3 cytokines. This could suggest that IL-1β is responsible for causing the UTI symptoms recognised by the patient. However, a similar rise in IL-1β later in the study does not lead to another self-declared UTI.

Patients UTI337 and 899 show relatively tight correlation of increases in the listed cytokines with occasions when the patient states they felt they had a UTI (Figure 54). However, the combination and magnitude of these is different between the patients and even within the individual patients. This makes it difficult to know if it is these increases that are responsible for the patients feeling like they have a UTI.

UTI414 and 569 are examples of patients feeling like they have a UTI for a long period of the study. In patient UTI414 this is from D2 to D4 and in UTI569, from D4 to D6 (Figure 54). It is clear in both patients, however, the immune activation appears to be greatly varied throughout this time. These examples further demonstrate the variability within the bladders of patients who feel they are suffering from a UTI.

These data outline the difficulty in narrowing down a single protein or combination of proteins which could be responsible for causing patients to feel symptoms. They also demonstrate how different bladder environments can be within this patient group during periods of reported symptoms.

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI236	IL-1β (pg/ml)	27.0	40.6	0.8	0.0	4.3	24.3	4.1	7.0	10.8	3.0	7.8	0.0
	IL-5 (pg/ml)	0.0	8.4	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-6 (pg/ml)	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Self-declared												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI337	IL-1β (pg/ml)	7.1	76.5	2.0	0.0	50.1	8.8	0.0	0.0	0.0	10.2	138.0	0.0
	IL-6 (pg/ml)	0.0	130.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	209.8	0.0
	IL-8 (pg/ml)	58.6	3664.1	13.9	0.0	43.9	120.3	48.8	31.6	0.0	369.0	2381.2	15.1
	IL-10 (pg/ml)	5.7	9.7	25.3	0.0	69.3	14.1	26.8	8.3	0.0	9.0	32.3	0.0
	Self-declared												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI414	IL-1β (pg/ml)	1.9	3.6	0.6	0.0	0.0	1.6	0.0	0.0	0.0	1.4	2.3	0.0
	IL-6 (pg/ml)	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-8 (pg/ml)	0.0	33.1	0.0	0.0	1.7	0.0	0.0	0.0	0.0	18.6	0.0	0.0
	IL-10 (pg/ml)	0.0	72.7	54.5	0.0	2.0	71.0	28.3	0.0	29.6	29.5	0.0	0.0
	Self-declared												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI569	IL-1β (pg/ml)	21.5	7.6	2.0	81.8	44.8	10.0	3.3	2.4	2.2	6.9	9.1	4.6
	IL-4 (pg/ml)	0.5	0.7	0.7	0.4	0.0	0.0	0.1	0.0	0.0	1.3	0.1	0.0
	IL-6 (pg/ml)	32.9	0.0	8.4	84.6	0.0	6.7	0.0	0.0	1.4	7.7	0.0	0.0
	IL-8 (pg/ml)	2.3	19.9	15.1	182.7	21.9	16.7	2.9	18.3	0.0	13.5	148.6	0.0
	IL-10 (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
	IL-12 (pg/ml)	10.1	0.0	0.0	0.0	0.0	25.7	0.0	0.0	0.0	2.3	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TNFα (pg/ml)	0.0	0.0	0.0	0.0	0.0	10.9	0.0	0.0	0.0	5.2	0.0	0.0
	Self-declared												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI899	IL-1β (pg/ml)	5.6	1.1	5.9	0.0	0.0	1.6	3.8	0.2	1.9	3.9	2.3	0.0
	IL-4 (pg/ml)	0.0	1.4	2.4	0.0	0.0	2.1	0.0	0.0	0.0	0.3	0.0	0.0
	IL-8 (pg/ml)	8.0	0.0	13.7	0.0	2.9	0.0	5.5	28.0	6.0	13.5	0.0	0.0
	Self-declared												

Figure 54. Patient case examples for trends in cytokine concentrations and patients self-declaring they feel they have a UTI at the time of sampling on the study questionnaire.

8.3.4. Antibiotic Treatment

It might be assumed that antibiotic treatment would cause an overall reduction in immune activation as bacteria are cleared from the bladder and symptoms are prevented. However, as concluded previous to this, it seems that in several of the study patients, treatment is not always successful at clearing the pathogenic colonisation or reliable at preventing symptoms. In line with these observations it was often seen that cytokine concentrations would show fluctuations even during periods of antibiotic treatment. Patients UTI218, 337, 414, 468, 562 and 899 will be used as case examples to highlight these observations.

UTI218 sees a large elevation in both IL-12 and IL-17 at D1 when the patient is treated with trimethoprim (Figure 55 and Appendix 13, p.227). Antibiotic treatment continues to the second donation, however, the protein concentrations are greatly decreased. This may suggest that the treatment was able to clear the pathogen causing this immune activation. *Enterococcus faecalis* is present in this patient at D1 but not present at D2, perhaps suggesting this is responsible (Figure 19, p.101). However, *Enterococcus faecalis* returns at D4 but does not appear to affect the cytokine concentrations. A similar observation in the cytokine concentrations can be made in patient UTI337 at D6 and D7 (Figure 55). During this time the patient is receiving cephalexin treatment (Appendix 16, p.230). However, unlike in patient UTI218 no such change in the isolated pathogens could be seen (Figure 19). These data suggest that the immune activation is not being directly affected by differing pathogens in the bladders of these patients.

In patient UTI899 elevations in IL-5, IL12 and IFNγ concentrations appear to align with antibiotic treatment courses (Figure 55). Patients UTI468 and 562 appear to have elevated concentrations of the listed cytokines at the first treatment course, but these elevations are not subsequently seen at the second course. In both patients the first occasion sees only the colonisation with *E. coli* and the second with polymicrobial colonisation (Figure 19). This might suggest that polymicrobial colonisation may lead to an overall lower immune activation in these patients, perhaps due to desensitisation of the immune response to increased activation by multiple pathogens. This is certainly not a consistent observation however, as for example, in patient UTI414, there are elevations in IL-6 and IL-10 at D2 where they have polymicrobial colonisation with both *P. mirabilis* and *C. albicans* (Figure 55 and Figure 19).

In section 8.3.1 it was suggested that treatment at times of increased immune activation within a patient may be a more successful means of clearing a bladder of long-term colonisation. However, the case examples given here suggest that this is not necessarily observed within patients. When looking at the colonisation status of these patients after periods of antibiotic treatment and high levels of immune activation there seems to be no distinct pattern in clearance of the bladder (Figure 19 and Figure 23, p.113). For example, patient UTI899 is treated with trimethoprim at D2 at the same time they have high levels of IL-5, IL12 and IFNγ within the bladder (Figure 55 and Appendix 35, p.249). This appears to give rise to colonisation with *E. coli* strain ST73, which remains in their bladder for the remainder of the study (Figure 23).

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI218	IL-12 (pg/ml)	27.2	6.2	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8	0.0
	IL-17A (pg/ml)	32.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI337	IL-1β (pg/ml)	7.1	76.5	2.0	0.0	50.1	8.8	0.0	0.0	0.0	10.2	138.0	0.0
	IL-5 (pg/ml)	0.0	25.7	0.0	0.0	0.0	58.6	23.5	2.1	0.5	0.8	0.0	0.0
	IL-6 (pg/ml)	0.0	130.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	209.8	0.0
	IL-8 (pg/ml)	58.6	3664.1	13.9	0.0	43.9	120.3	48.8	31.6	0.0	369.0	2381.2	15.1
	IL-12 (pg/ml)	0.0	0.0	0.0	0.0	0.0	28.9	0.0	0.0	0.0	0.0	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.7	0.0
	TNFα (pg/ml)	0.0	0.0	0.0	0.0	0.0	36.8	0.0	0.0	12.0	0.0	12.5	0.0
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI414	IL-6 (pg/ml)	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-10 (pg/ml)	0.0	72.7	54.5	0.0	2.0	71.0	28.3	0.0	29.6	29.5	0.0	0.0
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI468	IL-1β (pg/ml)	22.3	20.1	6.2	5.2	1.0	6.6	5.8	16.3	4.0	17.2	9.1	24.9
	IL-17A (pg/ml)	19.1	0.0	0.0	0.0	0.0	4.2	0.0	0.0	0.0	0.1	0.0	1.4
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI562	IL-1β (pg/ml)	0.0	1.9	0.4	0.0	0.0	0.7	0.9	0.8	0.0	1.1	0.8	0.8
	IL-5 (pg/ml)	16.3	12.3	0.0	0.0	11.3	4.4	3.0	0.0	13.5	5.0	8.1	5.0
	IL-8 (pg/ml)	6.4	42.7	13.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-10 (pg/ml)	0.0	11.6	6.0	0.0	0.0	9.6	0.7	0.0	0.0	0.0	0.0	0.0
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI899	IL-5 (pg/ml)	0.6	90.6	0.0	0.0	63.3	2.3	0.0	0.0	0.0	0.0	55.8	0.0
	IL-12 (pg/ml)	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IFNγ (pg/ml)	0.0	36.1	0.0	0.0	0.0	3.7	2.0	0.0	150.8	0.0	0.0	0.0
	Treated												

Figure 55. Patient case examples for trends in cytokine concentrations and antibiotic treatment for a UTI.

Prophylactic Treatment

As can be seen to some degree in Figure 55 in patients UTI337 and 414, long-term antibiotic treatment is not always effective at keeping the immune response from fluctuating. Patients UTI115, 524 and 675 will be used to further demonstrate this observation (Figure 56). All 3 patients were receiving prophylactic courses of antibiotics and all had regular isolation of *E. coli* from their study samples (Figure 23, p.113). Patient UTI115 received 2 prophylactic courses of cephalexin, separated by a short course of trimethoprim at D7 (Appendix 10, p.224). Patient UTI524 received nitrofurantoin until D4 then switched to a course of trimethoprim from D5 onwards (Appendix 23, p.237). Finally, patient UTI received prophylactic nitrofurantoin from D3 onwards (Appendix 28, p.242). So despite a variety of different prophylactic drugs being prescribed, huge fluctuations in immune activation can be seen within these patients (Figure 56). There appears to be no distinct pattern caused by the different antibiotics. This can be clearly demonstrated by patient UTI524, who received 2 adjacent courses of different antibiotics, yet appear to see a similar level of protein fluctuation for both (Figure 56). These data may suggest that prophylactic courses of antibiotics are not sufficient in preventing immune activation in patients with persistent bladder colonisation.

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI115	IL-1β (pg/ml)	0.0	1.3	1.3	0.9	0.7	3.6	13.4	1.2	1.3	8.3	4.0	0.0
	IL-4 (pg/ml)	0.0	0.0	0.0	0.0	0.2	3.1	2.1	0.0	0.0	0.0	0.0	0.0
	IL-5 (pg/ml)	0.0	7.5	6.2	0.0	0.0	9.7	11.0	0.0	1.3	0.4	0.0	0.0
	IL-6 (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-8 (pg/ml)	0.0	0.3	9.1	1.2	0.1	14.9	80.4	2.0	0.0	14.6	1.4	0.7
	IL-10 (pg/ml)	0.0	6.8	0.0	0.0	0.0	1.0	1.5	0.0	0.0	4.9	0.0	0.0
	IL-12 (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.0	0.0	16.5	0.0	0.0	4.7	0.4	0.0	0.0
	TNFα (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IFNγ (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI524	IL-1β (pg/ml)	0.0	0.4	0.0	0.0	0.0	1.3	0.9	0.6	0.0	0.0	1.6	5.6
	IL-4 (pg/ml)	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.2
	IL-5 (pg/ml)	37.2	24.0	0.0	0.0	0.0	4.7	0.0	0.0	0.0	67.0	4.6	0.0
	IL-6 (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-8 (pg/ml)	0.0	78.5	0.0	0.0	0.0	3.6	0.5	0.0	0.0	9.6	0.0	13.2
	IL-10 (pg/ml)	2.2	2.0	9.5	5.9	0.1	6.9	4.9	2.0	3.1	9.4	7.1	2.5
	IL-12 (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	7.7	1.8	20.4	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0
	TNFα (pg/ml)	0.0	3.6	0.0	0.0	0.0	15.3	19.2	0.0	1.7	32.2	15.0	0.0
	IFNγ (pg/ml)	0.0	0.0	0.0	0.0	0.0	59.8	0.0	0.0	0.0	35.9	42.0	0.0
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI675	IL-1β (pg/ml)	0.4	4.1	4.2	0.0	1.8	13.6	4.9	0.0	2.5	2.7	2.2	6.0
	IL-4 (pg/ml)	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.3	0.2	0.0
	IL-5 (pg/ml)	0.0	0.0	0.0	6.0	0.0	65.1	0.0	0.0	0.0	8.5	12.0	0.0
	IL-6 (pg/ml)	0.0	12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.1	2.0	0.0
	IL-8 (pg/ml)	0.0	20.7	12.2	0.0	5.5	20.1	35.6	0.0	0.5	14.3	28.4	0.0
	IL-10 (pg/ml)	0.0	8.8	9.3	5.3	8.3	17.1	16.6	10.7	19.6	19.3	18.9	3.7
	IL-12 (pg/ml)	6.7	14.0	0.0	7.1	0.0	19.1	15.1	5.3	24.2	0.7	0.0	0.0
	IL-17A (pg/ml)	9.9	1.2	40.1	8.0	17.6	6.5	47.5	13.1	0.0	14.7	44.7	14.5
	TNFα (pg/ml)	0.0	0.0	0.0	0.0	0.0	8.9	0.0	0.0	0.0	0.0	3.5	0.4
	IFNγ (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.9	0.0
	Treated												

Figure 56. Patient case examples for trends in cytokine concentrations and prophylactic antibiotic treatment for a UTI.

8.3.5. Timing of Cytokine Fluctuations

In section 8.3.2, patient UTI138 appeared to have rising levels of IL-10 at the donation before a positive HCP outcome was seen within the urine on 3 separate occasions (Figure 53, p181). Similar observations of changes in protein levels both before and after changes in bacterial load, HCP outcome, self-declared UTIs and treatment were seen in a large number of study patients. To demonstrate some of these observations, patients UTI337, 569, 675, 840 and 899 will be used as case examples.

Patient UTI337 appeared to have a full load of *E. coli* at D1 which was followed by a strong immune reaction at D2 (Figure 57). At D2 they also submit a positive urine sample to the NHS, declare they have a UTI and produce a urine with a positive HCP outcome. On this occasion it appears that the immune activation is in response to the diagnostic load of *E. coli* and sufficient at reducing colonisation load (Figure 21, p107). A similar pattern can also be seen after the full loads of *E. coli* at D5 and D10 (Figure 57). This suggests that in this patient the *E. coli* colonisation is triggering the immune response to activate in order to attempt to clear the infection and the temporal aspect of the study allows us to see this activation over time. This patient felt they had a UTI at D11 even after the *E. coli* has been cleared from the bladder, likely due to the strong immune response being seen here. This could suggest that symptoms may in some cases, only become apparent after the infection has already been cleared by the immune system, making antibiotic treatment superfluous.

Two periods of strong immune activation at D4 and D5 in patient UTI569 align with times when they took a positive urine sample to their GP (Figure 57). However, it is clear that the protein profiles were very different each time. Interestingly it was actually between these times when the patients saw diagnostic loads of *E. coli* and was treated for a UTI. This patient was fully colonised with *E. coli* strain ST3640 at D10 to D12 (Figure 21), they showed a positive HCP outcome and stated they felt they had a UTI at D10, by D11 the patient no longer suspected a UTI and by D12 the HCP outcome was no longer positive (Figure 57). This demonstrates the variation in clinical and subjective outcomes of a UTI with no apparent change in the bacterial colonisation.

As seen in patient UTI569 D4, a strong immune activation appears to precede colonisation with a diagnostic load of *E. coli* and a positive HCP outcome at D12 in patient UTI675 (Figure 57). This may suggest that these proteins could be able to predict these situations in this patients. However, their worth is questionable as the patient did not feel they had a UTI

at D12 nor did they take a urine sample to their GP (Appendix 28, p.242). Therefore, this is a case that the NHS would never have seen regardless, so predicting it would have likely caused confusion and perhaps unnecessary antibiotic treatment.

UTI 840 and 899 show examples of patients where the immune response appears to be peaking shortly after a patients state they have a UTI (Figure 57). Immune proteins appear to be found at relatively low levels when patients state they have a UTI, which is unexpected as it would be assumed patient symptoms would be caused by immune activation and inflammation. This may highlight the issue with accurately diagnosing the cause of patient symptoms in this patients group. As symptoms can often be diffuse and have alternative causes in older patients [29].

These data taken together suggest that there is no distinct pattern or trend in the measured cytokines with regards to predicting symptomatic infections, when using any of the possible definitions outlined here. There may be some trends which are patients specific, but would require continual monitoring to test their reliability to predict a symptomatic UTI in each separate patient.

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI337	IL-1β (pg/ml)	7.1	76.5	2.0	0.0	50.1	8.8	0.0	0.0	0.0	10.2	138.0	0.0
	IL-4 (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.4	3.5	0.0
	IL-5 (pg/ml)	0.0	25.7	0.0	0.0	0.0	58.6	23.5	2.1	0.5	0.8	0.0	0.0
	IL-6 (pg/ml)	0.0	130.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	209.8	0.0
	IL-8 (pg/ml)	58.6	3664.1	13.9	0.0	43.9	120.3	48.8	31.6	0.0	369.0	2381.2	15.1
	IL-10 (pg/ml)	5.7	9.7	25.3	0.0	69.3	14.1	26.8	8.3	0.0	9.0	32.3	0.0
	IL-12 (pg/ml)	0.0	0.0	0.0	0.0	0.0	28.9	0.0	0.0	0.0	0.0	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.7	0.0
	TNFα (pg/ml)	0.0	0.0	0.0	0.0	0.0	36.8	0.0	0.0	12.0	0.0	12.5	0.0
	Positive sample												
	Full *E. coli* load												
	HCP outcome												
	Self-declared												
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI569	IL-1β (pg/ml)	21.5	7.6	2.0	81.8	44.8	10.0	3.3	2.4	2.2	6.9	9.1	4.6
	IL-5 (pg/ml)	44.5	13.1	18.5	0.1	0.0	43.7	11.2	20.7	0.0	0.0	15.0	0.0
	IL-6 (pg/ml)	32.9	0.0	8.4	84.6	0.0	6.7	0.0	0.0	1.4	7.7	0.0	0.0
	IL-8 (pg/ml)	2.3	19.9	15.1	182.7	21.9	16.7	2.9	18.3	0.0	13.5	148.6	0.0
	IL-12 (pg/ml)	10.1	0.0	0.0	0.0	0.0	25.7	0.0	0.0	0.0	2.3	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	TNFα (pg/ml)	0.0	0.0	0.0	0.0	0.0	10.9	0.0	0.0	0.0	5.2	0.0	0.0
	Positive sample												
	Full *E. coli* load												
	HCP outcome												
	Self-declared												
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI675	IL-8 (pg/ml)	0.0	20.7	12.2	0.0	5.5	20.1	35.6	0.0	0.5	14.3	28.4	0.0
	IL-10 (pg/ml)	0.0	8.8	9.3	5.3	8.3	17.1	16.6	10.7	19.6	19.3	18.9	3.7
	IL-17A (pg/ml)	9.9	1.2	40.1	8.0	17.6	6.5	47.5	13.1	0.0	14.7	44.7	14.5
	IFNγ (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	15.9	0.0
	Positive sample												
	Full *E. coli* load												
	HCP outcome												
	Self-declared												
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI840	IL-1β (pg/ml)	3.3	3.6	15.3	0.0	3.9	6.2	6.0	3.2	1.2	1.3	0.0	0.0
	IL-4 (pg/ml)	0.6	0.0	2.9	0.0	0.2	0.0	0.0	0.0	0.0	0.6	0.0	0.0
	IL-5 (pg/ml)	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-8 (pg/ml)	0.0	0.0	7.4	0.0	0.0	3.2	16.1	16.8	0.0	1.9	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	9.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Positive sample												
	Full *E. coli* load												
	HCP outcome												
	Self-declared												
	Treated												

UTI number		D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
UTI899	IL-1β (pg/ml)	5.6	1.1	5.9	0.0	0.0	1.6	3.8	0.2	1.9	3.9	2.3	0.0
	IL-4 (pg/ml)	0.0	1.4	2.4	0.0	0.0	2.1	0.0	0.0	0.0	0.3	0.0	0.0
	IL-5 (pg/ml)	0.6	90.6	0.0	0.0	63.3	2.3	0.0	0.0	0.0	0.0	55.8	0.0
	IL-10 (pg/ml)	16.4	34.9	7.9	15.7	32.6	78.0	0.0	26.6	143.9	59.6	55.2	1.8
	IL-12 (pg/ml)	0.0	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	IL-17A (pg/ml)	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	18.3	0.0	0.0	0.0
	IFNγ (pg/ml)	0.0	36.1	0.0	0.0	0.0	3.7	2.0	0.0	150.8	0.0	0.0	0.0
	Positive sample												
	Full *E. coli* load												
	HCP outcome												
	Self-declared												
	Treated												

Figure 57. Patient case examples for trends in cytokine concentrations with regards to their timing in relation to the various symptomatic definitions.

8.4. Conclusions

In previous chapters, each of the individual study aspects have been analysed in detail. This chapter allowed for gathering all of these aspects together, allowing for cross sectional visualisation and data analysis. It was essential to perform this to see if different urinary components showed relevant correlation with one another.

By giving a quantification to each of the study samples it became possible to analyse the whole database together and begin to analyse potential differences within the different study cohorts. This provided 360 individual samples for analysis, thus giving the sample sizes needed to power statistical analyses. The total score assigned to each study sample provided this quantification and gave an indication of sample health. A higher total score indicated a generally poorer sample health.

By splitting the total scores down by the basic patient data it was possible to see that there were no significant differences between the sample health from diabetic and non-diabetic patients. Also no difference was seen between patients who chose different severities of their most common UTI symptoms. A statistical difference was however seen between total scores from male and female patients, with men showing a trend towards favourable sample health than women. One possible explanation for this is that the bacteria capable of travelling the relatively large distance from outside the body to the male bladder, are better adapted to survival within the urinary tract by avoiding activation of the patient's immune responses. This would give a lower total score from the host analysis perspective. Also this potential lack of activation may in turn prevent symptoms and thus give lower symptom scores, further lowering the total score.

Another statistical difference that could be obtained from the basic patient data was from breaking down the total scores by patient age groups. There scores rose with age from 65 to 79 years, after which they appeared to drop again. This could potentially be due to the older patients having better survival mechanisms and therefore perhaps being better able to cope with fluctuations in their homeostasis which UTIs may be responsible for. For example, they may have more efficient better immune activation perhaps preventing unnecessary inflammation and symptoms.

Splitting the total scores down by aspects of the clinical data analysis, it was possible to see additional interesting differences within the data. As would perhaps be expected all samples

positive for the different definitions of a symptomatic episode produced a poorer sample health. These were when patients declared they had a UTI at the time of sampling, a sample with a positive dipstick score from a HCPs perspective and, the definition used in this project, of when a positive urine sample was received by the NHS within 3 days of the study sample being collected. However, a positive HCP outcome on the dipstick contributes 7-10 points to the overall score, thus some of the difference seen could be partially weighted towards a positive outcome (Table 3, p.49). Also, on questionnaires where patients declared they had a UTI they are logically more likely to give higher scores to the symptom questions outlined in section 5.2. This again could contribute to a weighting of the samples total score. The positive urine sample was a simple binary score of yes or no, thus the significance seen here is much more organic and unbiased. This therefore supports the decision for it to be chosen as the definition of a symptomatic episode thus far.

Another interesting observation within the clinical data break down of the total scores was that no significant difference was seen between samples receiving antibiotic treatment and those that were. This suggests that whether a patient was receiving treatment or not did not impact the sample health score. This poses two possible explanations, the first being that treatment is effective at improving sample health, thus bringing the scores down to the level of those which were not being treated. Alternatively, it may suggest that antibiotic treatments are not being given effectively, as the patients receiving them have a similar sample health to those are not. Further investigation would be needed to deduce which of these was the cause of the lack of difference in total scores. For example, patients starting or stopping treatment could be regularly monitored throughout the transition to see if their total scores change.

Samples containing *E. coli* appeared to be associated with significantly poorer sample health scores than those without *E. coli* present. Due to the calculation including antibiotic resistance and motility data outcomes for only samples containing *E. coli*, this significance could be partially weighted. However, when looking at scores from samples that contained *E. coli* up to the diagnostic load compared to those with diagnostic loads, this significance could still be observed. Suggesting that *E. coli* presence in the urine does, in fact, cause a deterioration in patient and sample health. To assess the importance of this observation, other pathogens would need to be further analysed to see if this difference was *E. coli* specific or not. For this project *E. coli* was focussed on, however the bacterial isolation suggests that *Enterococcus faecalis* may be worth future analysis (Figure 17, p.99). In order to remove this sample bias, breaking down the total score to analyse the effect of motility on sample health

was performed only on samples containing *E. coli* (n=184). Samples containing motile *E. coli* gave a significantly lower total score, suggesting motility has a positive influence on sample health. One explanation for this could be that motile strains are able to subvert detection by the host, avoid immune activation and causing symptoms, all of which would lead to a higher total score. One potential mechanism for this could be by *E. coli* controlling flagella numbers, either per cell or within the population in order to avoid activating the immune system via the TLR5 pathway, as previously discussed in section 6.5 [131,140].

By analysing the total scores from only samples containing *E. coli*, it was possible to see that there was no significant difference in total scores between those with multiple drug resistances to the antibiotics tested and those resistant to just one drug or less. This could perhaps indicate that despite the survival advantage of being resistant to multiple antibiotics these strains are not necessarily responsible for a more aggressive disease phenotype.

In the previous chapter fluctuations in the urinary cytokine concentrations were analysed against the definition of symptoms that have been used throughout. This defined symptoms as samples in which a positive urine culture had been received by the NHS within 3 days either side of collection. There were no consistent correlations seen with the proteins and these periods of symptoms. It was therefore important to analyse all the cytokine concentration data with other potential ways of defining a symptomatic episode, such as a full load of *E. coli* in the urine, a positive HCP outcome, self-declared UTIs by patients on the questionnaires and antibiotic treatment for a UTI around the time of sampling. This analysis could potentially give information on trends in the immune system which could predict changes in the disease state. By visualising all of the protein concentrations with the different study aspects it was possible to see lots of small trends within each of the individual cytokines. However, these were generally very variable and not consistent or reliable around any of the other potential symptom definitions. The majority of trends would show peaks in protein concentration taking place after a potential symptomatic episode, which while interesting would not make them suitable for a predictive marker for symptomatic UTIs.

An interesting observation was made within the IL-8 cytokine data when comparing this to pathogens being identified within the urine. IL-8 appeared to show relatively few correlations with the *E. coli* load than were seen within the other tested cytokines. This protein appeared to be more strongly influenced by changes in the presence of other pathogens, most notably

Enterococcus faecalis. This suggests that genotypic and phenotypic analysis of *Enterococcus faecalis* could provide an important avenue of future analysis on the study's biobank.

It was suggested that perhaps timing treatment with periods of strong immune activation may be more effective at successfully clearing the bladder of long-term infection. However, inconsistent observations made this unclear. Further investigation would be needed to test whether this is a viable avenue to pursue.

This method of temporal analysis also made it possible to visualise changes in the immune response proteins during extended periods of treatment. It was clear to see in almost all of the measured cytokine profiles that prophylactic treatment with antibiotics did not prevent activation of an innate immune response. This is not surprising, as it has previously been seen in earlier analysis that there is still a great deal of microbial activity within the urine of patients taking long-term antibiotics to prevent UTIs.

Despite being unable to identify a clear predictive biomarker for a symptomatic episode, using any of the available definitions, performing cross-sectional analysis on all aspects of the study database allows for important observations to be made. This could suggest that looking cumulatively at all the aspects contributing to overall sample health may be more reliable at defining severity, than focussing on individual aspects of the clinical or bacterial outcomes.

Chapter 9. Discussion

The aims of this project were to investigate the changes in the elderly bladder over time from both the bacterial and a host aspect. In order to achieve this a longitudinal study was undertaken, sampling regular urine donations of 30 patients with a history of frequent recurrent UTIs. This involved acquiring full ethical approval as recruitment was done within an NHS setting in order to give a holistic view of the types of patients being seen within the clinics. Both men and women from Newcastle Upon Tyne and the surrounding areas were recruited to the study, with the average age being 75 years old.

The study completed in 82 weeks, which totalled equivalent to 5600 patient days. The study was run day-to-day by a small team of urology nurses and the chief investigator (author of this thesis). This therefore addresses the first aim of the project, which was to test the feasibility of such a study design in this age group. It has shown it is possible to complete such a demanding longitudinal pilot study in a patient group of this age. This in itself is a huge achievement, as pilot studies provide invaluable insight for potential future research and study design. In order to scale up such a study, what would be needed was either a proportionally larger research team or a significantly extended timeline. The willingness and engagement of patients, staff and researchers significantly aided the smooth and successful running of this clinical study.

A total of 12 urine samples and symptom questionnaires were collected from each patient 2.3 weeks apart on average. This allowed for a non-invasive method of observing changes in the patients' bladders over time with relatively good temporal resolution. The majority of previous studies analysing urinary constituents of UTI patients are single snapshot sampling [67,94,155,198] and those that have repeated sampling are with larger gaps between collection, a minimum of 4 weeks apart [185,201]. Blood samples were also taken for standard NHS urological blood testing and for DNA analysis. After completion of the study a large biobank of samples was created. This included 1,215 filtered urine samples, 405 unfiltered urine samples, 188 *E. coli* isolates and 30 blood samples. In addition to the urine, bacteria and blood, 360 symptom and treatment questionnaires were also collected. This database would allow for extensive bacterial, host and clinical analysis in order to address the aims of the project.

By analysing patient and clinical data, it was possible to gain perspective on the study population being analysed as well as highlighting some basic observations within the clinical and treatment data. For example, the most commonly identified symptoms patients gave for a UTI were increased frequency of urination and an increased urgency to urinate. These are also the most common lower urinary tract symptoms (LUTS) associated with normal ageing of the bladder, thus they are commonly misinterpreted as a UTI [29]. This can lead to inappropriate and ineffective treatment with antibiotics, compounding the issues with developing antibiotic resistances. This simple observation from the study questionnaires highlights the difficulties clinicians face in correctly interpreting symptoms patients declare. This issue is further highlighted when comparing numbers of positive urine samples received by the NHS in 12 months with patient estimates of how many UTIs they felt they suffered in the same amount of time. Patients would often overestimate the number of UTIs that they had suffered in the past year, compared the number actually being seen by the NHS. This further highlights a potential lack of reliability in self-diagnosis and medical history recall by these patients. Interestingly, the clinical and questionnaire data were able to highlight that prophylactic antibiotic treatments were not reliable at preventing increases in patient declared UTIs and increases in their symptoms scoring from the questionnaires. This lack of treatment efficacy was a common trend seen throughout the data analysis and will be addressed throughout this discussion.

One of the main aims of the study was to analyse changes from both the bacterial and host perspective around periods of symptoms. The study format aimed to capture events of transition between asymptomatic to symptomatic states. This could potentially allow methods of predicting such episodes in these patients, to give more effective treatment and minimise patient suffering. In order to do this a single definition of when a patient was suffering a symptomatic UTI was needed, this turned out to be one of the major difficulties during the study analysis. Patient declared symptoms were unreliable from a clinical perspective. Alternatively, using positive dipstick outcome or full diagnostic loads of bacteria to define a symptomatic UTI lacks any information on how the patient is actually feeling. For these reasons a symptomatic episode was defined as any study sample given within 3 days of a positive urine sample being received by the NHS. Indicating that a patient has taken a sample to their GP practice suspecting a UTI, the HCP has agreed, sent the urine for urinalysis and finally the NHS have stated it is a positive sample for a UTI. Thus this definition allowed for agreement between the patients, HCPs and NHS. By using this definition, a total of 22

periods of symptoms were recorded throughout the study, equivalent to 6.1% of all patient samples. These episodes were seen in 15 different study patients (50.0%). Due to these patients being recruited based on their medical history of frequent UTI, these values are perhaps lower than may have been originally expected. Therefore, providing less opportunities to visualise symptomatic changes within individuals, as the majority of these 15 patients only suffered one defined symptomatic episode in the 6 months of the study (73.3%).

E. coli was chosen to be the focus during these analyses as it is reported to be responsible for the vast majority of UTIs [100,101]. By isolating *E. coli* whenever it was found to be present in the urine, allowed bacterial phenotypic and genotypic analysis to be completed. *E. coli* was isolated from 51.1% of the urines collected during the study, with 27 of the 30 patients showing some form of colonisation at some point. Many of these patients showed long-term carriage of the pathogen. The majority of samples showed the growth of two or more pathogens (65.6%). Interestingly, the most commonly isolated pathogen from the study urines was *Enterococcus faecalis*, in 55.4% of all samples. This might suggest that analysis into the genetics and phenotype of *Enterococcus faecalis* would be a logical next step for this study analysis. The decision to focus on *E. coli* was supported by the literature, however looking back on the completed analysis *Enterococcus faecalis* appears to be the next logical avenue of research. Changes in the load and strain of this microbe could potentially explain some of the fluctuations we see within the bladders of these patients and may show further trends within the sample health.

By performing MLST sequencing on all isolated *E. coli* strains it was possible to find out which strains were commonly being isolated within these patients and what phylogenetic clades these fell into. The most commonly isolated sequence type was *E. coli* strain ST73, which fits with what is seen in the literature [198,212]. ST73 is associated with *E. coli* clade B2, which is the group most commonly associated with UPEC [90]. The vast majority of study isolates fell into this B2 clade (63.0%). However, clades D, F and B1 were also represented to a lesser extent. None of the study isolates were isolated from clade A, thought they have been isolated in UTI patients previously [124,203,228]. This is the clade most commonly associated with commensal gut *E. coli* strains [123]. This suggests very few of the study isolates are present in the urine due to gut contamination. However, it is still possible that the gut could act as a reservoir for UPEC strains before reaching the bladder [59,60].

Sequencing analysis also allowed observation of the stability of the different strains within the bladder and any changes in the colonising strain. By analysing these changes alongside the defined symptomatic episodes and periods of treatment, several observations could be made. It was clear from the data collected that antibiotic treatment is often an ineffective means of clearing the bacterial load from the bladder and in some cases also the symptoms. The inefficiency of treatment is exemplified in study patients receiving long-term prophylactic antibiotics; who would still complain of symptomatic episodes and experience fluctuations in bacterial load. Knowing the strains sequence type allowed observation of changes in the strain, which often occurred after rounds of treatment. In several cases this appeared to clear the way for more aggressive and symptomatic strains, for example in patient UTI569. This could give strength to the argument that some patients may benefit from being left untreated, with a harmless ABU strain colonising their bladder. Several studies have shown that the presence of certain strains of *E. coli* within the bladder can prevent, or significantly delay superinfection by a new pathogen, when being compared to ABU patients being given antibiotic treatment to clear the bladder [31,50–54,215]. It is clear that treatment in some patients is not only un reliable at 'clearing' the bladder, but may also be detrimental in terms of super-infections and on top of this could be contributing to the formation of antibiotic resistances, a huge problem at this time.

Whilst the data shown can tell us when changes in the strain are taking place it is not possible for us to know where the new strains are coming from. One suggestion may be that they are originating from a reservoir within the gut [59,60]. In order to test this hypothesis, perineal swabs could be used to sequence the bacteria found in this area to see if it matched the strain colonising the bladder [59]. Swabs were not included in this study, as it would be a significant burden on patients as they would need to regularly attend the clinic for the swabs to be taken correctly by a HCP. This may have negatively affected study recruitment, timeline and compliance. However, this may be a useful amend to the current ethics, if such a study was to be repeated in the future as this information could be useful for helping aid patient management and education. The study undertaken by Czaja *et al* (2009) suggests that the gut is the main source of bacteria, bladder isolation occasionally occurred without prior periurethral isolation, suggesting the bladder is a large reservoir for UPEC, which may or may not go on to cause a symptomatic infection [202]. Other potential reservoirs for uropathogenic bacteria in these patients include the vagina in women as well as previously reported intracellular bacterial communities (IBCs) [33,59,184]. IBCs are small colonies which

live within the epithelial cell lining of the bladder. These can lay dormant within the bladder for very long periods of time, but can also be seen to re-surface to colonise the bladder sporadically [33]. Such colonisation could explain how strains can appear to be cleared from the bladder and re-appear many weeks later in the urine [202]. IBCs are likely responsible for the bacterial reservoir within the bladder hypothesis mentioned above. For example, as is seen in patients UTI115 and 524. It could suggest that strains are present in the bladder for very long periods of time during an adult's lifespan and that age related deterioration of the bladder and immune defences leads to them no longer residing asymptomatically in certain people. How long these pathogens can remain in the human bladder for still needs to be investigated. These IBCs are likely a large contributor to UTI recurrence and the huge immune response variation seen in this study.

Sequencing of the *fliC* gene in all isolated *E. coli* samples allowed observation of situations whereby the strains were able to switch serotype of the expressed flagellin. Sequence alignment identified that most of the switches appeared to be full gene changes rather than changes due to single gene mutations. These urinary pathogens may be undergoing unilateral flagellar phase variation [213]. The reason for this is unclear, however one suggestion is that this may be a mechanism utilised by the infecting pathogen to subvert surveillance by the adaptive immune response and avoid clearance from the bladder. Another potential mechanism for avoiding immune clearance observed within the bacterial study data was related to the motility of the isolates. Motility after 6 hours was found in the minority of the *E. coli* isolates (32.6%). The pathogen may be able to maintaining some degree of motility within the population conferring a survival advantage and enabling ascension of the urinary tract, but at a low enough level to remain undetected and thus not be cleared by the innate immune activation. This is a theory currently being tested and early evidence appears to support this hypothesis (Picton, Lauvrak and Aldridge, unpublished data).

Phenotypic and genotypic analysis for the isolated *E. coli* samples compared to changes in symptomatic state showed no clear evidence for the strain, motility or bacterial load being responsible for causing such symptomatic episodes. This may suggest that bacteria themselves are not able to provide a reliable biomarker of change in symptoms. However, further analysis would be needed on other major pathogens residing within the urine to completely rule this out. This is supported by the fact that the majority of samples showed the growth of several different pathogens using these cultivation techniques, therefore indicating

that analysing the polymicrobial picture is essential and details could be missed by focussing on just one pathogen.

By performing blood analysis on parameters regularly requested for by urologists within the NHS, it was possible to identify that a large number of the study population were deficient in circulatory vitamin D. Association with increased risk of UTI and vitamin D deficiency has been previously shown [79–81]. This deficiency could provide an explanation for why some of this patient cohort are suffering from such urinary disorders. Therefore, a simple point of education could benefit these patients and potentially impact their disease status long-term. HCPs should perhaps be strongly recommending vitamin D supplementation to these patients with low levels of vitamin D. Whether this would have an impact on improved UTI outcome would need further investigation of supplemented patients over time, however early studies show that this may be a promising avenue for such patients [224]. The vitamin D status of the healthy controls was not assessed in this study due to ethical and time restrictions. However, this could provide an important insight and possible explanation into the observations made regarding the unexpected urinary cytokine concentrations and bacterial presence seen in these participants. For this reason, it would be recommended that future studies consider including blood collection from healthy controls.

Analysis of urinary constituents arising from the host allowed for observation of potential changes around symptomatic episodes and addressed the second aim of the study. This was to analyse potential changes in the colonising bacterial phenotype and genotype during periods of changing UTI symptoms and treatment. A library of 10 urinary cytokines were chosen based on their reported involvement with UTIs from the literature, some more extensively researched than others. These were IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, IL-17A, TNFα and IFNγ [54,94,147,150–155,159,160,162,165,173–181,220,221]. All chosen cytokines were measured within the study urine using commercially available ELISA kits.

Originally when submitting the ethics, it was decided that control urines would not be necessary as all results would be compared within each individual patient. This was felt to be the best method for looking for a predictive biomarker for symptomatic episodes. However, upon completion of the cytokine concentration analysis it became evident that there was a lack of correlation with symptoms and that the large variability in concentrations made it difficult to reliably draw conclusions without a healthy comparison. This led to the question of how the environments of healthy bladders compare to those of study patients being asked.

In order to address this, an amends to the ethical approval was submitted to recruit 15 healthy, age-similar control participants to the study. These participants were required to have no urinary complaints within the last 5 years. In hindsight this arm of the study could have been performed in parallel with the patient arm, which would be a recommendation if such a study was to be repeated. These urines allowed for a healthy baseline average to be calculated for each of the measured proteins, allowing normalisation of all patients samples for analysis.

The healthy urine samples from age-similar controls gave the opportunity to explore the normal bladder environment of people in this age group. The expected result was urine samples clear of any bacterial growth, normal dipstick outcomes and a close to absent immune response. However, low levels bacterial growth was seen in a vast majority of the healthy control samples, with one patient even showing diagnostic loads of *E. coli* in 2 of the 3 collected samples. One other healthy control gave a positive HCP score for all 3 urines. These 2 patients were excluded from the analysis as it was felt they were not healthy control samples, from a healthcare perspective. By taking an average of each of the measured proteins it was not only possible to obtains a baseline to which the patients' concentrations could be normalised, but it was also possible to get an overview of the healthy bladder host environment. Half of the measured proteins were seen at much lower levels in the healthy controls, which was expected. However, the other half appeared to be found at higher concentrations in the healthy controls. This was an unexpected observation and may suggest that mechanisms may be in place within the colonised patients which are repressing the release of certain immune response proteins, possibly explaining why they are less able to maintain a bladder clear of growth. Another suggestion is that the patients may be experiencing desensitisation of the immune response due to the long-term colonisation of the bladder. Further investigation would be needed to deduce which of these mechanisms is responsible.

When looking at cytokines with a concentration of over 10-fold higher than the lowest seen in each patient, it provided an overview of the levels of immune activation being seen within this patient cohort. This showed there was generally a low level of immune activation despite a high incidence of colonisation of these patients. This low level of immune response could be responsible for the inefficient clearing of the pathogens from the bladders of these patients. This is also a theory put forward by Sivick *et al* (2010) based in the observations they saw in the bladders of mice susceptible to UTIs [179]. One potential explanation for this could be due to gene silencing via DNA methylation within the bladder epithelium [229,230].

Silencing of the genes involved with the innate immune response and cytokine production could be responsible in part for the low levels of proteins being detected within the bladders of these patients. Which in turn may explain why the patients struggle to successfully clear these long-term infections from their bladders (Figure 58). In order to test this hypothesis, genomic DNA could be isolated from sloughed epithelial cells collected from the unfiltered urine stocks. This could then be used to perform DNA methylation testing of the genes of interest. DNA methylation is known to increase with age, thus could potentially have a significant impact on this patient group [229]. This theory of a dampened immune response in the elderly, is supported by comparing the data shown here with the much higher cytokine concentration values seen in younger women suffering from recurrent UTIs, aged 18 to 49, in the study completed by Czaja *et al* (2009) [202].

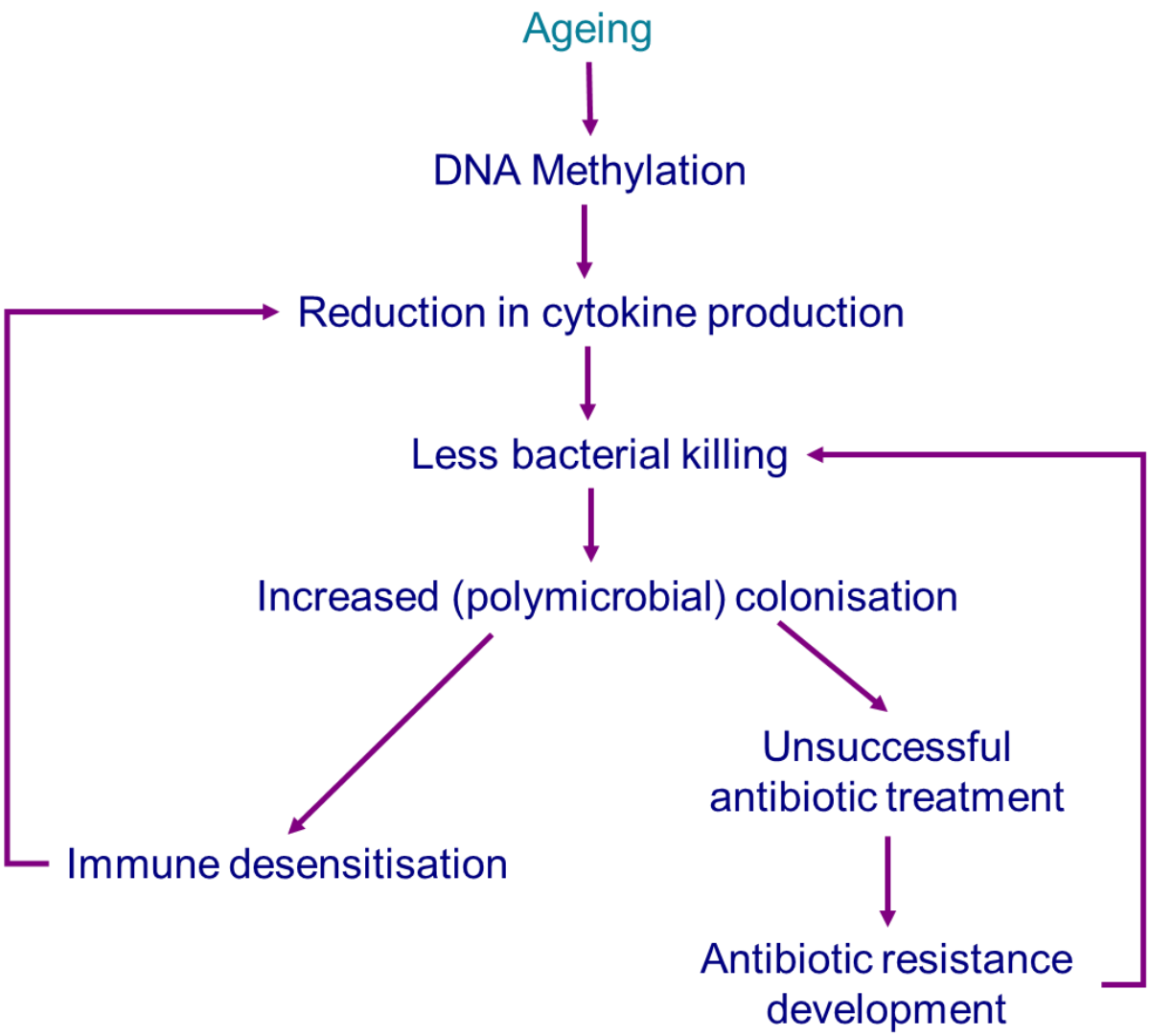


Figure 58. Possible model of perpetual colonisation within elderly patients suffering from ABU and recurrent UTIs.

By analysing all measured cytokines against all potential definitions for a symptomatic episode it was evident that there were no distinct patterns within the protein levels that would be able to reliably predict periods of symptomatic infection in these patients. What this temporal analysis did provide, however, is an indication of the enormous variability not only between patients but within individuals over time. This variation suggests that colonisation within the same patient group can produce extremely different immune responses. This may be explained by the huge variation in colonising pathogens seen between samples in most patients. This highly dynamic environment within the bladder likely provides an explanation for the inability of treatments to be effective and prevent re-infection and symptomatic episodes. This long-term carriage of several pathogens may also be causing a desensitisation of the immune response due to constant activation. Potentially providing another reason for the low levels of immune activation seen within the vast majority of study patients (Figure 58). The transient environment within the bladders of this patient group also suggests that it will be unlikely that a single or combination of urinary biomarkers will be identifiable for the purpose of predicting symptomatic episodes. The variability may also be being caused by changes in the bladder microbiome, much of which is known to be undetectable by standard culturing methods. The bladder is known to be colonised by a number of organisms even in healthy individuals [24,25]. Due to the lack of sensitivity of current urinalysis within the NHS, such fluctuations could be being missed. This may suggest that more sensitive methods of assessing bladder colonisation may be useful in some hard to treat patients, such as 16s rRNA sequencing [24,25].

An interesting observation made by analysing the host and bacterial data together was that IL-8 concentration appears to show stronger correlations with the presence of pathogens other than *E. coli* in the bladder. There appeared to be some observable relationships between IL-8 concentration and the presence of *Enterococcus faecalis*, suggested by qualitative analysis. This stood out as it was not an observation made for any of the other tested urinary cytokines. Therefore, a strong recommendation for future work is to perform the microbial analysis shown here for *E. coli* on *Enterococcus faecalis*.

The host analysis revealed some downfalls in the current antibiotic treatments being given to these patients, similar to what has already been discussed from the microbial analysis. Treatment courses often appeared to be ineffective at preventing fluctuations within the immune response. This was clearly demonstrated within patients who were taking long-term prophylactic antibiotics. Whereby these patients would show significant fluctuations in most

of the measured immune response proteins, suggesting that the bladder is not being kept clear of aggressive pathogens and that the immune system is being activated regularly despite treatment (Figure 58). These treatment courses were also seen to be ineffective at preventing symptomatic episodes, when using any of the possible symptom definitions. Therefore, this observation with respect to treatment, is again suggesting reduced benefits of treatment within these patients.

As well as extensive temporal analysis of the different samples within individual patients, it was important to be able to ask questions of the database of analysis as a whole. The method chosen for this was to assign each sample a 'total score' which takes into account all aspects of the analysis. Whereby a lower total score would indicate a healthier sample. This would then allow for statistical analysis to be run for different questions with respect to sample health. This method of analysis allowed differences to be identified between different patient cohorts. Interestingly, samples containing *E. coli* were related to poorer sample health and this health score was seen to deteriorate as the load of *E. coli* increased. To identify whether this was specific to *E. coli*, other pathogens would need to be analysed, such as the previously suggested microbial analysis of *Enterococcus faecalis*.

Total scores from samples positive for symptoms using all potential definitions, except treatment, showed a significant difference. A lack of agreement was observed between when a patient would declare they have symptoms compared to when a HCP or the NHS would define a symptomatic UTI. This may go back to the previously discussed reliability of patients reporting symptoms accurately and misdiagnosing themselves based on, potentially, unrelated symptoms related to the ageing bladder.

When looking at the sample health of those that were being treated with antibiotics and those that were not there appeared to be no difference in the scores from these 2 groups. This could either suggest that treatment is indeed effective at improving sample health by bringing the scores down to the level of those not being treated or that treatment is not significantly impacting the sample health score at all. If it is the latter, this supports the observations within this study data collected that have shown that antibiotics may be ineffective at clearing pathogens from the bladder, preventing fluctuations in immune activation, preventing symptomatic episodes long-term and perhaps even improving sample health in general within these patients (Figure 58). This calls into questions the worthwhile effectiveness of antibiotic treatment in these patients with long-term colonisation of the bladder, as they seem to be

unreliable at benefitting the patient in the long-term. In addition, ineffective treatment will promote the spread of antibiotic resistance within the bacterial community [231] (Figure 58). Multi-drug resistance has become an enormous global health problem. This highlights the real need for new antibiotics or more effective means of treatment in order to properly clear the bladder of harmful pathogens and break the cycle. This also demonstrates the importance of responsible antibiotic usage, which was an underpinning argument for undertaking this study. Reduction of unsuccessful antibiotic treatment could help eliminate one of the arms of the proposed model of perpetual colonisation within these patients outlined in Figure 58. Where possible antibiotic stewardship should be adopted as a meant to prevent unnecessary spread of resistances within the population [3,18]. A possible solution would be the use of short high-dose courses of a combination of antibiotics together. This could provide an avenue for future treatment, as not only would it minimise antibiotic resistance formation due to long treatment courses, but it may be more efficient at killing the wide range of bacteria, likely with different resistances, that appear to reside within the bladders of these patients.

By segmenting the patients' sample scored into the different age brackets an unexpected observation was made. There appeared to be an increase in the total scores up to the ages of 75-79, after which they appeared to reduce. This observation could suggest that there is a possible genetic component responsible. Whereby some patients are better able to cope with the disturbances within their urinary tract. Genetic links to UTIs are being heavily researched and are a clear direction for future analysis in this project. Peripheral blood samples were collected from each of the study patients at baseline. From these genomic DNA has begun to be isolated. The aim will be to complete sequence analysis upon this in order to look for previously reported single nucleotide polymorphisms (SNPs) which are knowns to influence UTI susceptibility and prognosis. Several SNPs have been described in the literature which can cause an increased risk of infection susceptibility, for example SNPs in different TLR genes [130]. For example, the Leu392X SNP in the ligand-binding domain of *TLR5* has been shown to lead to an increase risk of recurrent UTIs [232]. The Asp299Gly SNP in the *TLR4* gene has been shown to improve the cytokine response to lipopolysaccharide (LPS) [233]. This polymorphism has been suggested to have a protective effect against recurrent cystitis [232]. Future work will be to identify which patients, if any, possess some of these reported SNPS. As these genetic changes could potentially help explain some of the bacterial and host conclusions drawn from this study.

This clinical study has shown the feasibility of running a highly demanding longitudinal study on elderly patients with ABU. Detailed analysis of the bacterial and host responses has not been able to identify reliable biomarkers which could predict periods of symptoms. However, the data has revealed a large number of trends and observations within this patient group, specifically bringing into question the efficacy of treatment in these patients. The data shown provides evidence of shortfalls in the current antibiotic treatments being given to these patients. Suggesting that better treatment options are strongly needed in order to minimise the development of antibiotic resistance. This project has revealed the huge variability between the bladders of these patients highlighting the difficulties there would be in long-term monitoring and patient-guided management based on urinary information. The decision to focus on *E. coli* was originally supported by the literature. However, the vast polymicrobial colonisation of these patients, suggests that multiple pathogens must be the focus of future analysis in order to accurately deduce trends and monitor changes within the bladder in this age group. Moving forward other pathogens will need to be analysed in detail to better understand the bladder environment, as well as host-DNA analysis to assess what role genetics may be playing in the observations seen here. This study has provided a fascinating and detailed insight into the dynamic nature of the bladder environment in older people over time. Allowing for some unique and often unexpected observations to be made, as well as opening up several interesting avenues of future research.

Chapter 10. Appendices

Appendix 1. Strains used for MLST sequence alignment. Taken from McNally *et al* (2013) [90].

Strain	Sequence Type	BAPS Cluster	Pathotype	Accession Number
E. coli CE10	62	8	K1 ExPEC	NC_017646
E. coli DH1 –ECDH1	1,060	5	K12	CP001637.1
E. coli ME8659	1,060	5	K12	AP012030.1
E. coli DH10B	1,060	5	K12	NC_010473.1
E. coli W3110	10	5	K12	AC000091
E. coli MG1655	10	5	K12	U00096.2
E. coli BW2952	10	5	K12	CP001396.1
E. coli P12b	10	5	K12	NC_017663.1
E. coli H10407	48	5	ETEC	FN649414
E. coli UMNK88	100	5	K88	CP002729.1
E. coli REL606	93	5	B	CP000819.1
E. coli BL21 DE3	93	5	B	CP001509.3
E. coli ATCC9637	1,079	3	W	CP002185.1
E. coli SE11	156	3	Human commensal	AP009240.1
E. coli E23477A	1,132	3	ETEC	CP000800.1
E. coli IAI1	1,128	3	O:8	CU928160.2
E. coli 55989	678	1	EAEC	CU928145.2
E. coli C227_11	678	1	O104	AFRH00000000
E. coli 12009	17	1	O103 EHEC	AP010958.1
E. coli 11128	16	3	O111 EHEC	AP010960.1
E. coli 11368	21	3	O26 EHEC	AP010953.1
E. coli ATCC8739	1,120	5	K12	CP000946.1
E. coli HS	46	7	Human commensal	CP000802.1
E. coli CB9615	335	9	O55 EHEC	CP001846.1
E. coli EDL933	11	9	O157 EHEC	AE005174.2
E. coli Sakai	11	9	O157 EHEC	BA000007.2
E. coli TW14359	11	9	O157 EHEC	CP001368.1
E. coli EC4115	11	9	O157 EHEC	NC_011353.1
E. coli XuZhou21	11	9	O157 EHEC	NC_017906.1
E. coli RM12579	335	9	O55 EHEC	NC_017656.1
E. coli O42	414	6	EAEC	FN554766.1
E. coli UMNO26	597	6	O:7 ExPEC	CU928163.2
E. coli SMS35	354	8	Multidrug resistant	CP000970.1
E. coli E2348/69	15	4	O127 EPEC	FM180568.1
E. coli UTI18	131a	4	ExPEC	ERP001095
E. coli EC958	131b	4	ExPEC	CAFL01000001
E. coli NA114	131c	4	ExPEC	CP002797.1
E. coli P2U	131d	4	ExPEC	ERX159100
E. coli P5U	131d	4	ExPEC	ERX159106

Appendix 1. continued [90].

Strain	Sequence Type	BAPS Cluster	Pathotype	Accession Number
E. coli P2B	131d	4	ExPEC	ERX159099
E. coli UTI24	131a	4	ExPEC	ERP001095
E. coli UTI32	131a	4	ExPEC	ERP001095
E. coli UTI62	131a	4	ExPEC	ERP001095
E. coli UTI188	131a	4	ExPEC	ERP001095
E. coli UTI226	131a	4	ExPEC	ERP001095
E. coli UTI306	131a	4	ExPEC	ERP001095
E. coli UTI423	131a	4	ExPEC	ERP001095
E. coli UTI587	131a	4	ExPEC	ERP001095
E. coli SE15	131a	4	Human commensal	AP009378.1
E. coli LF82	135	4	AIEC	NC_011993.1
E. coli IHE3034	95	4	ST95 ExPEC	CP001969.1
E. coli UTI89	95	4	ST95 ExPEC	CP000243.1
E. coli S88	95	4	O45 ExPEC	CU928161.2
E. coli APEC01	95	4	APEC	CP000468.1
E. coli UM146	643	4	AIEC	CP002167.1
E. coli 536	127	4	O6 ExPEC	CP000247.1
E. coli LF82	135	4	AIEC	CU651637.1
E. coli NRG857c	135	4	AIEC	CP001855.1
E. coli ED1a	452	4	O81	CU928162.2
ABU83972	73	4	Asymptomatic	CP001671
E. coli CFT073	73	4	ExPEC	AE014075.1
E. coli Di14	73	4	ExPEC	CP002212.1
E. coli Di12	73	4	ExPEC	CP002211.1

Appendix 2. Patient consent form used in the study

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

The Freeman Hospital
High Heaton
Newcastle upon Tyne
NE7 7DN

Tel: 0191 233 6161
Fax: 0191 213 1968

Biomarkers for Urinary Tract Infection Study Patient Consent Form

Please indicate your understanding of the research study and your consent to take part by initialling (NOT ticking) each of the boxes below.

	Please Initial:
1) I confirm that I have read and understand the Participant Information Booklet (v2.0, December 2013), for this study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	☐
2) I give permission for my blood and urine samples to be collected, stored and used in scientific research by Newcastle University and their collaborators.	☐
3) If any of the research findings provide other information which may be relevant to me personally or my relatives such as risk of other illnesses, **I would / would not (delete as appropriate)** like my GP to be contacted to investigate this further.	☐
4) I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. I understand that if I withdraw from the study, all identifiable samples will be destroyed, but data collected up to my withdrawal will still be used.	☐
5) I consent that my medical notes may be viewed by Trust staff or Regulatory Authorities to ensure appropriate conduct of this study	☐
6) I give permission for information (contact telephone number, address, date of birth, gender, medications, medical history of urinary infections and antibiotic treatment) provided by me or found in my medical and other health related records to be supplied to and stored by researchers, including electronically, in a way that keeps my identity anonymous. I understand that my anonymised samples and data may be shared on a collaborative basis with researchers.	☐

Appendix 2. continued

7) I confirm that I offer my fluid samples as an unconditional gift and do not wish to place any restriction on the research that will be carried out on them, beyond the limits stated in the information, which I have already read. ☐

8) I give permission for long-term storage and use of my blood samples for health-related research purposes and relinquish all rights to these samples, which I am donating to the research community. ☐

9) I agree to take part in the above study. ☐

Patient's signature: _____ Date:_____

Full name of patient (please print): _____

Patient ID number (for NHS staff to complete): _____

Signature of person taking consent: _____ Date: _____

Full name of person taking consent (please print): _____

Thank you for agreeing to take part in this research.

Appendix 3. Patient case report form (CRF) used in the study

Participant Study Number: - ☐☐☐☐☐☐	Participant initials: ☐☐☐

Biomarkers for UTI study

Participant Case Report Form

CONFIDENTIAL

This study is funded by the Biomedical Research Centre (BRC) as part of the National Institute for Healthcare Research (NIHR)

Completed by:

Name: **Signature:**

Date ☐☐ Day ☐☐ Month ☐☐☐☐ Year

INSTRUCTION FOR COMPLETION

Please place a [**X**] or insert [**requested information**] in appropriate box

If you make any errors while completing this form, please strikethrough through the incorrect data with a horizontal line and initial and date any changes.

Please contact your local recruitment co-ordinator or Central Trial Office if you have any uncertainty regarding completion

CONTACTS

A. PARTICIPANT

Address	**1st line:**	
	2nd line:	
	Town:	
	County:	
	Postcode:	☐☐☐☐☐☐☐

D.O.B [DD/MM/YYYY]	☐☐☐☐☐☐☐☐ D D M M Y Y Y Y

Appendix 3. continued

Participant Study Number: -	Participant initials:

Sex [X]	Female	Male

Preferred telephone number	
Alternative telephone number	

Email Address					
Preferred means of Contact: **1 [most] to 5 [least]**	**Post**	**Email**	**Telephone (Landline)**	**Telephone (Mobile)**	**Text**

B. GENERAL PRACTITIONER		
Surname and Initials		

Practice Name	

Address	**1st line:**	
	2nd line:	
	Town:	
	County:	
	Postcode:	

Postcode	
Telephone number	

Appendix 3. continued

Participant Study Number: - ☐☐☐☐☐☐	Participant initials: ☐☐☐

Section 1: PARTICIPANT HISTORY						
1.	Diabetic?	Yes ☐	No ☐			
2.	*Women only* Oestrogen use	Systemic ☐	Vaginal ☐	None ☐		
3.	Urinary catheter	Indwelling ☐	CISC ☐	None ☐		
4.	Most frequent type of UTI	☐ No symptoms (bacteriuria only) ☐ Mild – Smelly/cloudy urine, frequency and pain ☐ Moderate – Some systemic flu-like symptoms ☐ Severe – Fever/rigors and loin pain				
5.	Serum Creatinine level?	☐ µmol/L OR ☐ mg/dL Don't Know ☐				
6.	Used *antibiotics* in the past month?	Yes ☐	No ☐			
6a.	*If yes,* please state the name(s) and dose(s) if known:	NAME	DON'T KNOW	DOSE (such as 250 mg three times a day)	DON'T KNOW	
		_____ ☐		_____	☐	
		_____ ☐		_____	☐	
		_____ ☐		_____	☐	
		_____ ☐		_____	☐	

Appendix 3. continued

Participant Study Number: - ☐☐☐☐☐☐	Participant initials: ☐☐☐

Section 2: URINARY HISTORY			
1.	Number of episodes of urinary tract infection experienced by participant in last 12 months	[Enter number = 00 - 99) ☐☐	
2.	Number of positive urine culture reports in last 12 months (clinician report from records available)	[Enter number = 00 – 99] ☐☐	
3.	Urinary tract status	Normal ☐ Abnormal ☐	Don't Know ☐
3a.	*If abnormal*	Structural defect ☐ Details (e.g. poor bladder emptying) _____ _____ _____ _____ Functional defect ☐ Details (e.g. scarred kidney) _____ _____ _____ _____	Don't Know ☐ Don't Know ☐

Appendix 4. Patient questionnaire used in the study

Patient ID: _____

Donation Number: _____

Today's Date: _____

Urinary Tract Infection Biomarker Study Questionnaire

A. Changes in symptoms

Please indicate whether you have had the following symptoms/problems in the past 48 hours (2 days) and how severe they were;

Please circle one number for each symptom

1. Frequency of urination (going to the toilet very often)

Did not have	Mild	Moderate	Severe
0	1	2	3

2. Urgency of urine (a strong and uncontrollable urge to pass urine)

Did not have	Mild	Moderate	Severe
0	1	2	3

3. Pain or burning when passing urine.

Did not have	Mild	Moderate	Severe
0	1	2	3

4. Not being able to empty your bladder completely/passing only small amounts of urine

Did not have	Mild	Moderate	Severe
0	1	2	3

5. Low back pain

Did not have	Mild	Moderate	Severe
0	1	2	3

6. Visible blood in your urine

Did not have	Mild	Moderate	Severe
0	1	2	3

7. Do you feel you are suffering now from symptoms of a urinary tract infection? Yes / No *(please circle)*

8. Please tell us any other ways that your urinary symptoms have changed in the last 2 days *(optional)*

_____

_____

B. Changes in antibiotic medicines

1. Have you taken any antibiotics **for a urinary tract infection** since you last sent in a urine sample (that is in the last 2 weeks approximately)? Yes / No

   (please circle)

   ***If yes*;**

   What was the name of the antibiotic?

   _____ ☐ don't know

   What was the dose and number of times you had to take it each day (such as 250 mg three times a day)?

   _____ ☐ don't know

   What was the date you took this medication?

   Start date: _____

   End date: _____ ☐ on-going

2. Have you taken antibiotics **for any other infection** since your last urine sample(that is in the last 2 weeks approximately)? Yes / No

   (please circle)

   ***If yes*;**

   What was the name of the antibiotic?

   _____ ☐ don't know

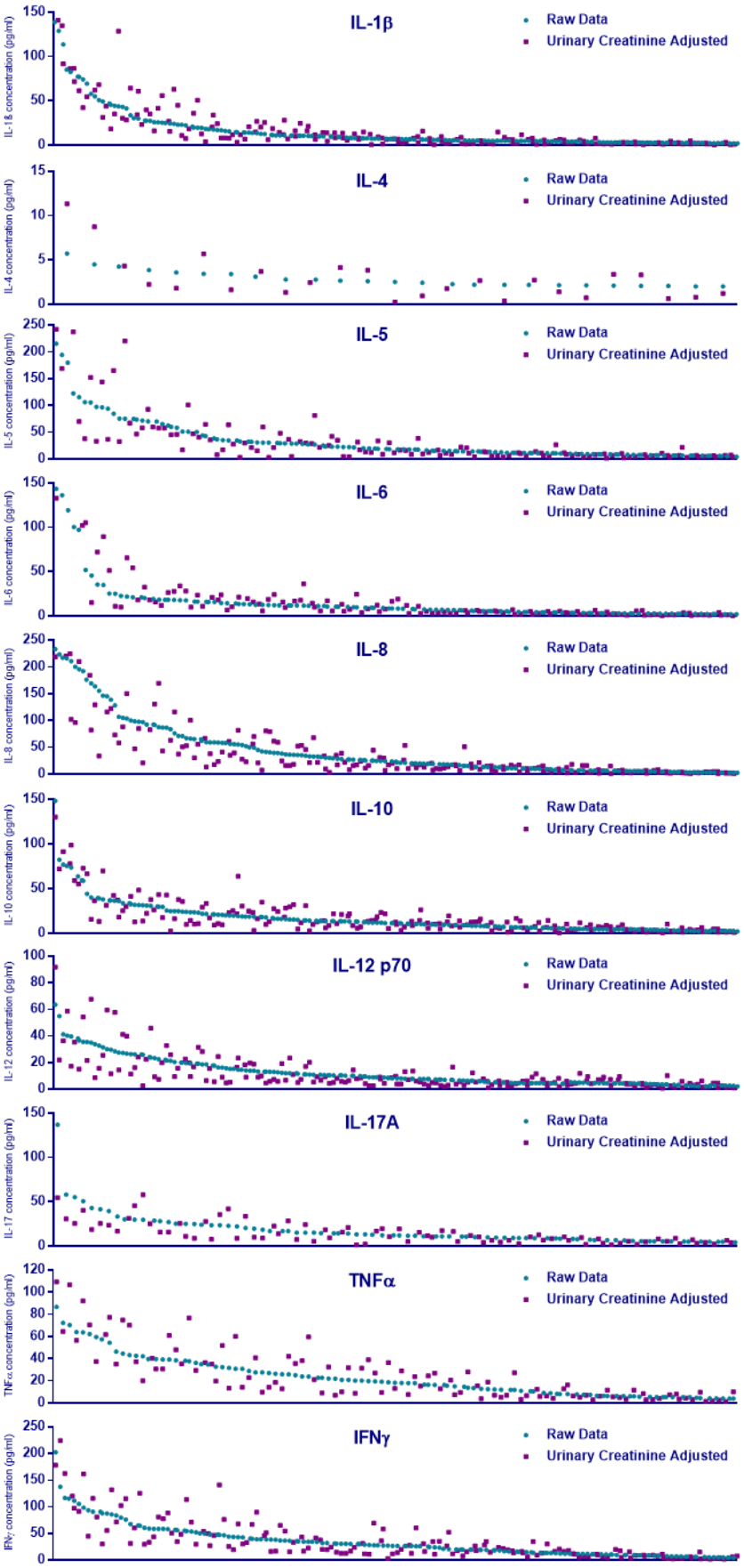
   What was the dose and number of times you had to take it each day (such as 250 mg three times a day)?

   _____ ☐ don't know

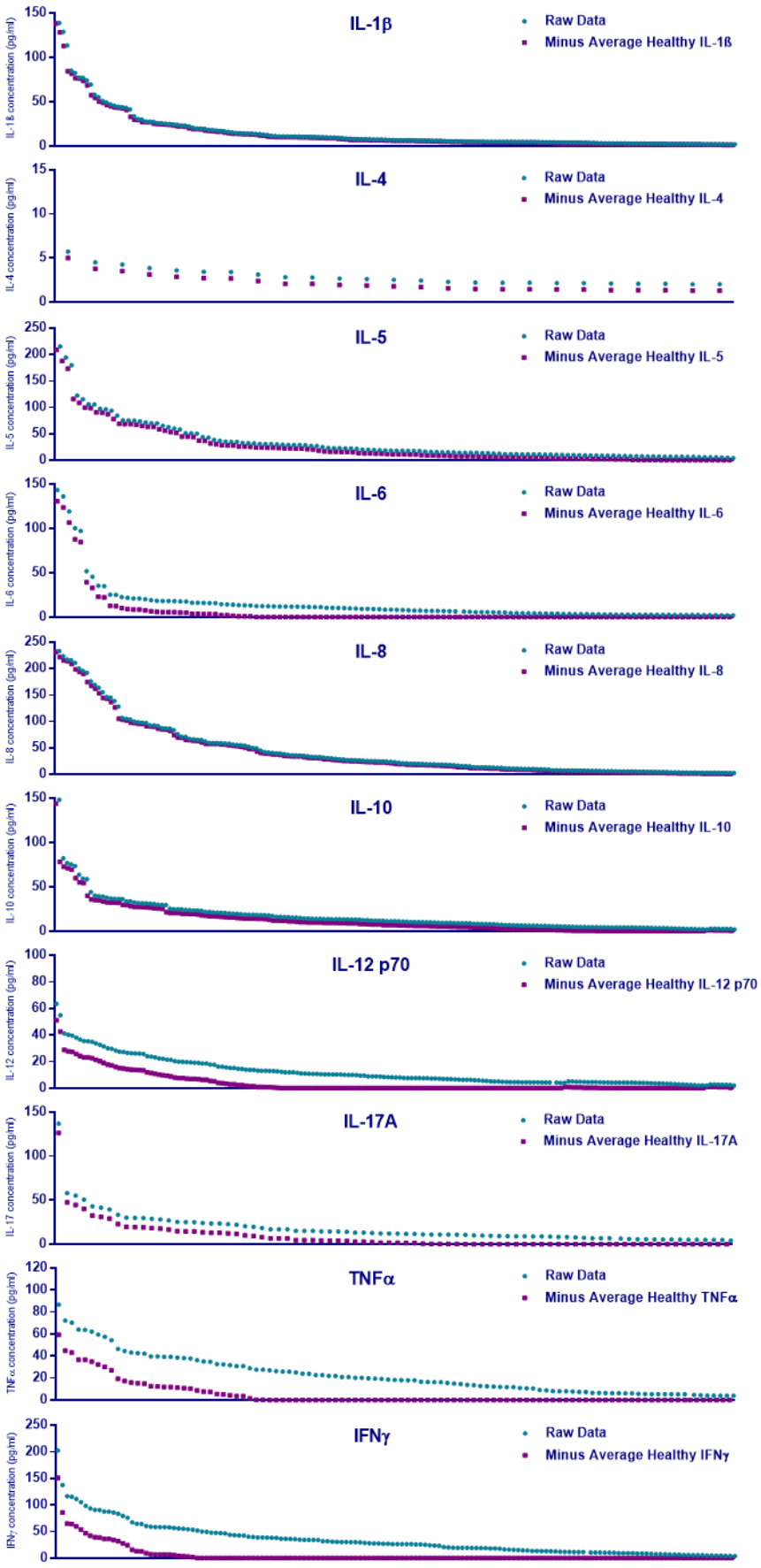
   What was the date you took this medication?

   Start date: _____

   End date: _____ ☐ on-going



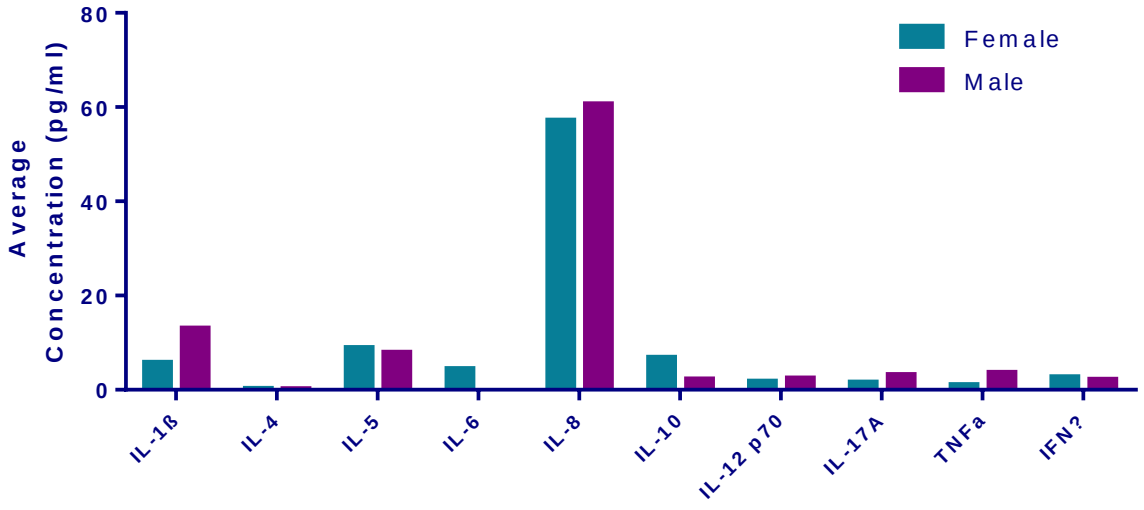
Appendix 5. All cytokine measurements which fell within the ELISA assay range shown as raw concentrations (green) and the corresponding values after being adjusted for urinary creatinine (purple). All values sorted from highest to lowest by the raw concentration data (green). Creatinine adjustments were done by multiplying the assayed protein concentration with the concentration of urinary creatinine measured.



Appendix 6. All cytokine measurements which fell within the ELISA assay range shown as raw concentrations (green) and the corresponding values after being adjusted to the healthy controls (purple). All values sorted from highest to lowest by the raw concentration data (green). Healthy averages were calculated by taking the mean of all concentrations of each individual protein within all healthy urine samples. This average was then subtracted from the raw values.

Figure	Comparison	P-value	N	Statistical Test
Figure 47	Patients vs Healthy controls	< 0.0001	405	Unpaired t test
Figure 48A	Male vs. Female	0.0012	360	Unpaired t test
Figure 48B	65-69 vs. 70-74	> 0.9999	360	One-way ANOVA
	65-69 vs. 75-79	< 0.0001		
	65-69 vs. 80-84	> 0.9999		
	65-69 vs. 85-89	> 0.9999		
	70-74 vs. 75-79	0.0004		
	70-74 vs. 80-84	> 0.9999		
	70-74 vs. 85-89	> 0.9999		
	75-79 vs. 80-84	0.0108		
	75-79 vs. 85-89	0.0226		
	80-84 vs. 85-89	> 0.9999		
Figure 48C	Non-diabetic vs. Diabetic	0.3662	360	Unpaired t test
Figure 48D	None vs. Mild	> 0.9999	360	One-way ANOVA
	None vs. Moderate	> 0.9999		
	None vs. Severe	> 0.9999		
	Mild vs. Moderate	> 0.9999		
	Mild vs. Severe	> 0.9999		
	Moderate vs. Severe	> 0.9999		
Figure 49A	No Self-declared UTI vs Self-declared UTI	< 0.0001	360	Unpaired t test
Figure 49B	Antibiotic treatment vs No antibiotic treatment	0.3045	360	Unpaired t test
Figure 49C	Positive urine sample vs No positive urine sample	0.0017	360	Unpaired t test
Figure 49D	Positive vs Negative HCP outcome	< 0.0001	360	Unpaired t test
Figure 50A	*E. coli* presence vs No *E. coli* presence	< 0.0001	360	Unpaired t test
Figure 50B	Diagnostic *E. coli* presence vs Non-Diagnostic *E. coli* presence	< 0.0001	360	Unpaired t test
Figure 50C	0 vs. $<10^5$ CFU/ml *E. coli*	0.0042	360	One-way ANOVA
	0 vs. $>10^5$ CFU/ml *E. coli*	< 0.0001		
	$<10^5$ vs. $>10^5$ CFU/ml *E. coli*	< 0.0001		
Figure 50D	Motile vs Non-Motile	0.0014	184	Unpaired t test

Appendix 7. Statistical analysis outcomes for total score analyses.



Appendix 8. Average cytokine concentrations split by females and males. No significant differences were seen between the sexes for any of the measured cytokines.

Patient Info	UTI number	UTI100											
	Age at recruitment	71											
	Sex	Male											
	Diabetic	Diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use												
	Estimated UTIs / Positive urines in 12 month	4 / 4											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	56											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	19											
	Abnormal Vitamin D												
	Sodum (mmol/L)	134											
	Abnormal Sodum												
	Potassium (mmol/L)	4.5											
	Abnormal Potassium												
	Urea (mmol/L)	5.3											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	3	2	0	0	2	0	0	1	0	2	0
	Urgency of urine	0	2	0	0	0	0	0	0	1	0	0	0
	Pain or burning upon urination	0	3	0	0	0	2	0	0	0	0	0	0
	Incomplete void/small volumes	0	2	0	1	0	2	0	0	0	0	0	0
	Low back pain	3	1	2	2	1	2	0	2	2	1	2	1
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	3	11	4	3	1	8	0	2	4	1	4	1
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior			N									
	Treated for UTI on day of sampling		N		N	N	N	N					
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	2000	0	0
	Bilrubin ('Mod' = Moderate)	Small	Small	-	Small	-	-	Small	-	Small	-	-	-
	Ketone (mg/dL)	0	5	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.020	1.030	1.010	1.030	1.015	1.015	1.025	1.030	1.030	1.015	1.020	1.015
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6	6	6	6	6.5	6	6	6	6	6	6.5
	Protein (mg/dL)	0	0	0	0	0	0	5	0	5	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	15	70	70	0	0	0	15	0	0	15	0	0
	HCP Outcome												
	Dip Stick Score	7	11	3	7	1	2	9	4	8	8	2	2
Protein Analysis	Urinary Creatinine (mg/dl)	259.14	208.82	40.16	230.49	242.38	130.10	300.45	167.51	186.12	70.70	145.60	154.09
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	1.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	66.00	5.85	13.79	0.00	6.41	275.76	10.42	0.00	1.28	0.00	5.08
	Healthy Adjusted IL-10 (pg/ml)	5.99	11.13	1.87	0.00	1.76	0.56	0.00	0.19	1.31	0.00	0.00	4.21
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	2.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	0	0	0	0	0	0	0	0
	Diagnostic levels of *E. coli*												
	MLST												
	E. coli clade												
	fliC Serotype												
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 9. Patient profile for UTI100. All study analysis combined. Green indicates a negative result and purple is a positive result. A blue filled box indicates it was not applicable, as the patient was male. N: Nitrofurantoin.

Patient Info	UTI number	UTI115											
	Age at recruitment	80											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	24 / 10											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	91											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	47											
	Abnormal Vitamin D												
	Sodum (mmol/L)	137											
	Abnormal Sodum												
	Potassium (mmol/L)	4.8											
	Abnormal Potassium												
	Urea (mmol/L)	6.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	3	0	0	0	0	0	0	0	0	0	0	0
	Urgency of urine	3	0	0	0	0	0	0	0	0	0	0	0
	Pain or burning upon urination	3	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	3	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	3	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	15	0	0	0	0	0	0	0	0	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	1	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling	C	C	C	C	C	C	T	C	C	C	C	C
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	Small	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	5	0	0	0
	Specific Gravity	1.010	1.005	1.010	1.015	1.010	1.005	1.010	1.010	1.010	1.020	1.005	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	10	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	200	0	0	0	0	10	0	0	0	0	0	0
	pH	6	6	6	6	6.5	6	6	6	5	6	6	6
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	15	0	0	0	125	70	500	0	0	125	0	0
	HCP Outcome												
	Dip Stick Score	6	1	0	1	5	8	6	0	1	6	1	0
Protein Analysis	Urinary Creatinine (mg/dl)	71.93	28.51	42.92	45.16	56.34	58.45	48.54	48.69	47.79	151.07	48.23	81.50
	Healthy Adjusted IL-1β (pg/ml)	0.00	1.34	1.33	0.91	0.75	3.59	13.37	1.23	1.27	8.29	4.04	0.01
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.18	3.12	2.05	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	7.52	6.23	0.00	0.00	9.67	11.00	0.00	1.29	0.35	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.34	9.12	1.20	0.14	14.91	80.42	1.97	0.00	14.59	1.37	0.69
	Healthy Adjusted IL-10 (pg/ml)	0.00	6.78	0.00	0.00	0.00	1.04	1.52	0.00	0.00	4.91	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.27	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	16.49	0.00	0.00	4.72	0.38	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	550	0	0	29500	>5*10^5	0	0	0	14300	100
	Diagnostic levels of *E. coli*												
	MLST			ST131			ST681	ST131				ST131	ST681
	E. coli clade			B2			B2	B2				B2	B2
	fliC Serotype			H5			H5	H5				H5	H5
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 10. Patient profile for UTI115. All study analysis combined. Green indicates a negative result and purple is a positive result. C: Cephalexin, T: Trimethoprim.

Patient Info	UTI number	UTI138											
	Age at recruitment	78											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	8 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	62											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	26											
	Abnormal Vitamin D												
	Sodum (mmol/L)	143											
	Abnormal Sodum												
	Potassium (mmol/L)	4.5											
	Abnormal Potassium												
	Urea (mmol/L)	7.1											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	2	1	2	1	3	2	2	2	2	2	2
	Urgency of urine	2	3	2	2	1	3	2	2	1	2	2	1
	Pain or burning upon urination	2	2	1	1	1	3	2	2	2	2	1	0
	Incomplete void/small volumes	1	3	1	0	0	3	2	2	2	2	1	2
	Low back pain	2	2	1	1	1	2	2	2	1	2	2	2
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	9	12	6	6	4	14	10	10	8	10	8	7
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	1	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	Small	Small	Small	-
	Ketone (mg/dL)	0	15	5	80	5	0	0	0	0	5	5	0
	Specific Gravity	1.020	1.020	1.025	1.025	1.025	1.020	1.020	1.020	1.010	1.020	1.020	1.015
	Non-Haemolysed Blood (Ery/uL)	10	10	10	0	0	0	0	0	10	0	0	10
	Haemolysed Blood (Ery/uL)	0	0	0	10	10	25	25	25	0	25	10	0
	pH	6	6	6	6	6	6	6	6	6.5	6	6	6
	Protein (mg/dL)	5	5	5	30	5	5	30	30	5	5	5	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	+	+	+	+	+	+	-	+	+	+	+	+
	Leukocytes (Leu/ul)	0	15	70	15	0	15	15	15	70	15	15	0
	HCP Outcome												
	Dip Stick Score	9	13	14	17	11	12	8	13	14	16	15	7
Protein Analysis	Urinary Creatinine (mg/dl)	87.34	152.57	132.93	138.65	148.14	115.45	132.74	147.65	110.10	114.78	159.94	118.17
	Healthy Adjusted IL-1β (pg/ml)	3.10	9.90	10.25	3.97	1.45	5.55	4.07	3.20	6.58	9.49	11.95	4.79
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.22	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	16.23	0.00	0.00	0.00	3.43	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	3.20	0.00	0.00	3.19	4.94	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	114.17	617.26	37.29	0.00	3.12	2.11	0.00	0.00	0.00	19.63	0.00
	Healthy Adjusted IL-10 (pg/ml)	25.78	27.41	17.68	4.83	19.85	32.42	14.48	0.00	7.04	15.86	4.13	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	6.89	23.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	1.68	19.37	6.48	0.00	3.64	13.57	2.24	0.00	3.94	0.00	4.72	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	6.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST	ST131	ST131	ST131	ST677	ST677	ST131	ST677	ST677	ST677	ST677	ST677	ST677
	E. coli clade	B2	B2	B2	B1	B1	B2	B1	B1	B1	B1	B1	B1
	fliC Serotype	H5	H12	H5	H21	H21	H5	H21	H21	H21	H21	H21	H21
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 11. Patient profile for UTI138. All study analysis combined. Green indicates a negative result and purple is a positive result.

Patient Info	UTI number	UTI139											
	Age at recruitment	74											
	Sex	Female											
	Diabetic	Diabetic											
	Urinary Tract Status	Abnormal: Vaginal prolapse											
	Most Frequent UTI	Mild											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	7 / 5											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	62											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	47											
	Abnormal Vitamin D												
	Sodum (mmol/L)	143											
	Abnormal Sodum												
	Potassium (mmol/L)	4.8											
	Abnormal Potassium												
	Urea (mmol/L)	5.2											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	1	0	0	0	0	2	0	0	0	2	0	0
	Urgency of urine	0	0	1	0	0	2	0	0	1	0	0	0
	Pain or burning upon urination	0	1	0	0	0	3	0	0	0	1	0	0
	Incomplete void/small volumes	0	0	0	0	1	1	1	2	1	1	0	0
	Low back pain	1	0	0	0	0	0	1	0	0	0	0	1
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	2	1	1	0	1	8	2	2	2	4	0	1
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	1	0	0	0	0	0	0
	Treated for UTI within 3 days prior					C							
	Treated for UTI on day of sampling								A				
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	100
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.010	1.010	1.005	1.010	1.010	1.010	1.010	1.010	1.005	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	10	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	25	0	0	0	0	0	0
	pH	6	6	6.5	6.5	6	7	6	6.5	6.5	7	6	6.5
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	15	70	70	0	0	500	125	70	70	125	0	0
	HCP Outcome												
	Dip Stick Score	2	3	4	2	2	9	4	4	4	7	1	2
Protein Analysis	Urinary Creatinine (mg/dl)	63.93	47.26	46.45	55.41	46.09	42.18	46.52	61.60	222.35	47.51	55.97	37.95
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.26	0.00	0.00	0.00	7.96	0.00	0.00	0.00	0.59	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.28	0.00	0.00	7.52	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	100	0	0	2000	200	0	0	0
	Diagnostic levels of *E. coli*												
	MLST					ST131			ST131	ST131			
	E. coli clade					B2			B2	B2			
	fliC Serotype					H4			H4	H4			
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 12. Patient profile for UTI139. All study analysis combined. Green indicates a negative result and purple is a positive result. C: Cephalexin, A: Amoxycillin.

Patient Info	UTI number	UTI218											
	Age at recruitment	69											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	7 / 8											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	78											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	27											
	Abnormal Vitamin D												
	Sodum (mmol/L)	136											
	Abnormal Sodum												
	Potassium (mmol/L)	5.1											
	Abnormal Potassium												
	Urea (mmol/L)	5.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	2	0	0	1	0	0	0	0	0	0	0
	Urgency of urine	0	2	0	0	1	0	0	0	0	0	0	0
	Pain or burning upon urination	0	2	0	0	1	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	0	6	0	0	3	0	0	0	0	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	0	0	1	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior		T										
	Treated for UTI on day of sampling	T											
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	Small	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.005	1.010	1.020	1.005	1.005	1.010	1.010	1.010	1.010	1.005	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	10	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	10	10	10	10	25	0	10	10	10	10	0	10
	pH	6	7	6.5	6	7	6.5	6.5	7	6	6	6.5	6
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	0	70	0	0	500	125	0	0	0	0	0	0
	HCP Outcome												
	Dip Stick Score	1	7	2	3	10	7	2	6	1	1	2	1
Protein Analysis	Urinary Creatinine (mg/dl)	43.36	34.57	120.45	96.63	63.05	28.23	76.94	103.14	36.45	64.90	55.79	48.36
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.00	10.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	27.22	6.25	8.97	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.78	0.00
	Healthy Adjusted IL-17A (pg/ml)	32.48	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	400	0	0	21900	0	0	0	0	0	0	0
	Diagnostic levels of *E. coli*												
	MLST		ST73			ST73							
	E. coli clade		B2			B2							
	fliC Serotype		H1			H1							
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 13. Patient profile for UTI218. All study analysis combined. Green indicates a negative result and purple is a positive result. T: Trimethoprim.

Patient Info	UTI number	UTI236											
	Age at recruitment	70											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	2 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	70											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	112											
	Abnormal Vitamin D												
	Sodum (mmol/L)	137											
	Abnormal Sodum												
	Potassium (mmol/L)	4.4											
	Abnormal Potassium												
	Urea (mmol/L)	6.8											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	1	1	0	0	0	0	0	0	0	0	0
	Urgency of urine	1	0	0	0	0	0	0	0	0	0	0	0
	Pain or burning upon urination	1	1	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	4	2	1	0	0	0	0	0	0	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	1	1	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior			P									
	Treated for UTI on day of sampling	N											
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.005	1.005	1.010	1.005	1.015	1.010	1.010	1.010	1.010	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	10	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	10	10	0	10	0	25	10	10	0	0	0	0
	pH	7.5	6	6.5	6	7	6.5	7	7	7	6	7	6.5
	Protein (mg/dL)	0	0	0	0	0	0	5	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	+	+	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	125	500	125	0	125	125	125	500	125	125	500	0
	HCP Outcome												
	Dip Stick Score	8	12	12	1	7	8	8	8	6	4	7	1
Protein Analysis	Urinary Creatinine (mg/dl)	84.26	68.94	46.56	46.90	52.10	107.54	309.85	86.71	52.57	54.01	93.92	57.39
	Healthy Adjusted IL-1β (pg/ml)	26.99	40.61	0.77	0.00	4.27	24.31	4.11	6.96	10.80	3.00	7.80	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	8.43	0.00	0.00	2.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	3.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	86.48	170.74	19.08	0.00	0.00	208.31	1018.41	82.87	0.00	8.14	217.02	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	100	0	0	0	0	0	0	0	0
	Diagnostic levels of *E. coli*												
	MLST				ST421								
	E. coli clade				B2								
	fliC Serotype				H7								
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 14. Patient profile for UTI236. All study analysis combined. Green indicates a negative result and purple is a positive result. N: Nitrofurantoin, P: Pivmecillinam hydrochloride.

Patient Info	UTI number	UTI266											
	Age at recruitment	71											
	Sex	Male											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Enlarged prostate, trabeculated bladder, diverticulum											
	Most Frequent UTI	Severe											
	Oestrogen use												
	Estimated UTIs / Positive urines in 12 month	2 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	84											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	36											
	Abnormal Vitamin D												
	Sodum (mmol/L)	139											
	Abnormal Sodum												
	Potassium (mmol/L)	5.3											
	Abnormal Potassium												
	Urea (mmol/L)	5.4											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	0	1	1	1	1	1	1	1	1	1	1
	Urgency of urine	2	0	1	1	0	0	1	0	1	0	1	1
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	1	0	1	1	1	1	1	1	1	1	1	0
	Low back pain	1	1	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	4	1	3	3	2	2	3	2	3	2	3	2
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	1	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior	T											
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	Small	-	-	Small	-	-	Small	Small	Small	Small
	Ketone (mg/dL)	5	0	5	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.015	1.020	1.030	1.025	1.015	1.020	1.020	1.010	1.020	1.020	1.025	1.030
	Non-Haemolysed Blood (Ery/uL)	0	0	10	10	10	0	0	0	10	0	0	0
	Haemolysed Blood (Ery/uL)	25	10	0	0	0	10	10	10	0	10	25	25
	pH	6.5	6	6	6	6	6.5	6	7	6	6.5	6	6
	Protein (mg/dL)	5	0	30	5	5	5	5	0	5	30	30	30
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	+	+	-	-	-	+	-	-	-	-	-
	Leukocytes (Leu/ul)	500	125	500	70	70	125	70	500	70	125	125	125
	HCP Outcome												
	Dip Stick Score	11	12	21	8	6	12	12	8	10	13	14	15
Protein Analysis	Urinary Creatinine (mg/dl)	132.25	193.97	192.29	154.32	127.75	197.83	155.85	66.09	195.65	144.33	203.20	221.03
	Healthy Adjusted IL-1β (pg/ml)	4.06	0.00	16.94	0.00	26.76	12.84	3.39	3.23	6.93	26.86	29.26	24.63
	Healthy Adjusted IL-4 (pg/ml)	0.00	3.77	0.56	0.00	0.00	4.99	1.93	0.00	0.00	0.78	0.22	0.00
	Healthy Adjusted IL-5 (pg/ml)	63.44	115.71	0.00	7.64	4.40	44.39	0.00	0.00	77.86	98.77	173.04	10.85
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	123.51	5.52	0.00	0.00	1.03	0.00	0.00	5.64	39.19	292.18
	Healthy Adjusted IL-8 (pg/ml)	5.15	167.79	724.60	59.23	11.89	287.29	346.33	7.02	77.51	275.09	1164.10	737.29
	Healthy Adjusted IL-10 (pg/ml)	0.00	11.13	13.95	17.41	6.26	7.55	7.67	6.54	0.00	9.13	2.45	0.10
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	22.57	11.44	0.00	0.00	17.68	0.00	0.00	0.00	51.15	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.51	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	0	0	0	0	0	0	0	0
	Diagnostic levels of *E. coli*												
	MLST												
	E. coli clade												
	fliC Serotype												
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 15. Patient profile for UTI266. All study analysis combined. Green indicates a negative result and purple is a positive result. A blue filled box indicates it was not applicable, as the patient was male. T: Trimethoprim.

Patient Info	UTI number	UTI337											
	Age at recruitment	75											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Vaginal prolapse, pessary in situ											
	Most Frequent UTI	Mild											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	8 / 8											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	117											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	18											
	Abnormal Vitamin D												
	Sodum (mmol/L)	146											
	Abnormal Sodum												
	Potassium (mmol/L)	3.6											
	Abnormal Potassium												
	Urea (mmol/L)	8.3											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	2	1	0	2	0	2	2	1	1	3	1
	Urgency of urine	1	2	1	0	2	0	2	0	1	1	2	1
	Pain or burning upon urination	0	2	0	0	2	0	1	0	0	0	2	0
	Incomplete void/small volumes	2	1	2	1	2	1	1	1	2	2	2	1
	Low back pain	1	1	1	1	2	0	1	1	1	0	2	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	6	8	5	2	10	1	7	4	5	4	11	3
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	0	0	1	0	0	0	0	1	1	0
	Treated for UTI within 3 days prior							C					
	Treated for UTI on day of sampling	T	C + T	C			C					C	C
	Treated for UTI within 3 days after				C								
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	Small	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.015	1.015	1.015	1.015	1.015	1.015	1.010	1.010	1.010	1.010	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	10	0	0	25	0	0	0	0	0	25	0
	pH	6	6	6	6	6.5	6	7	6.5	6	6	7	6.5
	Protein (mg/dL)	0	5	0	0	5	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	+	-	-	+	-	-	-	-	+	+	-
	Leukocytes (Leu/ul)	500	125	125	70	500	125	0	125	15	500	500	70
	HCP Outcome												
	Dip Stick Score	6	12	5	4	18	5	2	5	2	10	14	4
Protein Analysis	Urinary Creatinine (mg/dl)	104.34	92.74	58.91	57.34	134.01	88.16	71.66	105.33	78.31	65.49	101.57	72.13
	Healthy Adjusted IL-1β (pg/ml)	7.11	76.50	1.96	0.00	50.11	8.78	0.00	0.00	0.00	10.16	137.98	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.97	0.00	0.00	1.42	3.52	0.02
	Healthy Adjusted IL-5 (pg/ml)	0.00	25.73	0.00	0.00	0.00	58.65	23.49	2.10	0.49	0.83	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	130.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.74	209.85	0.00
	Healthy Adjusted IL-8 (pg/ml)	58.60	3664.05	13.92	0.00	43.90	120.33	48.83	31.56	0.00	369.03	2381.17	15.14
	Healthy Adjusted IL-10 (pg/ml)	5.69	9.66	25.27	0.00	69.27	14.07	26.83	8.29	0.00	8.97	32.29	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	28.90	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.73	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	36.78	0.00	0.00	12.02	0.00	12.45	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	7100	12000	200	>5*10^5	1000	1040	10300	6850	>5*10^5	1700	300
	Diagnostic levels of *E. coli*												
	MLST	ST73	ST73	ST73	ST73	ST73	ST59	ST73	ST73	ST73	ST73	ST73	ST73
	E. coli clade	B2	B2	B2	B2	B2	F	B2	B2	B2	B2	B2	B2
	fliC Serotype	H1	H1	H1	H1	H1	H7	H1	H1	H1	H1	H1	H1
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 16. Patient profile for UT337. All study analysis combined. Green indicates a negative result and purple is a positive result. T: Trimethoprim, C: Cephalexin.

Patient Info	UTI number	UTI343											
	Age at recruitment	77											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	12 / 7											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	65											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	19											
	Abnormal Vitamin D												
	Sodum (mmol/L)	139											
	Abnormal Sodum												
	Potassium (mmol/L)	3.9											
	Abnormal Potassium												
	Urea (mmol/L)	4.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	0	1	1	0	0	1	1	1	2	0	1
	Urgency of urine	3	1	1	1	1	1	0	1	0	1	0	0
	Pain or burning upon urination	2	0	0	1	0	0	0	0	0	2	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	2	1	3	2	2	1	1	1	2	2	1
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	7	3	3	6	3	3	2	3	2	7	2	2
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	1	0	0	0	0	0	1	0	0
	Treated for UTI within 3 days prior					C				T		C	
	Treated for UTI on day of sampling												C
	Treated for UTI within 3 days after										C		
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.005	1.010	1.010	1.005	1.010	1.010	1.010	1.005	1.010	1.010	1.005	1.005
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	25	0	10	10	10	10	10	10	25	25	10	10
	pH	7	7	7.5	7.5	7	7	7	7	7	6.5	7.5	7.5
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	+	+	+	-	-	+	+	+	-	-	-
	Leukocytes (Leu/ul)	125	70	125	70	0	0	500	125	70	125	0	0
	HCP Outcome												
	Dip Stick Score	9	10	13	13	3	3	13	13	12	7	5	5
Protein Analysis	Urinary Creatinine (mg/dl)	37.76	48.64	57.17	10.70	11.25	131.61	62.58	48.96	104.93	92.98	94.91	39.82
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	76000	>5*10^5	>5*10^5	>5*10^5	300	0	>5*10^5	>5*10^5	>5*10^5	>5*10^5	56000	0
	Diagnostic levels of *E. coli*												
	MLST	ST144	ST144	ST144	ST144	ST144		ST12	ST12	ST144	ST144	ST69	
	E. coli clade	B2	B2	B2	B2	B2		B2	B2	B2	B2	D	
	fliC Serotype	H6	H6	H6	H6	H6		H5	H5	H5	H6	H45	
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 17. Patient profile for UTI343. All study analysis combined. Green indicates a negative result and purple is a positive result. C: Cephalexin, T: Trimethoprim

Patient Info	UTI number	UTI365											
	Age at recruitment	68											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	16 / 9											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	63											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	27											
	Abnormal Vitamin D												
	Sodum (mmol/L)	138											
	Abnormal Sodum												
	Potassium (mmol/L)	4.2											
	Abnormal Potassium												
	Urea (mmol/L)	6.2											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	1	1	1	0	0	0	0	0	0	0	0
	Urgency of urine	0	0	0	0	0	0	0	0	0	0	0	0
	Pain or burning upon urination	0	0	1	0	0	0	1	0	0	0	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	0	1	2	1	0	0	1	0	0	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	1	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling	T	T	T	T	T	T						
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.005	1.010	1.010	1.010	1.005	1.005	1.005	1.005	1.010	1.005	1.005
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6.5	6	7	6.5	7.5	7.5	6.5	7	7	7.5	7
	Protein (mg/dL)	5	30	5	5	5	0	0	0	0	5	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	+	-	-	+	+	+	+	+	+	-	+
	Leukocytes (Leu/ul)	125	70	70	15	70	70	15	0	0	70	125	0
	HCP Outcome												
	Dip Stick Score	5	12	4	5	10	12	11	7	8	11	8	8
Protein Analysis	Urinary Creatinine (mg/dl)	77.74	56.96	71.38	106.36	121.54	100.03	64.18	92.33	79.37	79.98	67.79	61.21
	Healthy Adjusted IL-1β (pg/ml)	1.31	4.40	1.88	0.00	0.00	4.29	0.00	0.00	0.20	3.23	2.42	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	2.53	37.90	4.21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	>5*10^5	0	182000	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	0	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST		ST335		ST404	ST404	ST404	ST404	ST404	ST404	ST404		ST404
	E. coli clade		E		B2	B2	B2	B2	B2	B2	B2		B2
	fliC Serotype		H7		H5	H5	H5	H5	H37	H5	H5		H5
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 18. Patient profile for UTI365. All study analysis combined. Green indicates a negative result and purple is a positive result. T: Trimethoprim.

Patient Info	UTI number	UTI376											
	Age at recruitment	78											
	Sex	Male											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use												
	Estimated UTIs / Positive urines in 12 month	4 / 4											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	91											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	50											
	Abnormal Vitamin D												
	Sodum (mmol/L)	141											
	Abnormal Sodum												
	Potassium (mmol/L)	4.8											
	Abnormal Potassium												
	Urea (mmol/L)	6.2											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	1	0	1	1	1	1	0	0	0	0	0
	Urgency of urine	1	1	1	1	0	0	0	0	0	0	0	0
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	1	2	1	2	1	1	1	0	0	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.015	1.010	1.005	1.010	1.010	1.010	1.010	1.010	1.005	1.010	1.005	1.015
	Non-Haemolysed Blood (Ery/uL)	0	10	10	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	10	0	0	10	25	80	0	0	0	0	10	0
	pH	7.5	7.5	8.5	6	6.5	7	7.5	7.5	7	6.5	8.5	6.5
	Protein (mg/dL)	0	0	5	0	5	0	0	0	0	0	5	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	+	+	+	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	500	500	500	500	500	500	500	500	500	500	500	125
	HCP Outcome												
	Dip Stick Score	15	14	18	6	9	10	8	8	8	6	13	6
Protein Analysis	Urinary Creatinine (mg/dl)	79.11	149.21	112.50	101.29	112.13	63.68	79.85	93.26	63.14	70.28	78.84	104.57
	Healthy Adjusted IL-1β (pg/ml)	43.68	0.00	54.30	84.35	29.74	48.63	76.13	46.23	23.79	42.50	68.66	128.14
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	51.49	89.92	208.62	0.00	22.09	11.36	27.87	6.70	0.00	3.23	8.97	0.78
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	51.22	80.15	555.99	218.05	54.11	14.22	114.07	554.36	14.96	39.16	127.31	339.09
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	5.57	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	14.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	0	0	0	0	2550	0	0	200
	Diagnostic levels of *E. coli*												
	MLST									ST362			ST362
	E. coli clade									D			D
	fliC Serotype									H9			H9
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 19. Patient profile for UTI376. All study analysis combined. Green indicates a negative result and purple is a positive result. A blue filled box indicates it was not applicable, as the patient was male.

Patient Info	UTI number	UTI383											
	Age at recruitment	78											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Narrow urethral meatus, calcified uterine fibroid											
	Most Frequent UTI	Moderate											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	2 / 11											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	105											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	17											
	Abnormal Vitamin D												
	Sodum (mmol/L)	142											
	Abnormal Sodum												
	Potassium (mmol/L)	4.5											
	Abnormal Potassium												
	Urea (mmol/L)	5.1											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	1	1	2	1	1	2	2	1	1	1	1
	Urgency of urine	1	1	1	1	1	1	1	1	1	2	1	0
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	2	1	1	0	1	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	1	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	5	3	3	3	3	3	3	3	2	3	2	1
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	1	1	1	1	0	1	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling							MH	MH	MH	MH	MH	MH
	Treated for UTI within 3 days after						MH						
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.015	1.010	1.010	1.010	1.010	1.010	1.005	1.010	1.010	1.005	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	10	0	10	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	10	0	0	0	0	0	0	0	0
	pH	6	6	6	7	6	6.5	6.5	6	6	6	6	6.5
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	+	+	+	+	-	-	-	-	-
	Leukocytes (Leu/ul)	125	125	125	500	125	125	125	70	15	0	0	125
	HCP Outcome												
	Dip Stick Score	5	4	4	13	10	10	12	3	2	1	0	5
Protein Analysis	Urinary Creatinine (mg/dl)	101.90	54.43	57.05	80.64	64.24	65.04	63.91	41.75	42.11	66.86	68.79	31.44
	Healthy Adjusted IL-1β (pg/ml)	6.09	5.44	1.53	112.87	2.02	5.98	9.47	0.00	1.10	0.00	0.48	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.19	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.01	4.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	15.60	56.27	234.65	334.92	19.27	23.03	35.97	4.72	0.00	0.00	0.00	7.83
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	71000	23000	600	0	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST	ST12	ST12	ST12	ST12	ST12	ST12	ST12	ST12	ST12	ST12		ST12
	E. coli clade	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2		B2
	fliC Serotype	H5	H5	H5	H5	H5	H5	H5	H5	H5	H5		H5
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 20. Patient profile for UTI383. All study analysis combined. Green indicates a negative result and purple is a positive result. MH: Methenamine hippurate.

Patient Info	UTI number	UTI414											
	Age at recruitment	66											
	Sex	Female											
	Diabetic	Diabetic											
	Urinary Tract Status	Abnormal: Urethral Stenosis											
	Most Frequent UTI	Mild											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	12 / 9											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	72											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	30											
	Abnormal Vitamin D												
	Sodum (mmol/L)	130											
	Abnormal Sodum												
	Potassium (mmol/L)	4.2											
	Abnormal Potassium												
	Urea (mmol/L)	3.4											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	2	2	2	2	2	0	0	1	0	0	0
	Urgency of urine	0	2	2	3	2	2	1	0	0	0	0	0
	Pain or burning upon urination	0	2	2	2	1	1	0	0	0	0	0	0
	Incomplete void/small volumes	0	2	0	2	0	1	0	0	0	0	0	0
	Low back pain	0	2	1	2	1	1	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	2	10	7	11	6	7	1	0	1	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	1	1	0	1	0	0	0	0	0	0
	Treated for UTI within 3 days prior						C						
	Treated for UTI on day of sampling				C	C			C	C	C	C	C
	Treated for UTI within 3 days after		C										
Dipstick Analysis	Glucose (mg/dL)	250	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	Small	-	-	-	Small	-	-	-	-	-	-	-
	Ketone (mg/dL)	5	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.015	1.015	1.015	1.020	1.020	1.020	1.015	1.010	1.015	1.010	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6.5	6	6	6	6	6	6	6	6	6	6	6
	Protein (mg/dL)	0	0	0	5	5	5	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	15	0	0	0	15	15	0	0	0	70	0	0
	HCP Outcome												
	Dip Stick Score	10	1	1	3	8	5	1	0	1	3	0	0
Protein Analysis	Urinary Creatinine (mg/dl)	103.89	118.70	124.14	138.29	119.69	103.27	127.21	58.05	74.15	89.14	48.60	56.35
	Healthy Adjusted IL-1β (pg/ml)	1.89	3.58	0.58	0.00	0.00	1.58	0.00	0.00	0.00	1.39	2.34	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	33.05	0.00	0.00	1.71	0.00	0.00	0.00	0.00	18.57	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	72.72	54.47	0.00	2.03	71.03	28.27	0.00	29.56	29.45	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	19.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	6.90	0.00	0.00	39.34	0.00	6.93	5.26	9.05	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	2800	0	0	0	1000	0	300	100	0	950	0	0
	Diagnostic levels of *E. coli*												
	MLST	ST91				ST91		ST91	ST91		ST91		
	E. coli clade	B2				B2		B2	B2		B2		
	fliC Serotype	H4				H4		H4	H4		H4		
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 21. Patient profile for UTI414. All study analysis combined. Green indicates a negative result and purple is a positive result. C: Cephalexin.

Patient Info	UTI number	UTI468											
	Age at recruitment	78											
	Sex	Male											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Phimosis											
	Most Frequent UTI	Mild											
	Oestrogen use												
	Estimated UTIs / Positive urines in 12 month	5 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	88											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	42											
	Abnormal Vitamin D												
	Sodum (mmol/L)	140											
	Abnormal Sodum												
	Potassium (mmol/L)	4.5											
	Abnormal Potassium												
	Urea (mmol/L)	5.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	3	2	2	2	0	1	2	3	1	2	0	2
	Urgency of urine	3	2	2	2	0	2	2	2	1	2	1	1
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	1	1	2	1	0	1	1	2	1	1	1	1
	Low back pain	0	0	2	0	2	0	0	0	0	1	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	7	5	8	5	2	4	5	7	3	6	2	4
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling	N	N										
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	Small	Small	-	-	-	-
	Ketone (mg/dL)	5	0	0	5	0	0	0	5	0	0	0	5
	Specific Gravity	1.020	1.010	1.010	1.015	1.020	1.015	1.020	1.010	1.015	1.015	1.020	1.020
	Non-Haemolysed Blood (Ery/uL)	25	0	10	10	10	10	10	10	0	0	10	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	10	0	10
	pH	6	6.5	7	6	6.5	6	6.5	7	6	5	6	6.5
	Protein (mg/dL)	30	5	5	5	5	5	5	0	0	0	0	5
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	+	+	+	-	+	-	-	-	+	-	-	+
	Leukocytes (Leu/ul)	125	500	125	125	125	125	125	125	70	125	125	125
	HCP Outcome												
	Dip Stick Score	16	12	13	8	14	7	12	11	9	7	7	15
Protein Analysis	Urinary Creatinine (mg/dl)	195.39	89.35	103.85	77.42	145.96	126.00	151.90	142.90	168.28	113.31	142.30	162.66
	Healthy Adjusted IL-1β (pg/ml)	22.32	20.07	6.18	5.19	0.96	6.64	5.77	16.30	3.97	17.19	9.14	24.87
	Healthy Adjusted IL-4 (pg/ml)	0.41	0.06	0.00	1.54	1.88	1.45	0.00	0.04	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	22.48	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.90	0.00	0.00	0.00	0.00	0.00	0.00	2.06
	Healthy Adjusted IL-8 (pg/ml)	0.75	564.90	222.44	7.02	0.71	29.04	98.49	128.62	0.00	12.15	68.16	816.58
	Healthy Adjusted IL-10 (pg/ml)	3.98	0.07	3.92	0.00	25.51	14.38	3.86	1.58	12.05	11.83	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	9.81	0.00	0.00	27.80	2.21	9.21	0.00	0.00	7.57	1.49	0.00
	Healthy Adjusted IL-17A (pg/ml)	19.14	0.00	0.00	0.00	0.00	4.17	0.00	0.00	0.00	0.13	0.00	1.44
	Healthy Adjusted TNFα (pg/ml)	0.00	45.00	7.79	12.37	0.00	59.35	42.99	0.00	17.25	34.92	27.08	15.89
	Healthy Adjusted IFNγ (pg/ml)	12.78	38.94	63.88	0.00	0.00	0.00	24.44	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73
	E. coli clade	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2
	fliC Serotype	H1	H1	H1	H1	H1	H1	H1	H1	H1	H1	H1	H1
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 22. Patient profile for UTI468. All study analysis combined. Green indicates a negative result and purple is a positive result. A blue filled box indicates it was not applicable, as the patient was male. N: Nitrofurantoin.

Patient Info	UTI number	UTI524											
	Age at recruitment	67											
	Sex	Female											
	Diabetic	Diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	3 / 4											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	69											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	73											
	Abnormal Vitamin D												
	Sodum (mmol/L)	141											
	Abnormal Sodum												
	Potassium (mmol/L)	5.3											
	Abnormal Potassium												
	Urea (mmol/L)	4.8											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	3	2	2	2	1	2	2	1	1	1	2	1
	Urgency of urine	3	2	2	2	1	1	1	1	1	1	2	1
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	2	0	2	1	0	1	0	1	1	1	1	1
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	8	4	6	5	2	4	3	3	3	3	5	3
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling	N	N	N	N	T	T	T	T	T	T	T	T
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	Small	Small	Small
	Ketone (mg/dL)	5	0	0	0	0	0	0	0	0	0	0	5
	Specific Gravity	1.025	1.025	1.010	1.020	1.020	1.020	1.020	1.015	1.020	1.020	1.010	1.030
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6	6	5	6	6	6	6	6	6	6.5	6
	Protein (mg/dL)	0	30	5	0	5	0	0	5	0	5	0	30
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	0	0	0	0	0	0	0	0	0	0	0	15
	HCP Outcome												
	Dip Stick Score	4	5	1	3	3	2	2	2	2	6	4	12
Protein Analysis	Urinary Creatinine (mg/dl)	92.51	194.80	42.65	56.14	68.65	87.27	75.77	63.15	79.13	63.01	47.94	250.18
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.37	0.00	0.00	0.00	1.26	0.88	0.62	0.00	0.00	1.64	5.60
	Healthy Adjusted IL-4 (pg/ml)	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.67	0.21
	Healthy Adjusted IL-5 (pg/ml)	37.23	24.03	0.00	0.00	0.00	4.71	0.00	0.00	0.00	67.02	4.57	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	78.55	0.00	0.00	0.00	3.58	0.49	0.00	0.00	9.64	0.00	13.15
	Healthy Adjusted IL-10 (pg/ml)	2.18	1.96	9.49	5.93	0.13	6.93	4.89	2.05	3.07	9.43	7.08	2.45
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.00	7.66	1.75	20.39	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.01	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	3.60	0.00	0.00	0.00	15.29	19.17	0.00	1.67	32.20	14.96	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	59.75	0.00	0.00	0.00	35.90	42.03	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	3000	0	0	148000	0	3500	200	0	0	500	1500
	Diagnostic levels of *E. coli*												
	MLST		ST73			ST73		ST73	ST38			ST73	ST73
	E. coli clade		B2			B2		B2	D			B2	B2
	fliC Serotype		H1			H1		H1	H18			H1	H1
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 23. Patient profile for UTI524. All study analysis combined. Green indicates a negative result and purple is a positive result. N: Nitrofurantoin, T: Trimethoprim.

Patient Info	UTI number	UTI531											
	Age at recruitment	76											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Vaginal and urethral prolapse											
	Most Frequent UTI	None											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	2 / 2											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	69											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	62											
	Abnormal Vitamin D												
	Sodum (mmol/L)	143											
	Abnormal Sodum												
	Potassium (mmol/L)	4.8											
	Abnormal Potassium												
	Urea (mmol/L)	5.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	1	0	0	0	0	0	0	0	0	0	0
	Urgency of urine	0	2	0	0	0	0	0	0	0	0	0	0
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	1	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	0	4	0	0	0	0	0	0	0	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.005	1.005	1.005	1.005	1.005	1.005	1.005	1.010	1.005	1.005	1.005	1.005
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	10	0	10	0	10	0	10	0
	Haemolysed Blood (Ery/uL)	25	80	10	10	0	0	0	0	0	0	0	0
	pH	6.5	6.5	8.5	8	8	7	6	6	8	6.5	8	6
	Protein (mg/dL)	5	30	5	5	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	+	+	+	+	-	-	-	+	-	-	-	-
	Leukocytes (Leu/ul)	500	500	500	500	125	125	70	500	70	125	125	0
	HCP Outcome												
	Dip Stick Score	15	17	18	17	10	7	5	10	9	5	10	1
Protein Analysis	Urinary Creatinine (mg/dl)	62.35	47.87	21.79	21.66	15.87	9.85	9.00	48.37	17.32	16.99	22.31	18.56
	Healthy Adjusted IL-1β (pg/ml)	25.09	9.09	13.96	17.65	0.94	5.30	6.65	16.11	0.50	6.55	4.47	2.18
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	1.78	0.00	0.00	1.20	1.45	0.19	0.00
	Healthy Adjusted IL-5 (pg/ml)	12.95	0.00	30.12	15.69	2.09	25.90	23.79	28.69	0.00	22.31	0.00	15.63
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	33.34	45.41	32.08	19.25	0.00	1.26	0.00	100.14	0.00	5.45	11.51	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	2.69	20.62	1.38	0.00	0.00	14.27	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	13.43	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.24	0.00	0.00	0.00	2.77	0.00	0.00	0.00	2.72	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	13800	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST	ST420	ST420	ST420	ST420	ST420	ST420	ST583	ST420	ST420	ST420	ST420	ST420
	E. coli clade	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2
	fliC Serotype	H31	H31	H31	H31	H31	H5	H31	H31	H31	H31	H31	H31
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 24. Patient profile for UTI531. All study analysis combined. Green indicates a negative result and purple is a positive result.

Patient Info	UTI number	UTI536											
	Age at recruitment	73											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Moderate											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	5 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	112											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	24											
	Abnormal Vitamin D												
	Sodum (mmol/L)	129											
	Abnormal Sodum												
	Potassium (mmol/L)	5.0											
	Abnormal Potassium												
	Urea (mmol/L)	7.4											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	1	0	0	1	0	0	1	1	0	0	0	1
	Urgency of urine	0	0	0	0	0	0	0	0	1	0	0	0
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	0	0	1	0	0	0	0	0	0	0	0
	Low back pain	2	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	3	0	0	2	0	0	1	1	1	0	0	1
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	1	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after								T				
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	Small	-	-	-	-	-
	Ketone (mg/dL)	5	0	5	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.010	1.010	1.010	1.010	1.015	1.010	1.010	1.005	1.005	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	25	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6	6	6	6	6	6	6	6	6	6	6
	Protein (mg/dL)	30	5	30	5	5	30	5	0	5	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	+	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	500	15	125	125	70	15	70	0	15	0	0	15
	HCP Outcome												
	Dip Stick Score	15	3	7	5	4	5	7	0	4	1	0	2
Protein Analysis	Urinary Creatinine (mg/dl)	50.01	100.87	124.52	135.68	70.32	163.68	155.23	123.80	137.92	41.92	92.83	244.07
	Healthy Adjusted IL-1β (pg/ml)	14.73	0.00	0.13	0.72	0.00	0.00	1.18	0.00	0.69	0.00	0.00	1.16
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	3.29	8.46	24.31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.72	0.00
	Healthy Adjusted IL-10 (pg/ml)	2.50	0.08	7.68	5.30	9.70	0.00	0.44	0.00	6.11	0.00	0.00	0.60
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	7.28	0.00	1.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	22.77	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	7.54	11.31	0.00	0.00	0.00	11.95	0.00	0.00	4.45	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	112000	9750	19050	0	9350	1400	2000	100	0	1350	0	500
	Diagnostic levels of *E. coli*												
	MLST	ST73	ST355	ST355		ST73	ST355	ST355	ST355		ST69		ST355
	E. coli clade	B2	E	E		B2	E	E	E		D		E
	fliC Serotype	H1	H5	H5		H1	H5	H5	H5		H18		H5
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 25. Patient profile for UTI536. All study analysis combined. Green indicates a negative result and purple is a positive result. T: Trimethoprim.

Patient Info	UTI number	UTI562											
	Age at recruitment	65											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	Systemic											
	Estimated UTIs / Positive urines in 12 month	6 / 5											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	82											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	54											
	Abnormal Vitamin D												
	Sodum (mmol/L)	142											
	Abnormal Sodum												
	Potassium (mmol/L)	4.2											
	Abnormal Potassium												
	Urea (mmol/L)	6.1											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	0	0	0	1	0	0	1	1	1	1	0
	Urgency of urine	2	0	0	0	1	0	0	0	0	0	0	0
	Pain or burning upon urination	1	0	0	0	2	0	1	0	0	1	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	1	0	0	1
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	5	0	0	0	4	0	1	1	2	2	1	1
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	1	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after		T						T				
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	Small	Small	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.020	1.015	1.010	1.010	1.015	1.010	1.020	1.010	1.015	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6.5	6	5	6	6	6	6	6	6	6	6	6
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	+	+	-
	Leukocytes (Leu/ul)	70	125	15	15	0	15	0	15	15	70	15	15
	HCP Outcome												
	Dip Stick Score	7	9	4	2	0	3	0	4	2	9	7	2
Protein Analysis	Urinary Creatinine (mg/dl)	185.06	177.87	111.68	80.78	92.37	100.03	88.28	102.14	83.77	101.09	57.68	57.51
	Healthy Adjusted IL-1β (pg/ml)	0.00	1.93	0.44	0.00	0.00	0.67	0.88	0.79	0.00	1.13	0.78	0.78
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	16.30	12.32	0.00	0.00	11.31	4.38	2.98	0.01	13.52	4.97	8.06	5.04
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	6.38	42.71	12.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	11.64	5.96	0.00	0.00	9.64	0.65	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	>5*10^5	0	0	0	0	0	0	0	0	100	0
	Diagnostic levels of *E. coli*												
	MLST	ST625	ST625									ST1571	
	E. coli clade	B2	B2									unknown	
	fliC Serotype	H7	H7									H9	
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 26. Patient profile for UTI562. All study analysis combined. Green indicates a negative result and purple is a positive result. T: Trimethoprim.

Patient Info	UTI number	UTI569											
	Age at recruitment	77											
	Sex	Female											
	Diabetic	Diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	12 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	66											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	20											
	Abnormal Vitamin D												
	Sodum (mmol/L)	142											
	Abnormal Sodum												
	Potassium (mmol/L)	4.3											
	Abnormal Potassium												
	Urea (mmol/L)	6.6											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	2	1	3	3	2	2	2	2	2	2	2
	Urgency of urine	3	3	2	2	3	3	2	2	2	2	3	2
	Pain or burning upon urination	0	0	0	2	2	2	0	0	0	0	0	1
	Incomplete void/small volumes	2	1	1	2	2	2	0	2	1	0	2	2
	Low back pain	0	0	0	0	0	2	2	2	1	1	2	2
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	7	6	4	9	10	11	6	8	6	5	9	9
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	1	1	1	0	0	0	1	0	0
	Treated for UTI within 3 days prior			N		N							
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	Small	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.005	1.005	1.010	1.015	1.005	1.020	1.010	1.010	1.010	1.015	1.015	1.005
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	10	10	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	25	25	0	0	0	0	0	10	0
	pH	6.5	7	6.5	6.5	6	6	6.5	6.5	7	6	6	6
	Protein (mg/dL)	0	0	0	30	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	+	+	-
	Leukocytes (Leu/ul)	125	15	0	125	500	0	0	15	0	15	125	125
	HCP Outcome												
	Dip Stick Score	6	5	1	10	8	2	1	4	3	11	11	5
Protein Analysis	Urinary Creatinine (mg/dl)	33.09	66.71	86.38	104.87	40.12	92.84	89.04	105.93	73.16	159.78	145.06	39.62
	Healthy Adjusted IL-1β (pg/ml)	21.55	7.62	2.03	81.75	44.81	10.01	3.29	2.36	2.24	6.89	9.15	4.61
	Healthy Adjusted IL-4 (pg/ml)	0.55	0.73	0.68	0.36	0.00	0.00	0.08	0.00	0.00	1.33	0.14	0.00
	Healthy Adjusted IL-5 (pg/ml)	44.47	13.07	18.51	0.12	0.00	43.71	11.20	20.70	0.00	0.00	15.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	32.90	0.00	8.43	84.64	0.00	6.67	0.00	0.00	1.44	7.69	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	2.34	19.91	15.07	182.70	21.89	16.75	2.91	18.31	0.00	13.48	148.59	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	10.14	0.00	0.00	0.00	0.00	25.74	0.00	0.00	0.00	2.29	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	10.90	0.00	0.00	0.00	5.18	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	>5*10^5	200	0	5000	1000	>5*10^5	>5*10^5	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST					ST3640	ST442		ST3640	ST442	ST3640	ST3640	ST3640
	E. coli clade					B1	B1		B1	B1	B1	B1	B1
	fliC Serotype					H37	H21		H37	H6	H37	H37	H37
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 27. Patient profile for UTI569. All study analysis combined. Green indicates a negative result and purple is a positive result. N: Nitrofurantoin.

Patient Info	UTI number	UTI675											
	Age at recruitment	74											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Anterior Vaginal Prolapse											
	Most Frequent UTI	Mild											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	8 / 6											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	70											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	64											
	Abnormal Vitamin D												
	Sodum (mmol/L)	140											
	Abnormal Sodum												
	Potassium (mmol/L)	4.9											
	Abnormal Potassium												
	Urea (mmol/L)	7.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	2	3	3	2	2	2	2	2	1	2	2
	Urgency of urine	2	2	3	2	2	2	1	2	1	1	2	2
	Pain or burning upon urination	2	1	2	1	1	0	0	0	0	1	0	0
	Incomplete void/small volumes	2	2	2	2	3	2	1	2	1	2	2	2
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	8	7	10	8	8	6	4	6	4	5	6	6
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	1	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling			N	N	N	N	N	N	N	N	N	N
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	Mod	Mod
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.030	1.005	1.015	1.010	1.005	1.005	pol0	1.005	1.005	1.005	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	10	10	0	0	0	10	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6.5	6.5	6	6	7	6.5	7.5	6.5	7	6	7.5	6
	Protein (mg/dL)	30	0	0	0	5	5	5	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	+	-	-	-	-	-	-	-	-	-	-	+
	Leukocytes (Leu/ul)	15	0	0	0	0	0	0	0	125	15	15	500
	HCP Outcome												
	Dip Stick Score	14	2	1	0	4	4	6	2	7	3	10	14
Protein Analysis	Urinary Creatinine (mg/dl)	165.37	43.20	79.58	48.61	56.39	81.06	52.53	32.23	41.36	43.94	46.24	35.51
	Healthy Adjusted IL-1β (pg/ml)	0.45	4.13	4.18	0.00	1.80	13.64	4.91	0.00	2.46	2.72	2.25	5.98
	Healthy Adjusted IL-4 (pg/ml)	2.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.32	0.21	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	5.99	0.00	65.08	0.00	0.00	0.00	8.48	11.96	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	12.52	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.14	1.95	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	20.74	12.16	0.00	5.46	20.15	35.60	0.00	0.53	14.29	28.37	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	8.80	9.31	5.33	8.34	17.05	16.60	10.71	19.65	19.28	18.90	3.70
	Healthy Adjusted IL-12 p70 (pg/ml)	6.68	13.97	0.00	7.05	0.00	19.07	15.13	5.35	24.20	0.71	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	9.93	1.24	40.12	8.03	17.61	6.48	47.48	13.10	0.00	14.65	44.72	14.50
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	8.87	0.00	0.00	0.00	0.00	3.51	0.38
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.90	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	>5*10^5	200	0	0	1950	200	0	0	100	0	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST	ST69	ST69	ST69			ST69	ST69			ST69		ST69
	E. coli clade	D	D	D			D	D			D		D
	fliC Serotype	H18	H18	H6			H18	H18			H18		H18
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 28. Patient profile for UTI675. All study analysis combined. Green indicates a negative result and purple is a positive result. N: Nitrofurantoin.

Patient Info	UTI number	UTI726											
	Age at recruitment	75											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	10 / 5											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	66											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	21											
	Abnormal Vitamin D												
	Sodum (mmol/L)	141											
	Abnormal Sodum												
	Potassium (mmol/L)	5.4											
	Abnormal Potassium												
	Urea (mmol/L)	8.4											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	3	1	1	1	0	0	2	2	0	0	0	0
	Urgency of urine	3	3	3	1	2	2	2	1	0	1	0	0
	Pain or burning upon urination	0	0	0	1	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	3	2	3	1	0	2	0	0	0	0	0	1
	Low back pain	0	0	0	3	0	2	0	0	0	0	0	0
	Blood in urine	0	0	0	1	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	9	6	7	8	2	6	4	3	0	1	0	1
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	1	1	1	1	1	1	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling	N	N	N					T				
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	Small	-	Mod	Small	Small	Small	Small	Small	Small
	Ketone (mg/dL)	5	5	0	5	5	5	5	0	0	5	0	0
	Specific Gravity	1.020	1.020	1.020	1.020	1.030	1.030	1.020	1.015	1.030	1.020	1.025	1.020
	Non-Haemolysed Blood (Ery/uL)	0	10	0	10	10	0	10	10	0	10	0	10
	Haemolysed Blood (Ery/uL)	10	0	10	0	0	10	0	0	25	0	0	0
	pH	6.5	6	6.5	6	6	6	6.5	6	6	6	6	6
	Protein (mg/dL)	30	0	5	30	5	5	5	0	5	5	0	5
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	+	+	+	-	-	-	-	-
	Leukocytes (Leu/ul)	15	15	15	0	15	15	70	15	15	0	125	70
	HCP Outcome												
	Dip Stick Score	9	6	7	9	14	18	17	7	12	8	10	10
Protein Analysis	Urinary Creatinine (mg/dl)	258.67	147.42	202.06	232.90	142.04	190.89	29.69	256.53	293.85	203.31	300.39	108.32
	Healthy Adjusted IL-1β (pg/ml)	10.20	13.36	9.80	0.97	8.99	32.96	4.37	19.09	43.03	11.87	57.18	13.07
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.24	0.00	0.00	0.00	0.00	0.00	0.00	1.16	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	3.72	7.62	0.00	0.00	0.00	0.00	5.07	0.00	68.42	0.00	20.53	8.78
	Healthy Adjusted IL-6 (pg/ml)	8.43	3.53	12.74	106.61	0.00	5.21	0.00	22.31	87.62	22.91	9.23	0.00
	Healthy Adjusted IL-8 (pg/ml)	16.41	50.39	541.50	14.09	0.00	0.32	15.79	51.85	252.03	0.00	298.28	0.37
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.31	0.46	0.00	0.00	7.10	0.00	0.00	0.00	0.21	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	16.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.46	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	1050	900	0	>5*10^5	>5*10^5	0	>5*10^5	>5*10^5	50000	>5*10^5	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST		ST69	ST73		ST73	ST73		ST73	ST73	ST73	ST73	ST73
	E. coli clade		D	B2		B2	B2		B2	B2	B2	B2	B2
	fliC Serotype		H18	H1		H1	H1		H1	H1	H1	H1	H1
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 29. Patient profile for UTI726. All study analysis combined. Green indicates a negative result and purple is a positive result. N: Nitrofurantoin, T: Trimethoprim.

Patient Info	UTI number	UTI755											
	Age at recruitment	86											
	Sex	Male											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Trabeculated bladder & prostatic enlargement											
	Most Frequent UTI	Mild											
	Oestrogen use												
	Estimated UTIs / Positive urines in 12 month	3 / 1											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	200											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	67											
	Abnormal Vitamin D												
	Sodum (mmol/L)	139											
	Abnormal Sodum												
	Potassium (mmol/L)	4.3											
	Abnormal Potassium												
	Urea (mmol/L)	14.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	3	2	2	2	1	2	2	2	2	2	2	2
	Urgency of urine	2	1	2	1	1	1	1	1	2	2	2	2
	Pain or burning upon urination	1	0	1	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	2	2	1	1	1	1	1	1	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	8	5	6	4	3	4	4	4	4	4	4	4
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior	N											
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	5	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.010	1.015	1.005	1.015	1.005	1.005	1.020	1.015	1.010	1.010	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	10	0	0	0	0	0	10	0	0	0
	Haemolysed Blood (Ery/uL)	10	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6	6	6	6	6.5	6	6	6	6	6.5	6
	Protein (mg/dL)	5	5	5	0	5	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	70	70	0	70	0	0	0	0	0	0	0	0
	HCP Outcome												
	Dip Stick Score	6	4	3	4	2	2	1	2	2	0	1	0
Protein Analysis	Urinary Creatinine (mg/dl)	84.71	39.79	50.51	33.46	95.57	55.32	49.84	57.99	91.54	80.37	104.62	89.12
	Healthy Adjusted IL-1β (pg/ml)	2.40	4.03	2.50	2.41	0.85	1.49	1.25	0.00	0.03	1.45	1.98	1.61
	Healthy Adjusted IL-4 (pg/ml)	0.00	1.22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.94	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	8.20	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	27.51	0.00	0.00	0.00	13.65	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	11.59	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	0	7250	1800	100	1000	0	0	2000
	Diagnostic levels of *E. coli*												
	MLST						ST95	ST95	ST95	ST95			ST95
	E. coli clade						B2	B2	B2	B2			B2
	fliC Serotype						H7	H7	H7	H7			H7
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 30. Patient profile for UTI755. All study analysis combined. Green indicates a negative result and purple is a positive result. A blue filled box indicates it was not applicable, as the patient was male. N: Nitrofurantoin.

Patient Info	UTI number	UTI781											
	Age at recruitment	79											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Severe											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	12 / 5											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	94											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	70											
	Abnormal Vitamin D												
	Sodum (mmol/L)	136											
	Abnormal Sodum												
	Potassium (mmol/L)	4.7											
	Abnormal Potassium												
	Urea (mmol/L)	9.0											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	2	2	1	2	2	1	1	1	1	1	1	1
	Urgency of urine	1	2	2	1	1	1	0	1	1	1	1	1
	Pain or burning upon urination	1	1	0	1	0	0	1	0	0	0	0	0
	Incomplete void/small volumes	1	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	5	5	3	4	3	2	2	2	2	2	2	2
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	1	1	1	1	0	1	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	Small	-	-	-	Small
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.015	1.020	1.010	1.015	1.020	1.025	1.015	1.020	1.010	1.010	1.020
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	10	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6	6	6	6	6	6	6	6	6	6	6
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	+	-	-	-
	Leukocytes (Leu/ul)	15	0	15	0	0	0	125	0	125	0	15	15
	HCP Outcome												
	Dip Stick Score	2	1	4	0	1	2	8	4	11	0	2	7
Protein Analysis	Urinary Creatinine (mg/dl)	65.91	85.45	89.66	43.10	105.49	86.82	151.15	103.96	120.48	39.18	43.05	333.36
	Healthy Adjusted IL-1β (pg/ml)	0.00	1.21	0.00	0.00	0.00	0.00	0.00	0.00	2.26	0.00	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.27	0.00	0.00	0.00	0.21	0.24	0.00	0.00	1.48	1.70	0.50	0.00
	Healthy Adjusted IL-5 (pg/ml)	25.37	3.24	68.12	0.00	0.00	187.54	36.14	21.98	1.62	87.25	68.91	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	5.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	10.95	53.63	80.88	0.04	8.81	9.99	51.53	0.00	7.38	2.29	13.74	49.13
	Healthy Adjusted IL-10 (pg/ml)	0.00	3.33	5.04	16.97	0.00	0.45	39.89	4.84	0.00	35.87	27.25	14.96
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	5.45	14.84	0.00	0.00	17.31	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	13.07	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	31.91	0.00	0.00	0.00	53.90	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	12100	11500	1250	300	>5*10^5	>5*10^5	>5*10^5	135000	>5*10^5	0	0	700
	Diagnostic levels of *E. coli*												
	MLST	ST127	ST127	ST127	ST127	ST127	ST127	ST127	ST127	ST127			ST127
	E. coli clade	B2	B2	B2	B2	B2	B2	B2	B2	B2			B2
	fliC Serotype	H31	H31	H31	H31	H31	H31	H31	H31	H31			H31
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 31. Patient profile for UTI781. All study analysis combined. Green indicates a negative result and purple is a positive result.

Patient Info	UTI number	UTI834											
	Age at recruitment	67											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Severe											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	3 / 5											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	138											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	80											
	Abnormal Vitamin D												
	Sodum (mmol/L)	144											
	Abnormal Sodum												
	Potassium (mmol/L)	4.6											
	Abnormal Potassium												
	Urea (mmol/L)	10.5											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	1	0	1	1	1	2	0	0	2	2	2	2
	Urgency of urine	2	2	2	2	1	1	1	1	2	2	2	2
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	2	2	2	2	3	2	2	2	2	2	2	2
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	5	4	5	5	5	5	3	3	6	6	6	6
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	Small	-	-	-	-	Small	Small	Small	-	-
	Ketone (mg/dL)	5	0	5	5	0	0	0	0	0	0	0	0
	Specific Gravity	1.015	1.020	1.015	1.020	1.010	1.015	1.020	1.020	1.010	1.020	1.020	1.015
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	10	0	0	0	0	0
	pH	6	6	6	6	6	6	6	6	6	6	6	6
	Protein (mg/dL)	5	5	5	0	0	5	5	5	5	5	5	5
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	+	-	-	-	-
	Leukocytes (Leu/ul)	0	0	0	0	70	0	0	0	0	0	0	0
	HCP Outcome												
	Dip Stick Score	3	3	6	3	3	2	4	11	4	6	3	2
Protein Analysis	Urinary Creatinine (mg/dl)	128.40	163.54	152.06	106.95	35.27	154.28	164.19	134.77	110.41	139.58	129.29	131.27
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	22.81	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	2.94	0.00	0.00	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	9.08	20.57	0.00	0.00	0.00	0.00	0.00	15.56	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	85.86	6.44	0.00	0.00	33.94	46.89	0.00	0.00	64.87	27.81	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	0	0	0	0	0	0	0	0
	Diagnostic levels of *E. coli*												
	MLST												
	E. coli clade												
	fliC Serotype												
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 32. Patient profile for UTI834. All study analysis combined. Green indicates a negative result and purple is a positive result.

Patient Info	UTI number	UTI840											
	Age at recruitment	82											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use	Vaginal											
	Estimated UTIs / Positive urines in 12 month	10 / 7											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	98											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	38											
	Abnormal Vitamin D												
	Sodum (mmol/L)	141											
	Abnormal Sodum												
	Potassium (mmol/L)	4.4											
	Abnormal Potassium												
	Urea (mmol/L)	7.9											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	3	0	0	0	0	0	0	0	0	2	0
	Urgency of urine	0	2	1	0	1	0	1	0	0	0	1	0
	Pain or burning upon urination	0	2	0	0	1	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	2	0	0	0	1	1	0	1	0	2	0
	Low back pain	0	2	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	1	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	0	12	1	0	2	1	2	0	1	0	5	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	0	0	1	1	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling		N										
	Treated for UTI within 3 days after					N							
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.020	1.015	1.010	1.005	1.005	1.010	1.005	1.015	1.010	1.015	1.010	1.020
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	10	0	0	0	25	10	10	0	10	0	0	0
	pH	5	6	6	6	6	6	6	6	6	6	6	6
	Protein (mg/dL)	5	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	+	+	+	+	+	-	-
	Leukocytes (Leu/ul)	15	0	125	0	500	500	500	125	500	0	0	0
	HCP Outcome												
	Dip Stick Score	7	1	4	1	8	11	12	11	11	6	0	2
Protein Analysis	Urinary Creatinine (mg/dl)	147.97	28.46	50.67	60.81	19.80	93.88	49.83	122.91	69.99	103.57	84.81	127.86
	Healthy Adjusted IL-1β (pg/ml)	3.31	3.63	15.31	0.00	3.89	6.19	6.01	3.17	1.21	1.32	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.62	0.00	2.85	0.00	0.22	0.00	0.00	0.00	0.00	0.57	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	1.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.00	7.44	0.00	0.00	3.22	16.07	16.83	0.00	1.91	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	9.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	5900	2200	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	2200	1000
	Diagnostic levels of *E. coli*												
	MLST			ST354	ST354	ST354	ST354	ST354	ST354	ST354	ST73	ST354	ST354
	E. coli clade			F	F	F	F	F	F	F	B2	F	F
	fliC Serotype			H34	H34	H36	H36	H36	H36	H36	H2	H36	H36
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 33. Patient profile for UTI840. All study analysis combined. Green indicates a negative result and purple is a positive result. N: Nitrofurantoin.

Patient Info	UTI number	UTI891											
	Age at recruitment	76											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Moderate											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	3 / 2											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	85											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	13											
	Abnormal Vitamin D												
	Sodum (mmol/L)	143											
	Abnormal Sodum												
	Potassium (mmol/L)	4.2											
	Abnormal Potassium												
	Urea (mmol/L)	5.9											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	0	1	1	0	2	2	1	2	2	2	2	2
	Urgency of urine	1	2	1	1	0	1	2	2	2	0	2	0
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	0	1	0	0	0	0	0	1	0	0	0	0
	Low back pain	1	0	0	0	0	0	0	0	0	1	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	2	4	2	1	2	3	3	5	4	3	4	2
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.015	1.010	1.015	1.010	1.020	1.015	1.010	1.020	1.015	1.010	1.010	1.015
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	10	0	10	10	10	0
	Haemolysed Blood (Ery/uL)	25	10	0	0	0	10	0	25	0	0	0	0
	pH	6	6	6	6	6	6.5	7	6	6	7.5	7	6
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	70	0	0	125	0	70	15	125	15	70	125	125
	HCP Outcome												
	Dip Stick Score	6	1	1	4	2	6	5	8	4	7	7	5
Protein Analysis	Urinary Creatinine (mg/dl)	81.50	60.57	75.54	50.99	128.98	92.34	50.07	77.37	100.84	107.68	65.61	82.53
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.04	1.06	10.22	18.74	1.27
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	108.61	53.36	0.00	0.00	31.72	24.09	0.00	6.93	16.65	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	3.62	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	10.13	61.29	25.83	0.00	22.18	41.63	424.67	0.00	64.96	229.23	0.00
	Healthy Adjusted IL-10 (pg/ml)	10.25	19.36	13.00	0.00	0.00	35.22	32.10	0.87	0.00	0.00	20.57	6.18
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	23.10	0.00	0.00	0.00	13.67	0.00	0.00	0.00	2.92	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	5.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.11	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	600	0	0	0	0	0	0	0	0	0	0
	Diagnostic levels of *E. coli*												
	MLST		ST127										
	E. coli clade		B2										
	fliC Serotype		H31										
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 34. Patient profile for UTI891. All study analysis combined. Green indicates a negative result and purple is a positive result.

Patient Info	UTI number	UTI899											
	Age at recruitment	71											
	Sex	Female											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Moderate											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	4 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	81											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	70											
	Abnormal Vitamin D												
	Sodum (mmol/L)	138											
	Abnormal Sodum												
	Potassium (mmol/L)	4.6											
	Abnormal Potassium												
	Urea (mmol/L)	9.7											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	3	3	1	2	2	2	2	2	2	3	2	2
	Urgency of urine	2	3	1	2	1	1	2	2	2	3	2	2
	Pain or burning upon urination	0	2	0	0	0	0	0	0	0	2	0	0
	Incomplete void/small volumes	0	1	0	0	2	0	0	2	0	2	1	2
	Low back pain	0	2	1	2	1	2	2	2	2	3	2	2
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	5	11	3	6	6	5	6	8	6	13	7	8
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	1	0	0	0	0	0	0	0	1	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling		T										
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	Small	-	Small
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.010	1.010	1.020	1.010	1.015	1.015	1.015	1.010	1.015	1.020	1.020
	Non-Haemolysed Blood (Ery/uL)	0	0	10	0	10	10	10	0	10	0	10	0
	Haemolysed Blood (Ery/uL)	25	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6	6	6	6	6	6	6	6	6	6	6
	Protein (mg/dL)	0	0	5	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	+	-	+	+	+	+	+	+	+	+	+	+
	Leukocytes (Leu/ul)	70	0	70	0	0	0	0	0	0	15	0	0
	HCP Outcome												
	Dip Stick Score	10	0	10	7	6	7	7	6	6	11	8	10
Protein Analysis	Urinary Creatinine (mg/dl)	45.09	33.98	118.41	126.38	85.60	87.58	132.50	122.67	87.93	92.53	92.50	109.18
	Healthy Adjusted IL-1β (pg/ml)	5.64	1.05	5.92	0.00	0.00	1.62	3.79	0.23	1.91	3.91	2.26	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	1.41	2.38	0.00	0.00	2.05	0.00	0.00	0.00	0.32	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.58	90.57	0.00	0.00	63.26	2.26	0.00	0.00	0.00	0.00	55.84	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	8.02	0.00	13.71	0.00	2.90	0.00	5.54	28.00	6.05	13.50	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	16.39	34.88	7.86	15.71	32.58	78.05	0.00	26.55	143.94	59.62	55.17	1.78
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	2.93	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	1.24	0.00	18.29	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	36.07	0.00	0.00	0.00	3.73	1.99	0.00	150.77	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	>5*10^5	300	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5	>5*10^5
	Diagnostic levels of *E. coli*												
	MLST	ST69	ST602	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73	ST73
	E. coli clade	D	B1	B2	B2	B2	B2	B2	B2	B2	B2	B2	B2
	fliC Serotype	H18	H9	H1	H1	H1	H1	H1	H1	H1	H1	H1	H1
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

Appendix 35. Patient profile for UTI899. All study analysis combined. Green indicates a negative result and purple is a positive result. T: Trimethoprim.

Patient Info	UTI number	UTI924											
	Age at recruitment	86											
	Sex	Female											
	Diabetic	Pre-diabetic											
	Urinary Tract Status	Abnormal: Anterior Vaginal Prolapse											
	Most Frequent UTI	Severe											
	Oestrogen use	None											
	Estimated UTIs / Positive urines in 12 month	6 / 2											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	71											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	85											
	Abnormal Vitamin D												
	Sodum (mmol/L)	140											
	Abnormal Sodum												
	Potassium (mmol/L)	4.1											
	Abnormal Potassium												
	Urea (mmol/L)	5.1											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	1	1	2	1	1	1	1	1	1	1	1	1
	Urgency of urine	1	1	2	1	1	0	0	0	1	1	1	0
	Pain or burning upon urination	0	0	1	0	0	0	0	0	0	0	1	1
	Incomplete void/small volumes	1	1	1	1	1	0	0	1	1	1	1	0
	Low back pain	1	2	1	1	1	1	1	1	1	1	1	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	4	5	7	4	4	2	2	3	4	4	5	2
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	Small	Small	-	-
	Ketone (mg/dL)	5	5	5	5	5	5	0	0	0	0	5	0
	Specific Gravity	1.030	1.030	1.030	1.030	1.025	1.030	1.030	1.020	1.030	1.025	1.020	1.030
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6	6	6	6	6	5	6	6.5	6	6	6	6
	Protein (mg/dL)	5	5	5	30	5	5	30	5	5	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	0	0	125	15	15	0	0	0	15	15	15	0
	HCP Outcome												
	Dip Stick Score	6	6	10	9	7	7	6	4	10	8	5	4
Protein Analysis	Urinary Creatinine (mg/dl)	204.75	214.84	181.65	232.11	167.43	157.57	250.30	158.76	184.57	159.61	150.37	161.50
	Healthy Adjusted IL-1β (pg/ml)	1.65	2.84	19.11	9.95	10.57	0.00	3.99	5.28	4.08	4.71	10.10	7.16
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.38	0.01	0.00	0.32	0.93	0.15	0.00	0.51	1.36
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	11.54	0.00	0.00	0.00	28.01	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.05	0.00	0.00	57.16	2.84
	Healthy Adjusted IL-10 (pg/ml)	5.29	10.18	2.69	0.00	21.35	9.25	0.00	0.11	33.65	9.42	11.91	14.64
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.91	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	6.38	0.00	0.00	0.00	12.33	0.45	0.00	1.73
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	1150	12100	122000	1100	0	0	100	0	0	2000	0	0
	Diagnostic levels of *E. coli*												
	MLST	ST12	ST12	ST73	ST73			ST80			ST677		
	E. coli clade	B2	B2	B2	B2			B2			B1		
	fliC Serotype	H5	H5	H1	H1			H7			H5		
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

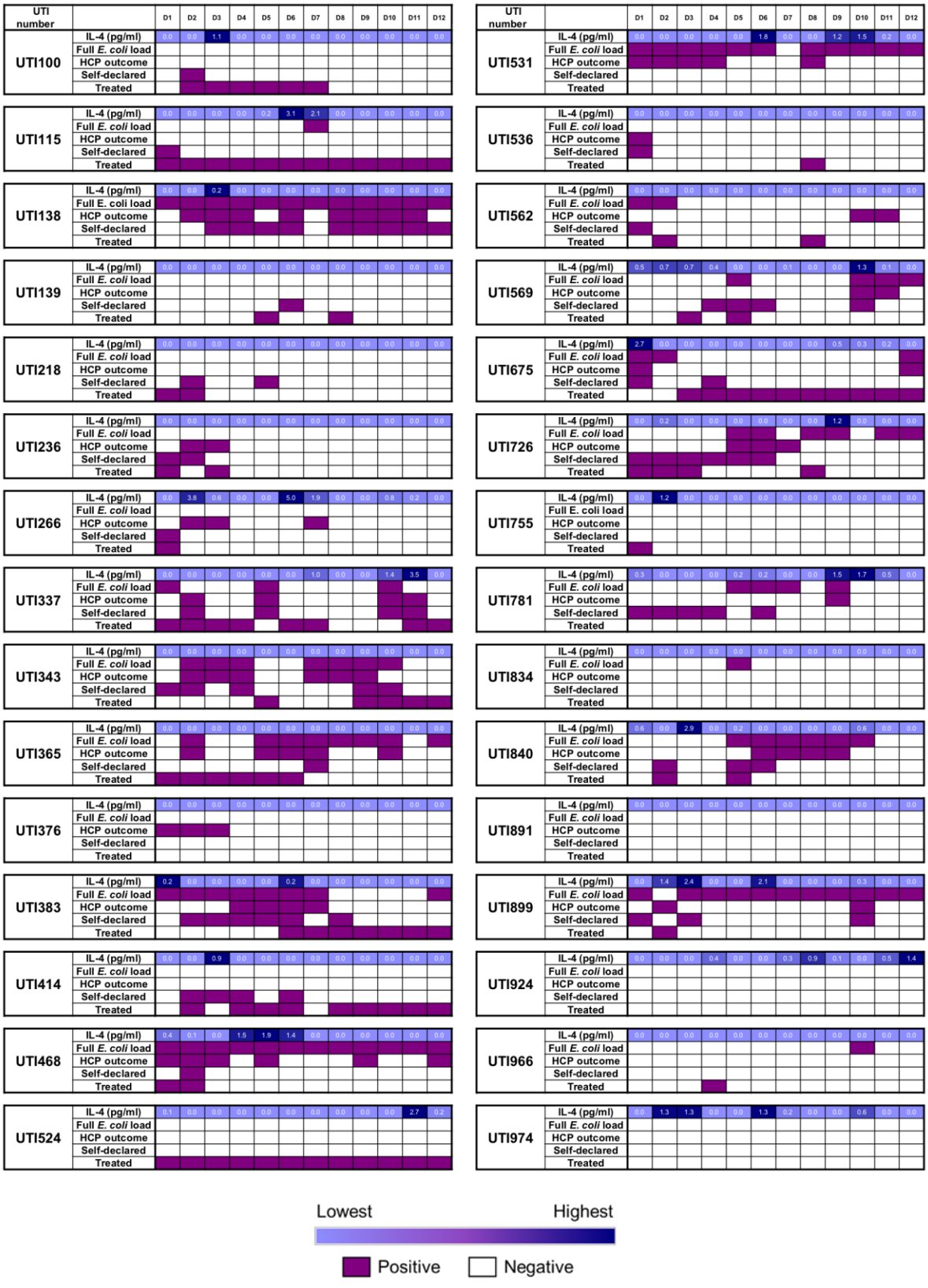
Appendix 36. Patient profile for UTI924. All study analysis combined. Green indicates a negative result and purple is a positive result.

Patient Info	UTI number	UTI966											
	Age at recruitment	79											
	Sex	Male											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Abnormal: Prostatic enlargement											
	Most Frequent UTI	Mild											
	Oestrogen use												
	Estimated UTIs / Positive urines in 12 month	6 / 4											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	87											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	25											
	Abnormal Vitamin D												
	Sodum (mmol/L)	125											
	Abnormal Sodum												
	Potassium (mmol/L)	4.9											
	Abnormal Potassium												
	Urea (mmol/L)	6.0											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	1	1	2	0	1	0	2	2	2	0	0	0
	Urgency of urine	0	0	2	2	2	0	2	0	0	0	0	0
	Pain or burning upon urination	0	0	1	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	1	0	0	0	0	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	2	1	5	2	3	0	4	2	2	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior				N								
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.005	1.005	1.010	1.005	1.005	1.005	1.010	1.005	1.005	1.010	1.030	1.020
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	6.5	5	6	6.5	6	6	6	6.5	6.5	6	6	6
	Protein (mg/dL)	5	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	70	70	125	125	125	0	125	0	15	500	0	125
	HCP Outcome												
	Dip Stick Score	6	5	4	6	5	1	4	2	4	5	4	5
Protein Analysis	Urinary Creatinine (mg/dl)	14.38	18.90	28.91	30.04	29.37	28.76	56.97	18.72	21.78	261.66	145.53	48.00
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.37	4.12	4.92	1.08	1.93	73.43	0.00	1.48	23.36	0.00	22.26
	Healthy Adjusted IL-4 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	0.00	5.26	6.37	0.00	0.00	71.26	0.00	0.00	0.00	0.00	94.35
	Healthy Adjusted IL-10 (pg/ml)	0.00	9.95	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-17A (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	2550	0	1000	0	0	> 5x10^5	0	0
	Diagnostic levels of *E. coli*												
	MLST					ST69		ST95			ST73		
	E. coli clade					D		B2			B2		
	fliC Serotype					H18		H7			H1		
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

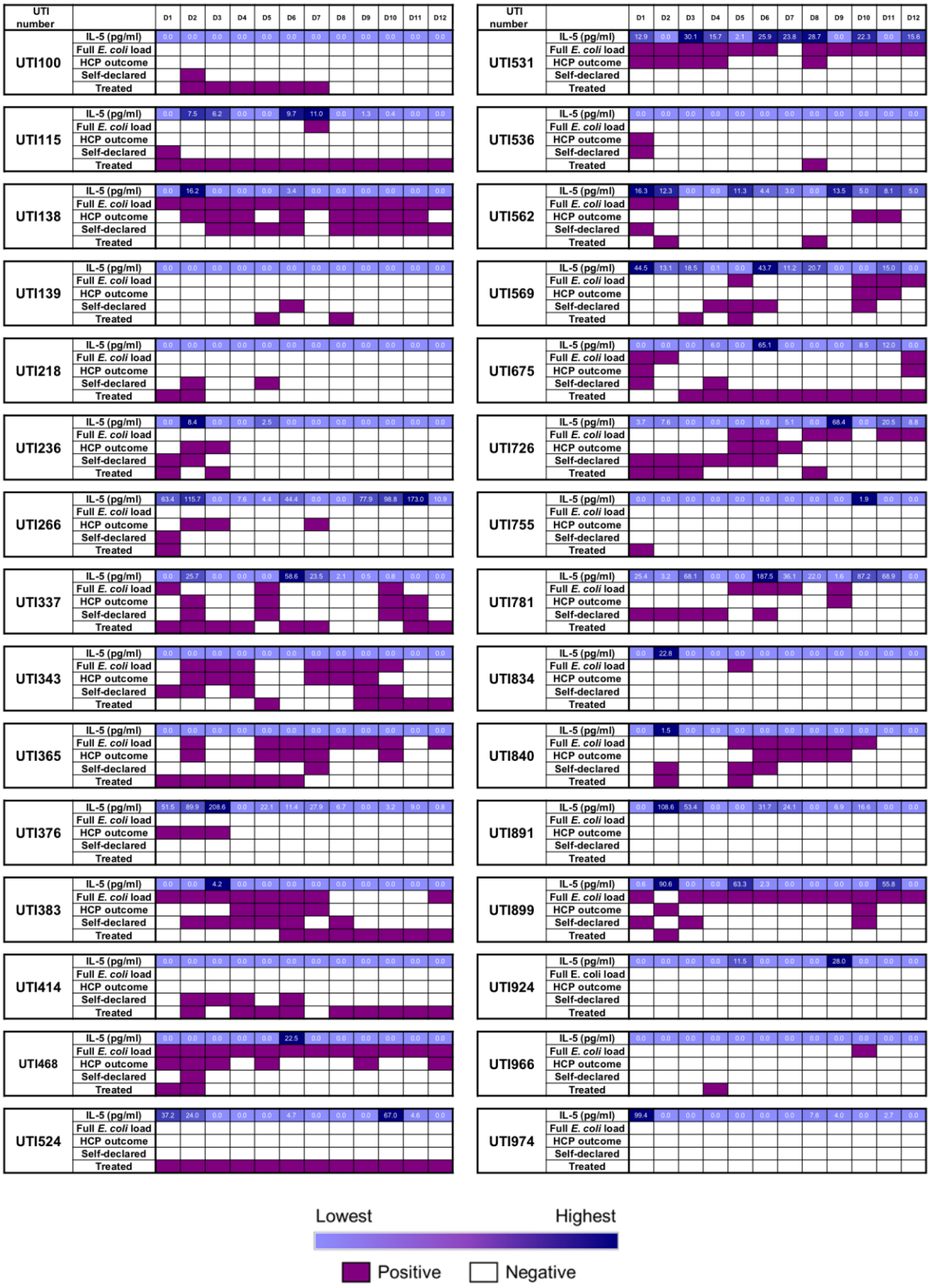
Appendix 37. Patient profile for UTI966. All study analysis combined. Green indicates a negative result and purple is a positive result. A blue filled box indicates it was not applicable, as the patient was male. N: Nitrofurantoin.

Patient Info	UTI number	UTI974											
	Age at recruitment	74											
	Sex	Male											
	Diabetic	Non-diabetic											
	Urinary Tract Status	Normal											
	Most Frequent UTI	Mild											
	Oestrogen use												
	Estimated UTIs / Positive urines in 12 month	12 / 3											
	Donation Number	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Clinical and Blood Data	Serum Creatinine (umol/L)	101											
	Abnormal Serum Creatinine												
	Vitamin D levels (nmol/L)	118											
	Abnormal Vitamin D												
	Sodum (mmol/L)	142											
	Abnormal Sodum												
	Potassium (mmol/L)	4.1											
	Abnormal Potassium												
	Urea (mmol/L)	6.1											
	Abnormal Urea												
	Positive urine sample within 3 days prior												
	Positive urine sample on day of sampling												
	Positive urine sample within 3 days after												
	Negative urine sample for a UTI within 3 days												
Questionnaire Info	Frequency of urination	1	2	1	1	1	1	0	0	0	0	0	0
	Urgency of urine	2	2	0	1	1	1	0	0	0	0	0	0
	Pain or burning upon urination	0	0	0	0	0	0	0	0	0	0	0	0
	Incomplete void/small volumes	1	1	1	1	0	0	0	0	0	0	0	0
	Low back pain	0	0	0	0	0	0	0	0	0	0	0	0
	Blood in urine	0	0	0	0	0	0	0	0	0	0	0	0
	Symptom score (out of 18)	4	5	2	3	2	2	0	0	0	0	0	0
	Feel they have a UTI now												
	Feel they have a UTI now (not smell)	0	0	0	0	0	0	0	0	0	0	0	0
	Treated for UTI within 3 days prior												
	Treated for UTI on day of sampling												
	Treated for UTI within 3 days after												
Dipstick Analysis	Glucose (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Bilrubin ('Mod' = Moderate)	-	-	-	-	-	-	-	-	-	-	-	-
	Ketone (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Specific Gravity	1.010	1.015	1.010	1.010	1.010	1.005	1.005	1.010	1.010	1.010	1.005	1.010
	Non-Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	10	0	0	0	0
	Haemolysed Blood (Ery/uL)	0	0	0	0	0	0	0	0	0	0	0	0
	pH	7	6.5	6	6.5	6	7	6.5	7.5	6.5	7.5	6.5	7
	Protein (mg/dL)	0	0	0	0	0	0	0	0	0	0	0	0
	Urobilinogen (mg/dL)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Nitrate	-	-	-	-	-	-	-	-	-	-	-	-
	Leukocytes (Leu/ul)	0	70	125	70	0	0	15	0	0	0	0	0
	HCP Outcome												
	Dip Stick Score	2	5	4	4	0	3	4	4	1	3	2	2
Protein Analysis	Urinary Creatinine (mg/dl)	35.57	39.85	61.50	34.34	66.00	30.48	25.54	74.99	46.52	60.07	39.97	92.96
	Healthy Adjusted IL-1β (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-4 (pg/ml)	0.00	1.28	1.28	0.00	0.00	1.33	0.17	0.00	0.00	0.63	0.00	0.00
	Healthy Adjusted IL-5 (pg/ml)	99.44	0.00	0.00	0.00	0.00	0.00	0.00	7.58	4.02	0.00	2.72	0.00
	Healthy Adjusted IL-6 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-8 (pg/ml)	0.00	1.07	3.89	0.00	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-10 (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IL-12 p70 (pg/ml)	0.74	42.47	13.83	0.00	0.00	3.32	21.43	0.00	0.00	0.00	10.91	11.78
	Healthy Adjusted IL-17A (pg/ml)	4.60	126.30	30.99	0.00	0.00	0.00	0.00	0.00	0.00	28.98	0.00	0.00
	Healthy Adjusted TNFα (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Healthy Adjusted IFNγ (pg/ml)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.11
Pathogen Analysis	*Escherichia coli*												
	Enterococcus faecalis												
	Streptococcus agalactiae												
	Proteus mirabilis												
	Staphylococcus aureus												
	Candida albicans												
	E. coli CFU/ml	0	0	0	0	0	0	0	0	0	0	0	0
	Diagnostic levels of *E. coli*												
	MLST												
	E. coli clade												
	fliC Serotype												
	Motile												
	Amoxycillin resistance												
	Co-amoxiclav resistance												
	Cephalexin resistance												
	Trimethoprim resistance												
	Nitrofurantoin resistance												
	Ciprofloxacin resistance												

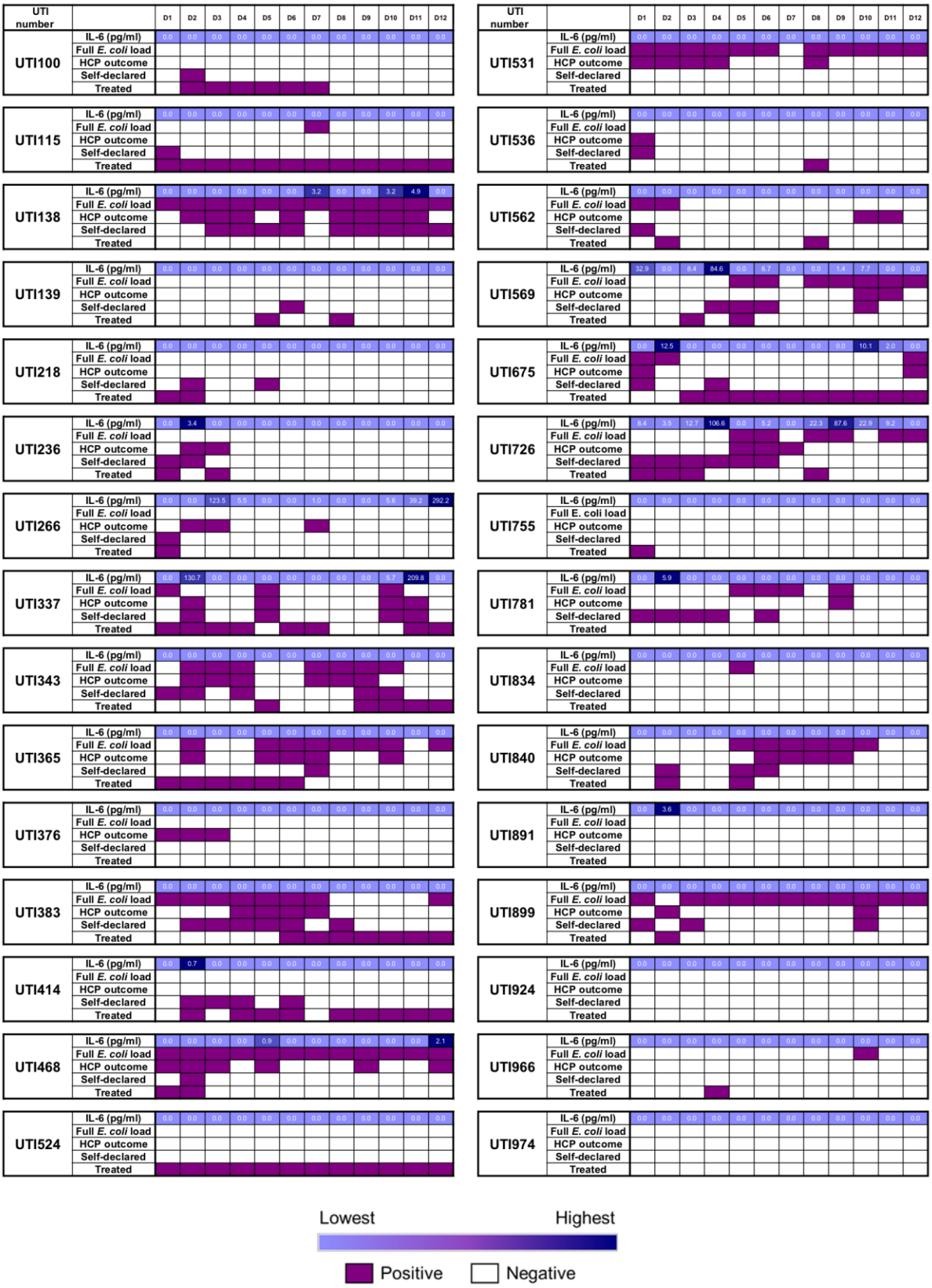
Appendix 38. Patient profile for UTI974. All study analysis combined. Green indicates a negative result and purple is a positive result. A blue filled box indicates it was not applicable, as the patient was male.



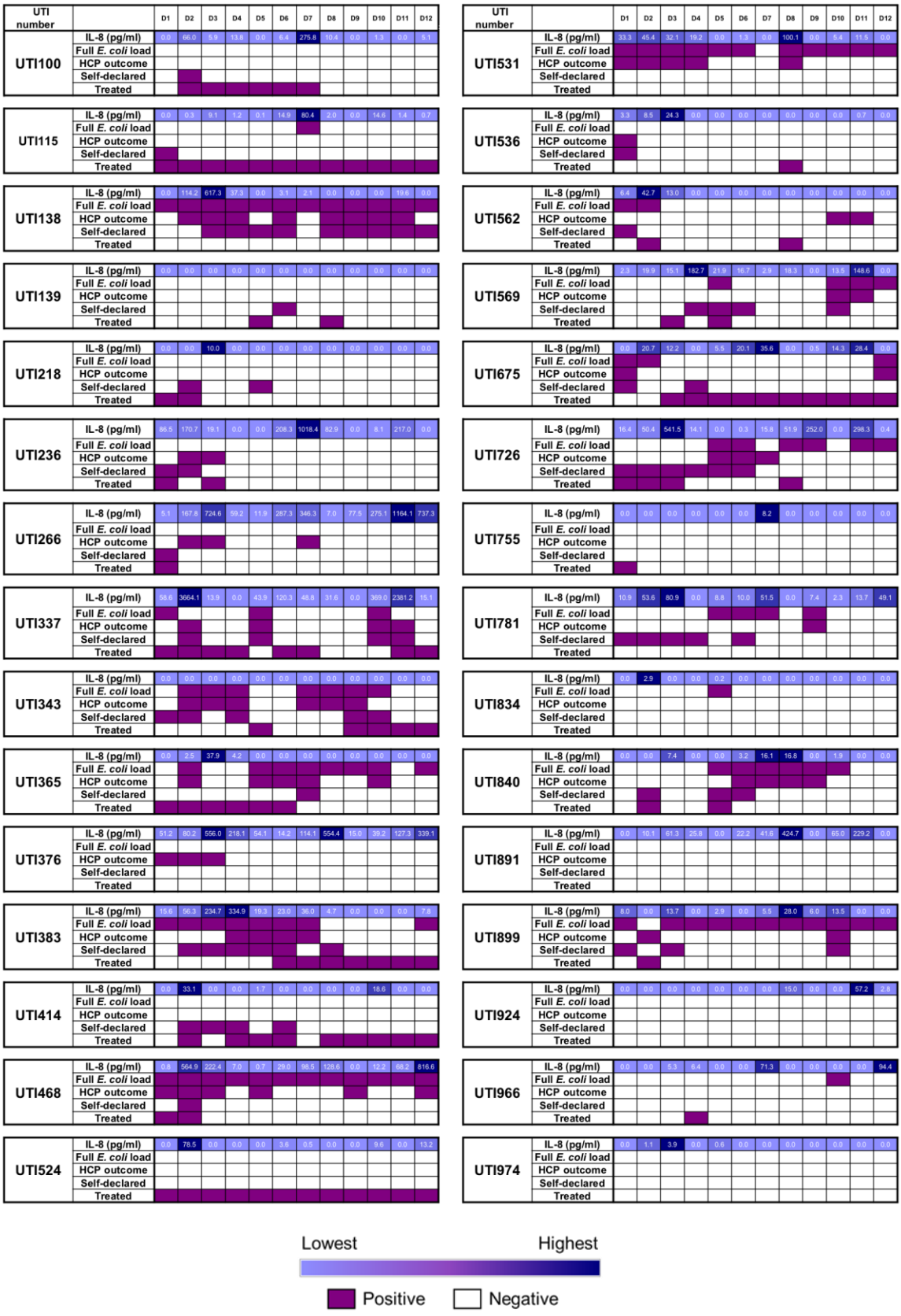
Appendix 39. IL-4 concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



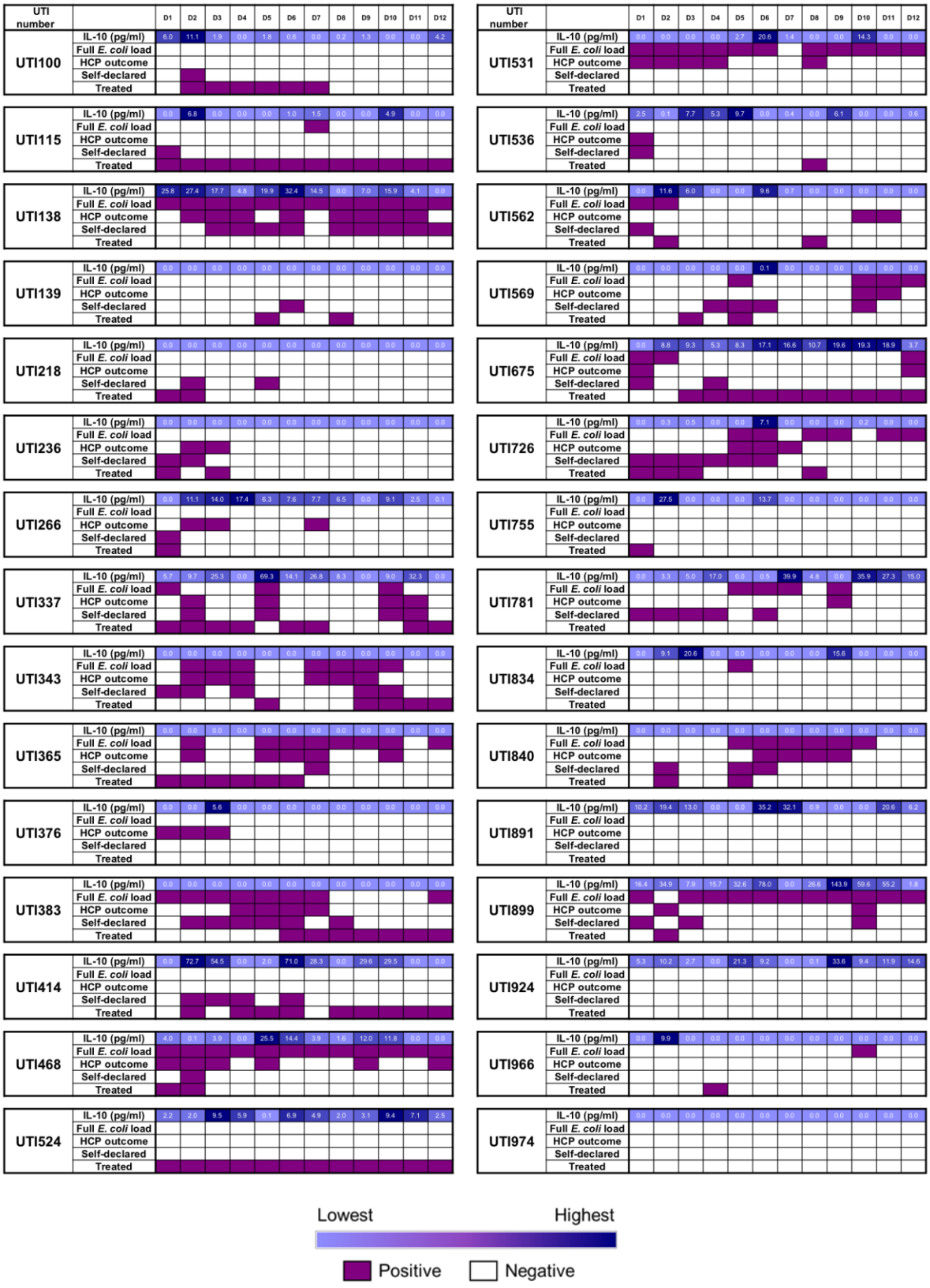
Appendix 40. IL-5 concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



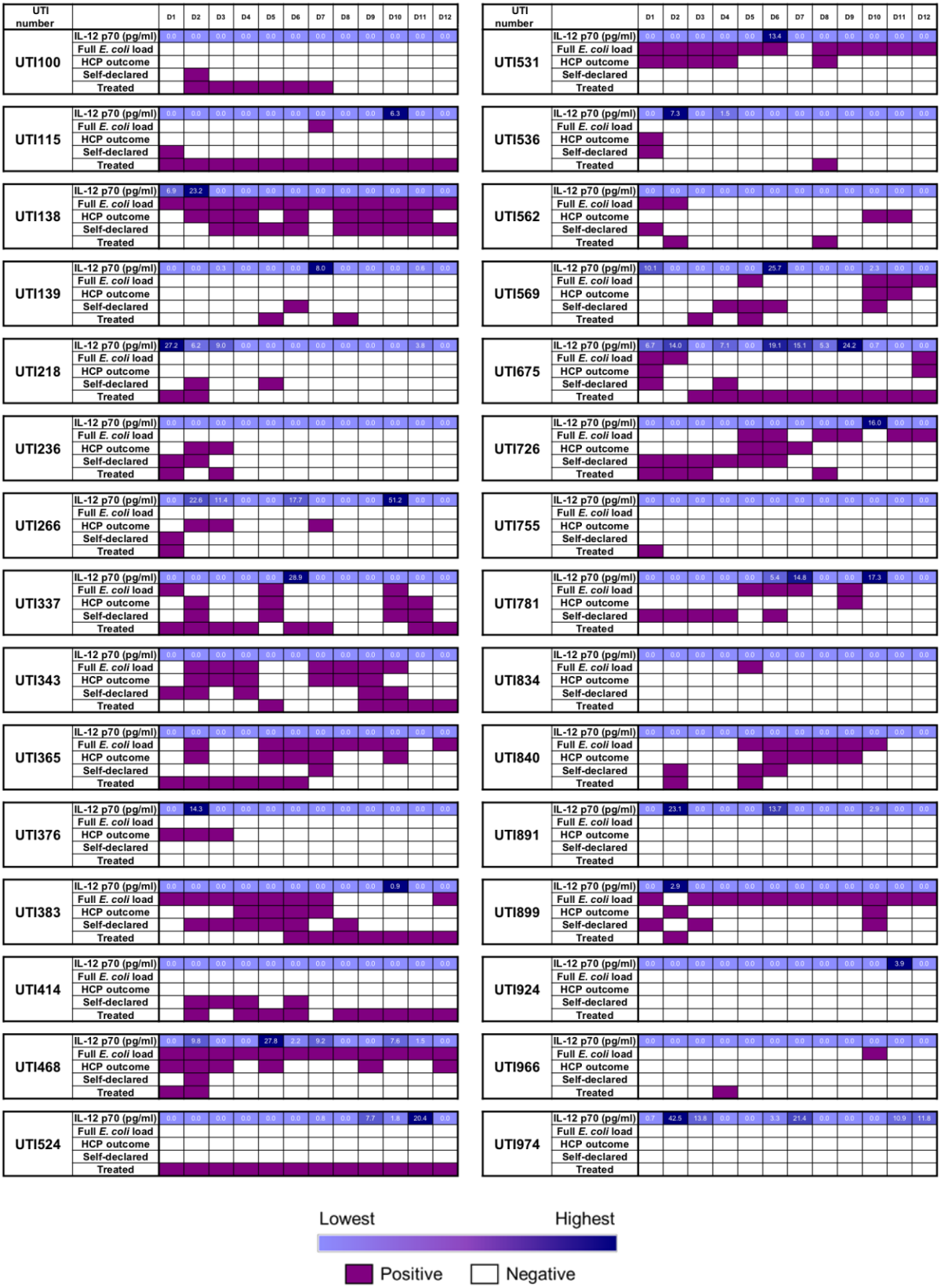
Appendix 41. IL-6 concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



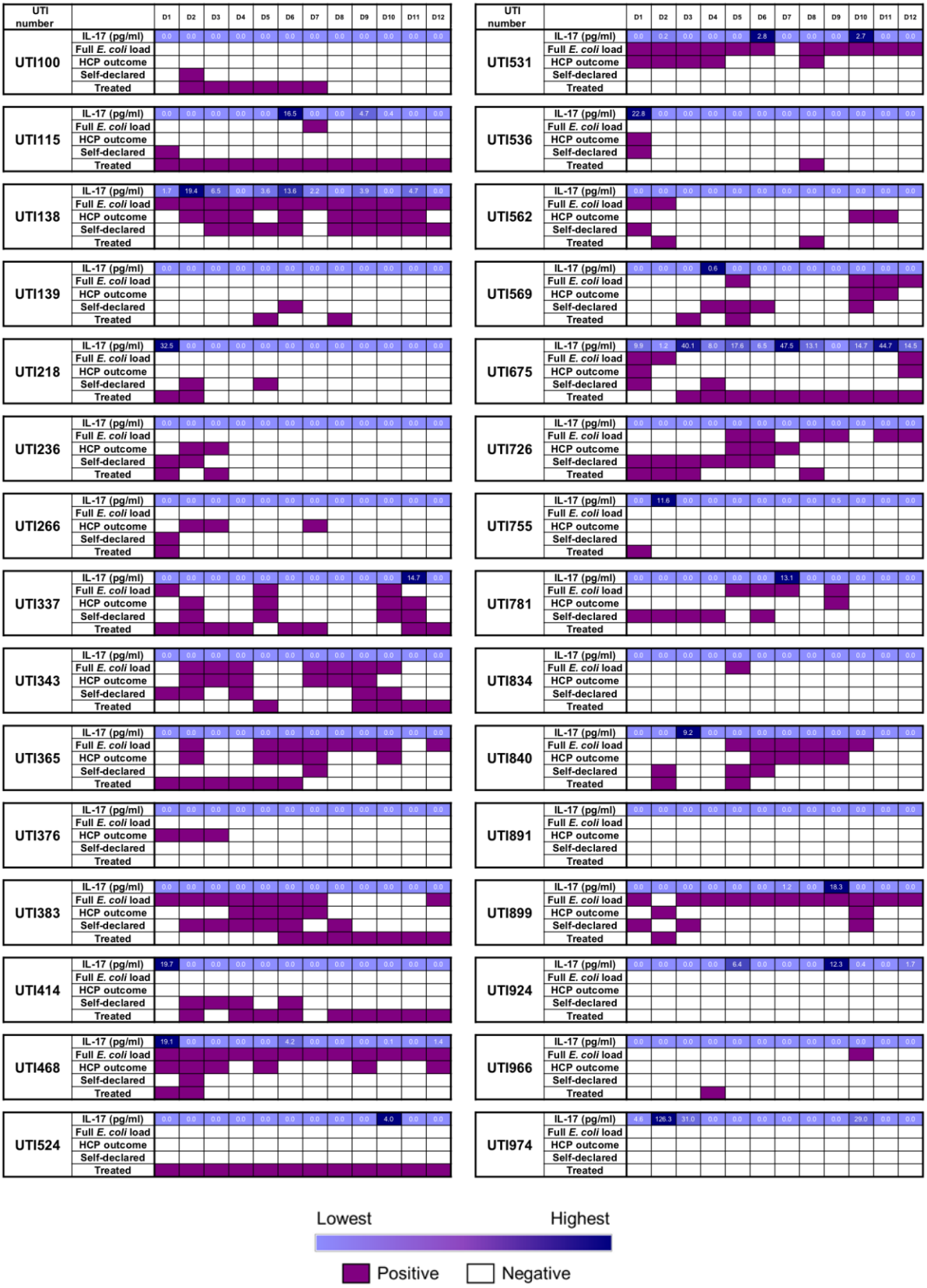
Appendix 42. IL-8 concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



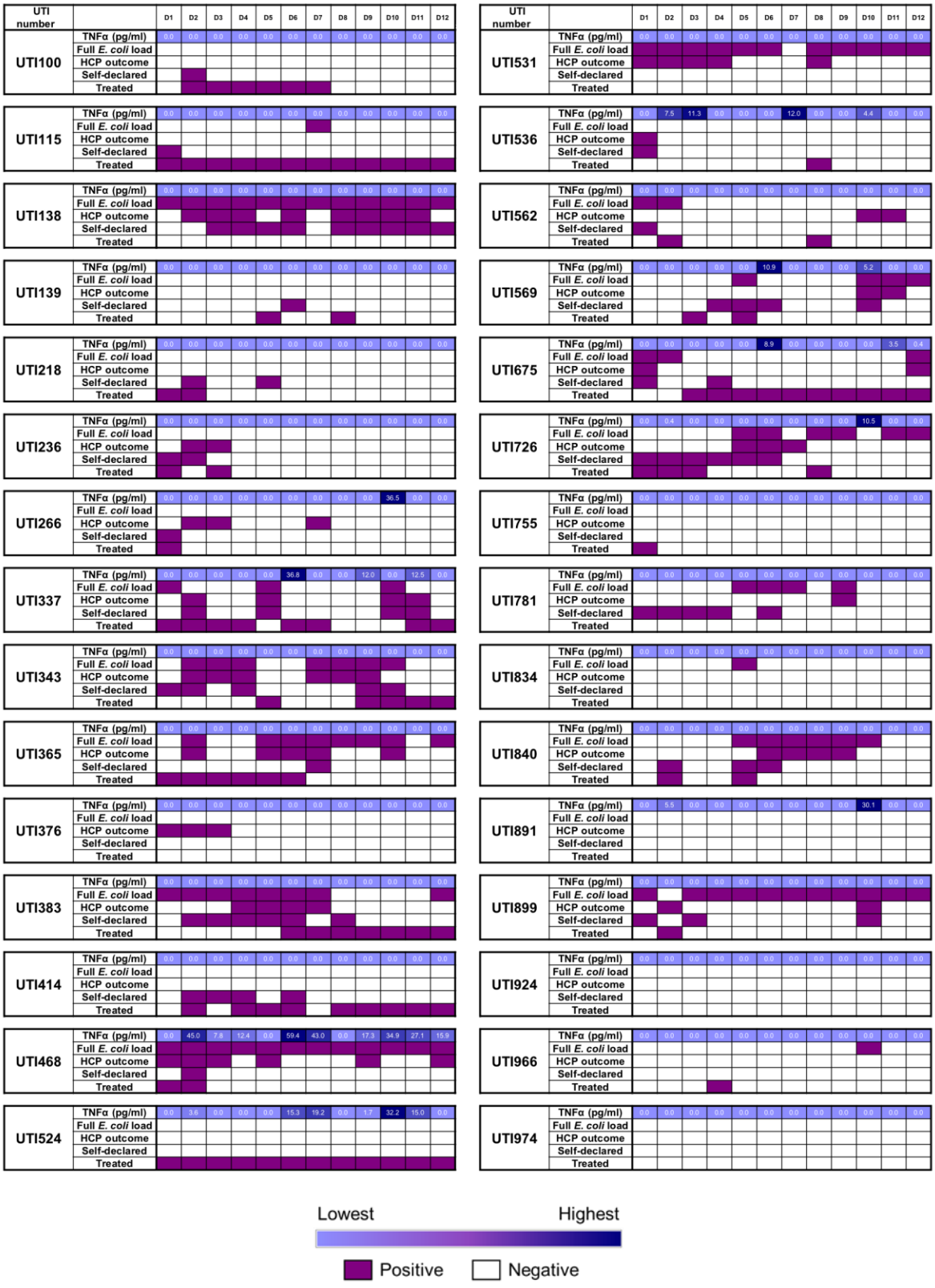
Appendix 43. IL-10 concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



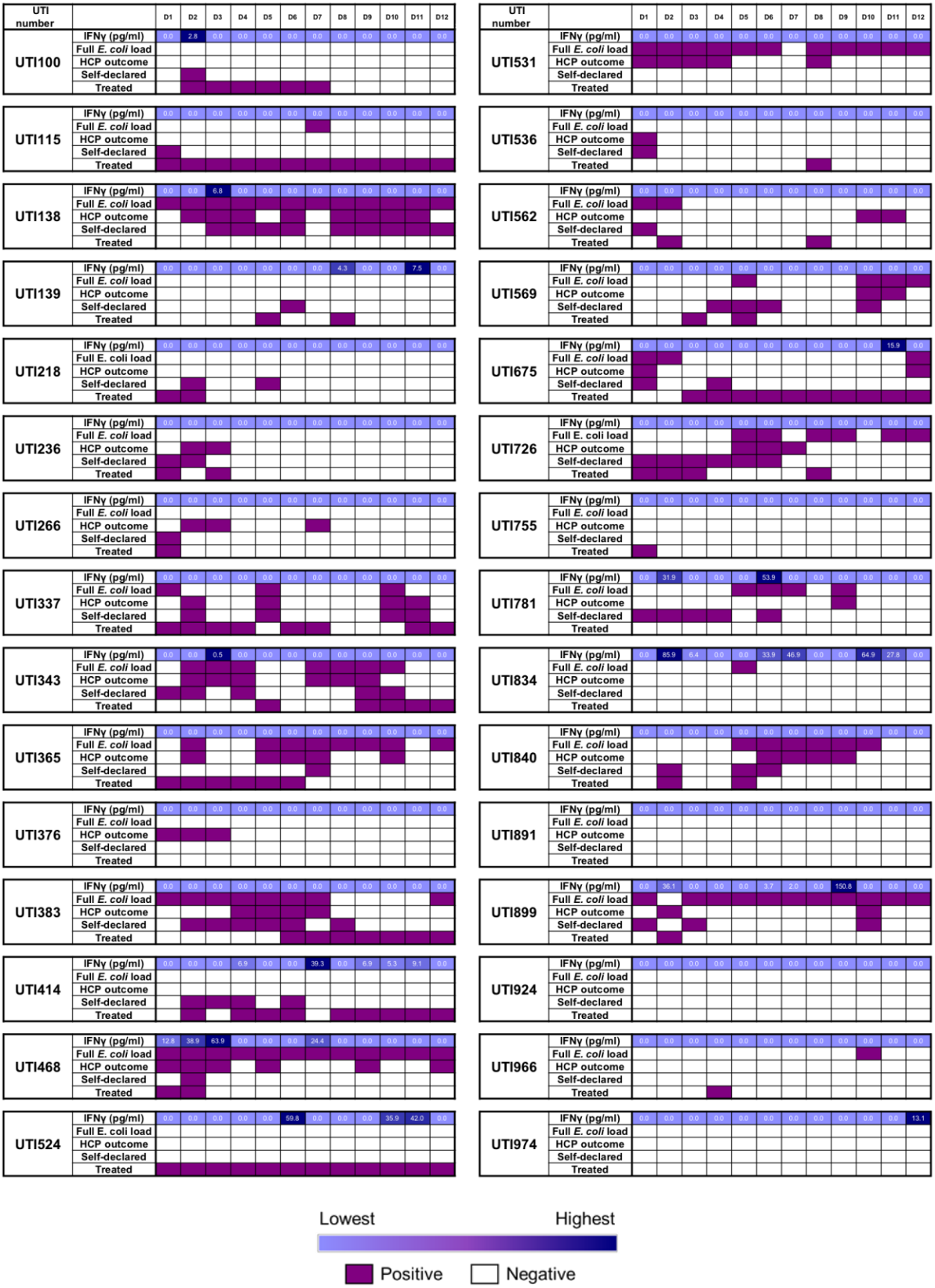
Appendix 44. IL-12 p70 concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



Appendix 45. IL-17 concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



Appendix 46. TNFα concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.



Appendix 47. IFNγ concentrations compared to *E. coli* load, HCP outcome and periods of antibiotic treatment. 'Full *E. coli* load' indicates samples containing diagnostic levels of *E. coli*. 'HCP outcome' shows if the dipstick returned a positive result for both nitrites and leukocytes. 'Self-declared' indicates where patients felt they had a UTI at the time of sampling from the questionnaire. A filled box in the 'treated' row indicates if the patients' received antibiotic treatment within 3 days of the study donation.

Chapter 11. Bibliography

1. Nicolle, L. E. Urinary tract infection in the elderly. *J. Antimicrob. Chemother.* **33,** 99–109 (1994).

2. Turner, D. *et al.* Cost effectiveness of management strategies for urinary tract infections: results from randomised controlled trial. *BMJ* **340,** c346 (2010).

3. Garau, J., Nicolau, D. P., Wullt, B. & Bassetti, M. Antibiotic stewardship challenges in the management of community-acquired infections for prevention of escalating antibiotic resistance. *J. Glob. Antimicrob. Resist.* **2,** 245–253 (2014).

4. Fowler, C. J., Griffiths, D. & de Groat, W. C. The neural control of micturition. *Nat. Rev. Neurosci.* **9,** 453–66 (2008).

5. Elbadawi, A. Functional anatomy of the organs of micturition. *Urol. Clin. North Am.* **23,** 177–210 (1996).

6. Pollock, J. S., Ryan, M. J., Samson, W. K. & Brooks, D. P. Water and electrolyte homeostasis brings balance to physiology. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307,** R481–3 (2014).

7. Luca, L. De, David, R. & Menani, J. in *Neurobiology of Body Fluid Homeostasis: Transduction and Integration* 267–274 (CRC Press/Taylor & Francis, 2014). doi:doi:10.1201/b15544-16

8. Cannon, W. B. Organization For Physiological Homeostasis. *Physiol. Rev.* **9,** 399–431 (1929).

9. Kurts, C., Panzer, U., Anders, H.-J. & Rees, A. J. The immune system and kidney disease: basic concepts and clinical implications. *Nat. Rev. Immunol.* **13,** 738–53 (2013).

10. FitzGerald, M. P., Stablein, U. & Brubaker, L. Urinary habits among asymptomatic women. *Am. J. Obstet. Gynecol.* **187,** 1384–1388 (2002).

11. Latini, J. M., Mueller, E., Lux, M. M., Fitzgerald, M. P. & Kerder, K. J. Voiding Frequency in a Sample of Asymptomatic American Men. *J. Urol.* **172,** 980–984 (2004).

12. Hickling, D. R., Sun, T.-T. & Wu, X.-R. Anatomy and Physiology of the Urinary Tract: Relation to Host Defense and Microbial Infection. *Microbiol. Spectr.* **3,** 10.1128/microbiolspec.UTI–0016–2012 (2015).

13. Hooton, T. M. *et al.* Perineal anatomy and urine-voiding characteristics of young women with and without recurrent urinary tract infections. *Clin. Infect. Dis.* **29,** 1600–1601 (1999).

14. Nicolle, L. E. Epidemiology of urinary tract infections. *Clin. Microbiol. Newsl.* **24,** 135–140 (2002).

15. Lepor, H. Pathophysiology of Benign Prostatic Hyperplasia in the Aging Male Population. *Rev. Urol.* **7,** S3–S12 (2005).

16. Nicolle, L. E. Complicated urinary tract infection in adults. *Can. J. Infect. Dis. Med. Microbiol.* **16,** 349–360 (2005).

17. Lukacz, E. S. *et al.* A healthy bladder: A consensus statement. *Int. J. Clin. Pract.* **65,** 1026–1036 (2011).

18. Grabe, M. *et al. Guidelines on Urological Infections. European Association of Urology* (2016).

19. Andersson & Arner, A. Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol. Rev.* **84,** 935–986 (2004).

20. Nicolle, L. E., McIntyre, M., Zacharias, H. & MacDonell, J. A. Twelve-month Surveillance of Infections in Institutionalized Elderly Men. *J. Am. Geriatr. Soc.* **32,** 513–519 (1984).

21. National Center for Biotechnology Information. Cystitis: Overview. *PubMed Health* (2013). Available at: http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0072587/.

22. Grover, S., Srivastava, A., Lee, R., Tewari, A. K. & Te, A. E. Role of inflammation in bladder function and interstitial cystitis. *Ther. Adv. Urol.* **3,** 19–33 (2011).

23. Fouts, D. E. *et al.* Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J. Transl. Med.* **10,** 1–17 (2012).

24. Siddiqui, H., Nederbragt, A. J., Lagesen, K., Jeansson, S. L. & Jakobsen, K. S. Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. *BMC Microbiol.* **11,** 1–12 (2011).

25. Wolfe, A. J. *et al.* Evidence of Uncultivated Bacteria in the Adult Female Bladder. *J. Clin. Microbiol.* 1376–1383 (2012). doi:10.1128/JCM.05852-11

26. Foxman, B. Epidemiology of urinary tract infections. *Am. J. Med.* **113,** 5S–13S (2002).

27. Schwan, W. R. Flagella allow uropathogenic Escherichia coli ascension into murine kidneys. *Int. J. Med. Microbiol.* **298,** 441–447 (2008).

28. Scottish Intercollegiate Guidelines Network. *Scottish Intercollegiate Guidelines Network (SIGN). Management of suspected bacterial urinary tract infection in adults. (SIGN publication no. 88)* (2012).

29. Boscia, J. A. *et al.* A lack of association between bacteriuria and symptoms in the elderly. *Am. J. Med.* **81,** 979–982 (1986).

30. Nicolle, L. E. Bacteriuria in the elderly : a review Pathogenesis. *Geriatr. Nephrol. Urol.* **1,** 163–172 (1992).

31. Kunin, C. M. *Urinary Tract Infections: Detection, Prevention and Management*. (Williams & Wilkins., 1997).

32. Foxman, B. Recurring Urinary Tract Infection: Incidence and Risk Factors. *Am. J. Public Health* **80,** 331–333 (1990).

33. Rosen, D. A., Hooton, T. M., Stamm, W. E., Humphrey, P. A. & Hultgren, S. J. Detection of Intracellular Bacterial Communities in Human Urinary Tract Infection. *PLoS Med.* **4,** 1949–1958 (2007).

34. Albert, X. *et al.* Antibiotics for preventing recurrent urinary tract infection in non-pregnant women. *Cochrane Database Syst. Rev*. (2004).

35. Public Health England. *Management of infection guidance for primary care for consultation and local adaptation About Public Health England*. (2016).

36. Falagas, M. E., Kastoris, A. C., Kapaskelis, A. M. & Karageorgopoulos, D. E. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrumm β-lactamase producing, Enterobacteriaceae infections: a systematic review. *Lancet Infect. Dis.* **10,** 43–50 (2010).

37. MHRA. *Public Assessment Report, Decentralised Procedure, FOSFOMYCIN 3G GRANULES FOR ORAL SOLUTION*. (2013).

38. Gupta, K., Hooton, T. M. & Stamm, W. E. Increasing Antimicrobial Resistance and the Management of Uncomplicated Community-Acquired Urinary Tract Infections. *Ann. Intern. Med.* **135,** 41–50 (2001).

39. The National Institute for Health and Care Excellence (NICE). *Infection - Prevention and control of healthcare-associated infections in primary and community care*. (2012).

40. Nicolle, L. E., Bjornson, J., Harding, G. K. M. & MacDonell, J. A. Bacteriuria in Elderly Institutionalized Men. *N. Engl. J. Med.* **309,** 1420–1425 (1983).

41. Nicolle, L. E., Mayhew, W. J. & Bryan, L. Prospective randomized comparison of therapy and no therapy for asymptomatic bacteriuria in institutionalized elderly women. *Am. J. Med.* **83,** 27–33 (1987).

42. Trautner, B. W. Asymptomatic bacteriuria: when the treatment is worse than the disease. *Nat Rev Urol* **9,** 85–93 (2012).

43. Davies, J. & Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev*. **74,** 417–433 (2010).

44. Schito, G. C. *et al.* The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *Int. J. Antimicrob. Agents* **34,** 407–413 (2009).

45. Kahlmeter, G. Prevalence and antimicrobial susceptibility of pathogens in uncomplicated cystitis in Europe. The ECO.SENS study. *Int. J. Antimicrob. Agents* **22,** (2003).

46. Gupta, K., Sahm, D. F., Mayfield, D. & Stamm, W. E. Antimicrobial Resistance Among Uropathogens that Cause Community-Acquired Urinary Tract Infections in Women: A Nationwide Analysis. *Clin. Infect. Dis.* **33,** 89–94 (2001).

47. Tancrede, C. Role of human microflora in health and disease. *Eur. J. Clin. Microbiol. Infect. Dis.* **11,** 1012–1015 (1992).

48. Seelig, M. S. The role of antibiotics in the pathogenesis of Candida infections. *Am. J. Med.* **40,** 887–917 (2016).

49. Weinberger, M., Sweet, S., Leibovici, L., Pitlik, S. D. & Samra, Z. Correlation between candiduria and departmental antibiotic use. *J. Hosp. Infect.* **53,** 183–186 (2003).

50. Cai, T. *et al.* The role of asymptomatic bacteriuria in young women with recurrent urinary tract infections: To treat or not to treat? *Clin. Infect. Dis.* **55,** 771–777 (2012).

51. Nordenstam, G. R., Brandberg, C. Å., Odén, A. S., Svanborg Edén, C. M. & Svanborg, A. Bacteriuria and Mortality in an Elderly Population. *N. Engl. J. Med.* **314,** 1152–1156 (1986).

52. Lindberg, U., Claesson, I., Hanson, L. Å. & Jodal, U. Asymptomatic bacteriuria in schoolgirls. *J. Pediatr.* **92,** 194–199 (1978).

53. Sundén, F., Håkansson, L., Ljunggren, E. & Wullt, B. Escherichia coli 83972 Bacteriuria Protects Against Recurrent Lower Urinary Tract Infections in Patients With Incomplete Bladder Emptying. *J. Urol.* **184,** 179–185 (2016).

54. Köves, B. *et al.* Rare Emergence of Symptoms during Long-Term Asymptomatic Escherichia coli 83972 Carriage without an Altered Virulence Factor Repertoire. *J. Urol.* **191,** 519–528 (2016).

55. Dulawa, J. Urinary tract infection. *Rocz. Akad. Med. Bialymstoku* **49,** 182–184 (2004).

56. Curns, A., Holman, R., Sejvar, J., Owings, M. & Schonberger, L. INfectious disease hospitalizations among older adults in the united states from 1990 through 2002. *Arch. Intern. Med.* **165,** 2514–2520 (2005).

57. Hooton, T. M. Recurrent urinary tract infection in women. *Int. J. Antimicrob. Agents* **17,** 259–268 (2001).

58. Nielubowicz, G. R. & Mobley, H. L. T. Host-pathogen interactions in urinary tract infection. *Nat. Rev. Urol.* **7,** 430–441 (2010).

59. Gruneberg, R. N. Relationship of infecting urinary organism to the faecal flora in patients with symptomatic urinary infection. *Lancet* **2,** 766–768 (1969).

60. Yamamoto, S. *et al.* Genetic Evidence Supporting the Fecal-Perineal-Urethral Hypothesis in Cystitis Caused by Escherichia Coli. *J. Urol.* **157,** 1127–1129 (1997).

61. Kucheria, R., Dasgupta, P., Sacks, S. H., Khan, M. S. & Sheerin, N. S. Urinary tract infections: new insights into a common problem. *Postgrad. Med. J.* **81,** 83–6 (2005).

62. Ruben, F. L. *et al.* Clinical infections in the noninstitutionalized geriatric age group: methods utilized and incidence of infections. The Pittsburgh Good Health Study. *Am. J. Epidemiol.* **141,** 145–157 (1995).

63. Kaye, D. Urinary tract infections in the elderly. *Bull. N. Y. Acad. Med.* **56,** 209–220 (1980).

64. Cowart, S. L. & Stachura, M. A. X. E. in *Clinical Methods: The History, Physical, and Laboratory Examinations* (eds. Walker, H., Hall, W. & Hurst, J.) (Butterworths, 1990).

65. Roxe, D. M. in *Clinical Methods: The History, Physical, and Laboratory Examinations* (eds. Walker, H., Hall, W. & Hurts, J.) **3rd editio,** (Butterworths, 1990).

66. Semeniuk, H. Evaluation of the Leukocyte Esterase and Nitrite Urine Dipstick Screening Tests for Detection of Bacteriuria in Women with Suspected Uncomplicated Urinary Tract Infections. *J. Clin. Microbiol.* **37,** 3051–3052 (1999).

67. Sundvall, P.-D. *et al.* Interleukin-6 concentrations in the urine and dipstick analyses were related to bacteriuria but not symptoms in the elderly: a cross sectional study of 421 nursing home residents. *BMC Geriatr.* **14,** 88 (2014).

68. Eigbefoh, J. O., Isabu, P., Okpere, E. & Abebe, J. The diagnostic accuracy of the rapid dipstick test to predict asymptomatic urinary tract infection of pregnancy. *J. Obstet. Gynaecol. (Lahore).* **28,** 490–5 (2008).

69. Seeler, R. A. Hemolysis due to gram-negative urinary tract infection. *Birth Defects Orig. Artic. Ser.* **13,** 425–431 (1977).

70. Palocaren, T. *et al.* Beta haemolytic group A streptococci causing urinary tract infection. *Br. J. Urol.* **74,** 444–446 (1994).

71. Hall, K. K. & Lyman, J. A. Updated Review of Blood Culture Contamination. *Clin. Microbiol. Rev.* **19,** 788–802 (2006).

72. Gupta, K. *et al.* International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin. Infect. Dis.* **52,** (2011).

73. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk susceptibility tests*. (1997).

74. Ellington, M. J. *et al.* Routine Use of Microbial Whole Genome Sequencing in Diagnostic and Public Health Microbiology. *PLoS Pathog.* **8,** 1–9 (2012).

75. Perrone, R. D., Madias, N. E. & Levey, A. S. Serum creatinine as an index of renal function: new insights into old concepts. *Clin. Chem.* **38,** 1933–1953 (1992).

76. Morrison, G. in *Clinical Methods: The History, Physical, and Laboratory Examinations* 890–894 (Butterworths, 1990).

77. Rastegar, A. in *Clinical Methods: The History, Physical, and Laboratory*

Examinations 884–887 (Butterworths, 1990).

78. Hosten, A. O. in *Clinical Methods, The History, Physical, and Laboratory Examinations* (ed. Butterworths) 874–878 (1990).

79. Nseir, W., Taha, M., Nemarny, H. & Mograbi, J. The association between serum levels of vitamin D and recurrent urinary tract infections in premenopausal women. *Int. J. Infect. Dis.* **17,** e1121–e1124 (2013).

80. Kwon, Y. E. *et al.* Vitamin D deficiency is an independent risk factor for urinary tract infections after renal transplants. *Medicine (Baltimore).* **94,** e594 (2015).

81. Tekin, M. *et al.* The Association between Vitamin D Levels and Urinary Tract Infection in Children. *Horm. Res. Paediatr.* **83,** 198–203 (2015).

82. Singhal, N., Kumar, M., Kanaujia, P. K. & Virdi, J. S. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. *Front. Microbiol.* **6,** 791 (2015).

83. Valentine, N., Wunschel, S., Wunschel, D., Petersen, C. & Wahl, K. Effect of Culture Conditions on Microorganism Identification by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry. *Appl. Environ. Microbiol.* **71,** 58–64 (2005).

84. Riley, L. W. *Molecular epidemiology of infectious diseases: principles and practices*. (ASM Press, 2004).

85. Maiden, M. C. J. *et al.* Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc. Natl. Acad. Sci. U. S. A.* **95,** 3140–3145 (1998).

86. Zhang, L. & Foxman, B. Molecular epidemiology of Escherichia coli mediated urinary tract infections. *Front. Biosci.* **8,** (2003).

87. Mandlik, A. *et al.* RNA-seq-based monitoring of infection-linked changes in Vibrio cholerae gene expression. *Cell Host Microbe* **10,** 165–174 (2011).

88. Wirth, T. *et al.* Sex and virulence in Escherichia coli: An evolutionary perspective. *Mol. Microbiol.* **60,** 1136–1151 (2006).

89. Tartof, S. Y., Solberg, O. D., Manges, A. R. & Riley, L. W. Analysis of a Uropathogenic Escherichia coli Clonal Group by Multilocus Sequence Typing. *J. Clin. Microbiol.* **43,** 5860–5864 (2005).

90. McNally, A., Cheng, L., Harris, S. R. & Corander, J. The evolutionary path to extraintestinal pathogenic, drug-resistant Escherichia coli is marked by drastic reduction in detectable recombination within the core genome. *Genome Biol. Evol.* **5,** 699–710 (2013).

91. Foxman, B. *et al.* Urinary tract infection among women aged 40 to 65: Behavioral and sexual risk factors. *J. Clin. Epidemiol.* **54,** 710–718 (2001).

92. Kaśs, E. H. Asymptomatic infections of the urinary tract. *Trans. Assoc. Am. Physicians*

69, 56–64 (1956).

93. Barnett, B. J. & Stephens, D. S. Urinary tract infection: An overview. *Am. J. Med. Sci.* **314,** 245–249 (1997).

94. Hedges, S. *et al.* Comparison of Urine and Serum Concentrations of Interleukin-6 in Women with Acute Pyelonephritis or Asymtomatic Bacteriuria. *J. Infect. Dis.* **166,** 653–656 (1992).

95. Lewis, D. A. *et al.* The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front. Cell. Infect. Microbiol.* **3,** 41 (2013).

96. Kunin, C. M. The natural history of recurrent bacteriuria in schoolgirls. *N. Engl. J. Med.* **282,** 1443–1448 (1970).

97. Hooton, T. M. *et al.* A prospective study of asymptomatic bacteriuria in sexually active young women. *N. Engl. J. Med.* **343,** 992–997 (2000).

98. Grabe, M. *et al. Guidelines on Urological Infections*. (2015).

99. Campbell, N. A., Reece, J. B. & Mitchell, L. G. in *Campbell Biology* (The Benjamin Cummings Publishing Company, 2002).

100. Kaper, J. B. Pathogenic Escherichia coli. *Int. J. Med. Microbiol.* **295,** 355–356 (2004).

101. Stamm, W. An Epidemic of Urinary Tract Infections? *The New England Journal of Medicine* **345,** 1055–1057 (2001).

102. Bishop, B. L. *et al.* Cyclic AMP-regulated exocytosis of *Escherichia coli* from infected bladder epithelial cells. *Nat. Med.* **13,** 625–630 (2007).

103. Spurbeck, R. R. *et al.* Fimbrial Profiles Predict Virulence of Uropathogenic Escherichia coli Strains : Contribution of Ygi and Yad Fimbriae . *Infect. Immun.* **79,** 4753–4763 (2011).

104. Martinez, J. J., Mulvey, M. A., Schilling, J. D., Pinkner, J. S. & Hultgren, S. J. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *EMBO J.* **19,** 2803–2812 (2000).

105. Wright, K. J., Seed, P. C. & Hultgren, S. J. Development of intracellular bacterial communities of uropathogenic Escherichia coli depends on type 1 pili. *Cell. Microbiol.* **9,** 2230–2241 (2007).

106. Jones, C. H. *et al.* FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the Enterobacteriaceae. *Proc. Natl. Acad. Sci. U. S. A.* **92,** 2081–2085 (1995).

107. Johnson, J. R. Virulence factors in Escherichia coli urinary tract infection. *Clin. Microbiol. Rev.* **4,** 80–128 (1991).

108. Lindberg, F., Lund, B., Johansson, L. & Normark, S. Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. *Nature* **328,** 84–87 (1987).

109. Abraham, S. N., Sun, D., Dale, J. B. & Beachey, E. H. Conservation of the D-mannose-adhesion protein among type 1 fimbriated members of the family Enterobacteriaceae. *Nature* **336,** 682–684 (1988).

110. Leffler, H. & Svanborg-Edén, C. Glycolipid receptors for uropathogenic Escherichia coli on human erythrocytes and uroepithelial cells. *Infect. Immun.* **34,** 920–929 (1981).

111. Leffler, H. & Edén, C. S. Chemical identification of a glycosphingolipid receptor for Escherichia coli attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. *FEMS Microbiol. Lett.* **8,** 127–134 (1980).

112. Krogfelt, K. A., Bergmans, H. & Klemm, P. Direct evidence that the FimH protein is the mannose-specific adhesin of Escherichia coli type 1 fimbriae. *Infect. Immun.* **58,** 1995–1998 (1990).

113. Bahrani-mougeot, F. K. *et al.* Type 1 fimbriae and extracellular polysaccharides are preeminent uropathogenic Escherichia coli virulence determinants in the murine urinary tract. *Mol. Microbiol.* **45,** 1079–1093 (2002).

114. Connell, I. *et al.* Type 1 fimbrial expression enhances Escherichia coli virulence for the urinary tract. *Proc. Natl. Acad. Sci. U. S. A.* **93,** 9827–9832 (1996).

115. Henderson, J. P. *et al.* Quantitative Metabolomics Reveals an Epigenetic Blueprint for Iron Acquisition in Uropathogenic Escherichia coli. *PLoS Pathog.* **5,** 1–11 (2009).

116. Dhakal, B. K. & Mulvey, M. A. The UPEC Pore Forming Toxin α-Hemolysin Triggers Proteolysis of Host Proteins to Disrupt Cell Adhesion, Inflammatory and Survival Pathways. *Cell Host Microbe* **11,** 58–69 (2012).

117. Whitfield, C. & Valvano, M. a. Biosynthesis and expression of cell-surface polysaccharides in gram-negative bacteria. *Adv. Microb. Physiol.* **35,** 135–246 (1993).

118. Dobrindt, U., Hochhut, B., Hentschel, U. & Hacker, J. Genomic islands in pathogenic and environmental microorganisms. *Nat. Rev. Microbiol.* **2,** 414–425 (2004).

119. Bingen, E. *et al.* Phylogenetic Analysis of Escherichia coli Strains Causing Neonatal Meningitis Suggests Horizontal Gene Transfer from a Predominant Pool of Highly Virulent B2 Group Strains. *J. Infect. Dis.* **117,** 642–650 (1998).

120. Wise, R. *et al.* Antimicrobial resistance is a major threat to public health. *BMJ* **317,** 609 LP – 610 (1998).

121. Kraker, M. E. A. De *et al.* The changing epidemiology of bacteraemias in Europe : trends from the European Antimicrobial Resistance Surveillance System. *Clin. Microbiol. Infect.* **19,** 860–868 (2012).

122. Clermont, O., Bonacorsi, S. & Bingen, E. Rapid and simple determination of the Escherichia coli phylogenetic group. *Appl. Environ. Microbiol.* **66,** 4555–4558 (2000).

123. Herzer, P. J., Inouye, S., Inouye, M. & Whittam, T. S. Phylogenetic Distribution of Branched Rna-Linked Multicopy Single-Stranded-Dna Among Natural Isolates of Escherichia-Coli. *J. Bacteriol.* **172,** 6175–6181 (1990).

124. Toval, F. *et al.* Characterization of Escherichia coli isolates from hospital inpatients or outpatients with urinary tract infection. *J. Clin. Microbiol.* **52,** 407–418 (2014).

125. Boyd, E. F. & Hartl, D. L. Chromosomal Regions Specific to Pathogenic Isolates of Escherichia coli Have a Phylogenetically Clustered Distribution Chromosomal Regions Specific to Pathogenic Isolates of Escherichia coli Have a Phylogenetically Clustered Distribution. *J. Bacteriol.* **180,** 1159–1165 (1998).

126. Abraham, C. & Medzhitov, R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. *Gastroenterology* **140,** 1729–1737 (2011).

127. Janeway Jr., C. Introduction: The role of innate immunity in the adaptive immune response. *Semin. Immunol.* **10,** 349–350 (1998).

128. Janeway, C. A. & Medzhitov, R. Innate immune recognition. *Annu. Rev. Immunol.* **20,** 197–216 (2002).

129. Takeda, K., Kaisho, T. & Akira, S. Toll-like receptors. *Annu. Rev. Immunol.* **21,** 335–376 (2003).

130. Ragnarsdottir, B., Lutay, N., Gronberg-Hernandez, J., Koves, B. & Svanborg, C. Genetics of innate immunity and UTI susceptibility. *Nat Rev Urol* **8,** 449–468 (2011).

131. Hayashi, F. *et al.* The innate immune response to bacterial flagellin is mediated by Toll- like receptor 5. *Nature* **410,** 1099–103. (2001).

132. Baeuerle, P. A. & Baltimore, D. I kappa B: a specific inhibitor of the NF-kappa B transcription factor. *Science (80-.).* **242,** 540–546 (1988).

133. Rhee, S. H., Keates, A. C., Moyer, M. P. & Pothoulakis, C. MEK Is a Key Modulator for TLR5-induced Interleukin-8 and MIP3-alpha Gene Expression in Non-transformed Human Colonic Epithelial Cells. *J. Biol. Chem.* **279,** 25179–25188 (2004).

134. Zhang, D. *et al.* A Toll-like Receptor That Prevents Infection by Uropathogenic Bacteria. *Science (80-.).* **303,** 1522 LP – 1526 (2004).

135. Macnab, R. Genetics and biogenesis of bacterial flagella. *Annu. Rev. Genet.* **261,** 131–158 (1992).

136. Minamino, T. & Namba, K. Self-assembly and type III protein export of the bacterial flagellum. *J. Mol. Microbiol. Biotechnol.* **7,** 5–17 (2004).

137. Turner, L., Ryu, W. S. & Berg, H. C. Real-time imaging of fluorescent flagellar filaments. *J. Bacteriol.* **182,** 2793–2801 (2000).

138. Kim, M., Bird, J. C., Van Parys, A. J., Breuer, K. S. & Powers, T. R. A macroscopic scale model of bacterial flagellar bundling. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 15481–15485 (2003).

139. Smith, K. D. *et al.* Toll-like receptor 5 recognizes a conserved site on flagellin required for protofilament formation and bacterial motility. *Nat. Immunol.* **4,** 1247–1253

(2003).

140. Gewirtz, A. T., Navas, T. a, Lyons, S., Godowski, P. J. & Madara, J. L. Cutting Edge: Bacterial Flagellin Activates Basolaterally Expressed TLR5 to Induce Epithelial Proinflammatory Gene Expression. *J. Immunol.* **167,** 1882–1885 (2001).

141. Wright, K. J., Seed, P. C. & Hultgren, S. J. Uropathogenic Escherichia coli Flagella Aid in Efficient Urinary Tract Colonization Uropathogenic Escherichia coli Flagella Aid in Efficient Urinary Tract Colonization. *Infect. Immun.* **73,** 7657–7668 (2005).

142. Lane, M. C., Alteri, C. J., Smith, S. N. & Mobley, H. L. T. Expression of flagella is coincident with uropathogenic Escherichia coli ascension to the upper urinary tract. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 16669–16674 (2007).

143. O'Neill, L. A. J., Golenbock, D. & Bowie, A. G. The history of Toll-like receptors-redefining innate immunity. *Nat. Rev. Immunol.* **13,** 453–460 (2013).

144. Elner, V. M. *et al.* Neutrophil chemotactic factor (IL-8) gene expression by cytokine-treated retinal pigment epithelial cells. *Am. J. Pathol.* **136,** 745–50 (1990).

145. Yang, D., Biragyn, A., Kwak, L. W. & Oppenheim, J. J. Mammalian defensins in immunity: More than just microbicidal. *Trends Immunol.* **23,** 291–296 (2002).

146. Wimley, W. C. Describing the Mechanism of Antimicrobial Peptide Action with the Interfacial Activity Model. *ACS Chem. Biol.* **5,** 905–917 (2010).

147. Zhang, J.-M. & An, J. Cytokines, Inflammation and Pain. *Int. Anesthesiol. Clin.* **45,** 27–37 (2007).

148. Zaiou, M. Multifunctional antimicrobial peptides: Therapeutic targets in several human diseases. *J. Mol. Med.* **85,** 317–329 (2007).

149. Paul, W. E. & Seder, R. A. Lymphocyte responses and cytokines. *Cell* **76,** 241–251 (1994).

150. Cohen, M. C. & Cohen, S. Cytokine function: a study in biologic diversity. *Am. J. Clin. Pathol.* **105,** 589 LP – 598 (1996).

151. Baggiolini, M., Dewald, B. & Moser, B. Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. *Adv. Immunol.* **55,** 97–179 (1994).

152. Beg, A. A., Finco, T. S., Nantermet, P. V & Baldwin, A. S. Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of I kappa B alpha: a mechanism for NF-kappa B activation. *Mol. Cell. Biol.* **13,** 3301–3310 (1993).

153. Davidoff, R., Yamaguchi, R., Leach, G. E., Park, E. & Lad, P. M. Multiple urinary cytokine levels of bacterial cystitis. *J. Urol.* **157,** 1980–1985 (1997).

154. Martins, S. M., Darlin, D. J., Lad, P. M. & Zimmern, P. E. Interleukin-1B: A clinically relevant urinary marker. *J. Urol.* **151,** 1198–1201 (1994).

155. Rodhe, N., Löfgren, S., Strindhall, J., Matussek, A. & Mölstad, S. Cytokines in urine

in elderly subjects with acute cystitis and asymptomatic bacteriuria. *Scand. J. Prim. Health Care* **27,** 74–79 (2009).

156. Le Gros, G., Ben-Sasson, S. Z., Seder, R., Finkelman, F. D. & Paul, W. E. Generation of interleukin 4 (IL-4)-producing cells in vivo and in vitro: IL-2 and IL-4 are required for in vitro generation of IL-4-producing cells. *J. Exp. Med.* **172,** 921–9 (1990).

157. Swain, S. L., Weinberg, A. D., English, M. & Huston, G. IL-4 directs the development of Th2-like helper effectors. *J. Immunol.* **145,** 3796–3806 (1990).

158. Noelle, R. J., Daum, J., Bartlett, W. C., McCann, J. & Shepherd, D. M. Cognate interactions between helper T cells and B cells. V. Reconstitution of T helper cell function using purified plasma membranes from activated Th1 and Th2 helper cells and lymphokines. *J.Immunol.* **146,** 1118–1124 (1991).

159. Asadi Karam, M. R., Oloomi, M., Mahdavi, M., Habibi, M. & Bouzari, S. Vaccination with recombinant FimH fused with flagellin enhances cellular and humoral immunity against urinary tract infection in mice. *Vaccine* **31,** 1210–1216 (2013).

160. Zhou, X., Fragala, M. S., McElhaney, J. E. & Kuchel, G. A. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Curr. Opin. Clin. Nutr. Metab. Care* **13,** 541–547 (2010).

161. Hannan, T. J., Mysorekar, I. U., Hung, C. S., Isaacson-Schmid, M. L. & Hultgren, S. J. Early severe inflammatory responses to uropathogenic E. coli predispose to chronic and recurrent urinary tract infection. *PLoS Pathog.* **6,** 29–30 (2010).

162. Esuvaranathan, K. *et al.* Interleukin-6 Production by Bladder Tumors is Upregulated by BCG Immunotherapy. *J. Urol.* **154,** 572–575 (1995).

163. Xing, Z. *et al.* IL-6 is an antiinflammatory cytokine required for controlling local or systemic acute inflammatory responses. *J. Clin. Invest.* **101,** 311–320 (1998).

164. Schindler, R. *et al.* Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* **75,** 40 LP – 47 (1990).

165. De Man, P. *et al.* Interleukin-6 induced at mucosal surfaces by gram-negative bacterial infection. *Infect. Immun.* **57,** 3383–3388 (1989).

166. Hedges, S. *et al.* Uroepithelial cells are part of a mucosal cytokine network. *Infect. Immun.* **62,** 2315–2321 (1994).

167. Masure, S., Proost, P., Van Damme, J. & Opdenakker, G. Purification and identification of 91-kDa neutrophil gelatinase. Release by the activating peptide interleukin-8. *Eur. J. Biochem.* **198,** 391–398 (1991).

168. Ko, Y. C. *et al.* Elevated interleukin-8 levels in the urine of patients with urinary tract infections. *Infect. Immun.* **61,** 1307–1314 (1993).

169. Agace, W. W. The role of the epithelial cell in Escherichia coli induced neutrophil migration into the urinary tract. *Eur. Respir. J.* **9,** 1713 LP – 1728 (1996).

170. Haraoka, M. *et al.* Neutrophil recruitment and resistance to urinary tract infection. *J. Infect. Dis.* **180,** 1220–1229 (1999).

171. Hang, L. *et al.* Macrophage inflammatory protein-2 is required for neutrophil passage across the epithelial barrier of the infected urinary tract. *J. Immunol.* **162,** 3037–3044 (1999).

172. Frendéus, B. *et al.* Interleukin 8 Receptor Deficiency Confers Susceptibility to Acute Experimental Pyelonephritis and May Have a Human Counterpart. *J. Exp. Med.* **192,** 881–890 (2000).

173. Chan, C. Y., St. John, A. L. & Abraham, S. N. Mast Cell Interleukin-10 Drives Localized Tolerance in Chronic Bladder Infection. *Immunity* **38,** 349–359 (2013).

174. Duell, B. L. *et al.* Innate Transcriptional Networks Activated in Bladder in Response to Uropathogenic *Escherichia coli* Drive Diverse Biological Pathways and Rapid Synthesis of IL-10 for Defense against Bacterial Urinary Tract Infection. *J. Immunol.* **188,** 781–792 (2012).

175. D'Andrea, A. *et al.* Production of natural killer cell stimulatory factor (interleukin 12) by peripheral blood mononuclear cells. *J. Exp. Med.* **176,** 1387–98 (1992).

176. Gazzinelli, R. T., Hieny, S., Wynn, T. a, Wolf, S. & Sher, A. Induction of Interferon Y By an Intracellular Parasite and. *Immunology* **90,** 6115–6119 (1993).

177. Tripp, C. S., Gately, M. K., Hakimi, J., Ling, P. & Unanue, E. R. Neutralization of IL-12 decreases resistance to Listeria in SCID and C.B-17 mice. Reversal by IFN-gamma. *J. Immunol.* **152,** 1883–1887 (1994).

178. Kirjavainen, P. V. *et al.* Abnormal immunological profile and vaginal microbiota in women prone to urinary tract infections. *Clin. Vaccine Immunol.* **16,** 29–36 (2009).

179. Sivick, K. E., Schaller, M. A., Smith, S. N. & Mobley, H. L. T. The innate immune response to uropathogenic Escherichia coli involves IL-17A in a murine model of urinary tract infection. *J. Immunol.* **184,** 2065–75 (2010).

180. Rosenblum, M. G. & Donato, N. J. Tumor necrosis factor α: A multifaceted peptide hormone. *Crit. Rev. Immunol.* **9,** 21–44 (1989).

181. Kishimoto, T., Akira, S. & Taga, T. Interleukin-6 and its receptor: a paradigm for cytokines. *Science (80-.).* **258,** 593 LP – 597 (1992).

182. Nobles, C. *et al.* Correlation of urine and plasma cytokine levels among reproductive-aged women. *Eur. J. Clin. Invest.* **45,** 460–465 (2015).

183. Helbig, S. *et al.* Diagnosis and Inflammatory Response of Patients with Candiduria. *Mycoses* **56,** 61–69 (2013).

184. Anderson, G. G., Martin, S. M. & Hultgren, S. J. Host subversion by formation of intracellular bacterial communities in the urinary tract. *Microbes Infect.* **6,** 1094–1101 (2004).

185. Hernández, J. G., Sundén, F., Connolly, J., Svanborg, C. & Wullt, B. Genetic control of the variable innate immune response to asymptomatic bacteriuria. *PLoS One* **6,** (2011).

186. Thomas, V., Shelokov, A. & Forland, M. Antibody-Coated Bacteria in the Urine and the Site of Urinary-Tract Infection. *N. Engl. J. Med.* **290,** 588–590 (1974).

187. Winberg, J., Andersen, H. J., Hanson, L. A. & Lincoln, K. Studies of Urinary Tract Infections in Infancy and Childhood. *Br. Med. J.* **2,** 524–527 (1963).

188. Percival, A., Brumfitt, W. & De Louvois, J. Serum-antibody levels as an indication of clinically inapparent pyelonephritis. *Lancet* **284,** 1027–1033 (1964).

189. Ratner, J. J., Thomas, V. L., Barbara, A. & Forland, M. Bacteria-Specific Antibody in the Urine of Patients with Acute Pyelonephritis and Cystitis. *J. Infect. Dis.* **143,** 404–412 (1981).

190. Wagner, J. a, Williams, S. a & Webster, C. J. Biomarkers and surrogate end points for fit-for-purpose development and regulatory evaluation of new drugs. *Clin. Pharmacol. Ther.* **81,** 104–107 (2007).

191. Atkinson A.J., J. *et al.* Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* **69,** 89–95 (2001).

192. Burska, A., Boissinot, M. & Ponchel, F. Cytokines as Biomarkers in Rheumatoid Arthritis. *Mediators Inflamm.* 1–24 (2014).

193. Lee, J. W. *et al.* Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm. Res.* **23,** 312–328 (2006).

194. Wagner, J. a. Overview of biomarkers and surrogate endpoints in drug development. *Dis. Markers* **18,** 41–6 (2002).

195. FDA. *Guidance for Industry: Pharmacogenomic Data Submissions. Guidance for Industry* **23,** (2005).

196. Gábor, G., Tóth, F., Ózsvári, L., Abonyi-Tóth, Z. & Sasser, R. G. Early Detection of Pregnancy and Embryonic Loss in Dairy Cattle by ELISA Tests. *Reprod. Domest. Anim.* **42,** 633–636 (2007).

197. Nicolle, L. E., Brunka, J., Orr, P., Wilkins, J. & Harding, G. K. M. Urinary immunoreactive interleukin-1α and interleukin-6 in bacteriuric institutionalized elderly subjects. *J. Urol.* **149,** 1049–1053 (1993).

198. Croxall, G. *et al.* Molecular epidemiology of extraintestinal pathogenic Escherichia coli isolates from a regional cohort of elderly patients highlights the prevalence of ST131 strains with increased antimicrobial resistance in both community and hospital care settings. *J. Antimicrob. Chemother.* **66,** 2501–2508 (2011).

199. Clark, G. *et al.* Genomic analysis uncovers a phenotypically diverse but genetically homogeneous Escherichia coli ST131 clone circulating in unrelated urinary tract infections. *J. Antimicrob. Chemother.* (2012).

200. Croxall, G. *et al.* Increased human pathogenic potential of Escherichia coli from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples. *J. Med. Microbiol.* 102–109 (2016).

201. Sundén, F. & Wullt, B. Predictive value of urinary interleukin-6 for symptomatic urinary tract infections in a nursing home population. *Int. J. Urol.* 168–174 (2015).

202. Czaja, C. A. *et al.* Prospective Cohort Study of Microbial and Inflammatory Events Immediately Preceding Escherichia coli Recurrent Urinary Tract Infection in Women. *J. Infect. Dis.* **200,** 528–536 (2009).

203. Chen, S. L. *et al.* Genomic diversity and fitness of E. coli strains recovered from the intestinal and urinary tracts of women with recurrent urinary tract infection. *Sci. Transl. Med.* **5,** (2013).

204. European Committee on Antimicrobial Susceptibility Testing. *Antimicrobial susceptibility testing EUCAST disk diffusion method*. (2015).

205. Larkin, M. A. *et al.* Clustal W and Clustal X version 2.0. *Bioinformatics* **23,** 2947–2948 (2007).

206. de Jager, W., Bourcier, K., Rijkers, G. T., Prakken, B. J. & Seyfert-Margolis, V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol.* **10,** 52 (2009).

207. Seminerio, J. L., Aggarwal, G. & Sweetser, S. 26-Year-Old Man With Recurrent Urinary Tract Infections. *Mayo Clin. Proc.* **86,** 557–560 (2011).

208. Hidron, A. I. *et al.* Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Annual Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect. Control Hosp. Epidemiol.* **29,** 996–1011 (2008).

209. Wang, L., Rothemund, D., Curd, H. & Reeves, P. R. Species-wide variation in the Escherichia coli flagellin (H-antigen) gene. *J Bacteriol* **185,** (2003).

210. Wiedemann, B., Kliebe, C. & Kresken, M. The epidemiology of β-lactamases. *J. Antimicrob. Chemother.* **24,** 1–22 (1989).

211. Lecointre, G., Rachdi, L., Darlu, P. & Denamur, E. Escherichia coli molecular phylogeny using the incongruence length difference test. *Mol. Biol. Evol.* **15,** 1685–1695 (1998).

212. Alhashash, F. *et al.* Increase in bacteraemia cases in the East Midlands region of the UK due to MDR Escherichia coli ST73: High levels of genomic and plasmid diversity in causative isolates. *J. Antimicrob. Chemother.* **71,** 339–343 (2016).

213. Feng, L. *et al.* A genomic islet mediates flagellar phase variation in Escherichia coli strains carrying the flagellin-specifying locus flk. *J. Bacteriol.* **190,** 4470–4477 (2008).

214. Von Wintersdorff, C. J. H. *et al.* Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Front. Microbiol.* **7,** 1–10 (2016).

215. Hansson, S., Jodal, U., Lincoln, K. & Svanborg-Eden, C. Untreated asymptomatic bacteriuria in girls: II--Effect of phenoxymethylpenicillin and erythromycin given for intercurrent infections. *Bmj* **298,** 856–859 (1989).

216. Bent, S., Simel, D. L. & Fihn, S. D. Does This Woman Have an Acute Uncomplicated Urinary Tract Infection? *J. Am. Med. Assoc.* **287,** 2701–2710 (2013).

217. Odden, M. C., Shlipak, M. G. & Tager, I. B. Serum creatinine and functional limitation in elderly persons. *Journals Gerontol. - Ser. A Biol. Sci. Med. Sci.* **64,** 370–376 (2009).

218. LaClair, R. E. *et al.* Prevalence of Calcidiol Deficiency in CKD: A Cross-Sectional Study Across Latitudes in the United States. *Am. J. Kidney Dis.* **45,** 1026–1033 (2016).

219. Bowker, L. K., Briggs, R. S., Gallagher, P. J. & Robertson, D. R. Raised blood urea in the elderly: a clinical and pathological study. *Postgrad. Med. J.* **68,** 174–9 (1992).

220. Mosmann, T. R. Properties and functions of interleukin-10. *Adv. Immunol.* **56,** 1–26 (1994).

221. Trinchieri, G. Interleukin-12: A proinflammatory cytokine with immuneregulatory functions that bridge innate resistence and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 251–176 (1995).

222. Arndt, T. Urine-creatinine concentration as a marker of urine dilution: Reflections using a cohort of 45,000 samples. *Forensic Sci. Int.* **186,** 48–51 (2009).

223. Hawn, T. R. *et al.* Genetic variation of the human urinary tract innate immune response and asymptomatic bacteriuria in women. *PLoS One* **4,** (2009).

224. Jorde, R. *et al.* Prevention of urinary tract infections with vitamin D supplementation 20 , 000 IU per week for five years . Results from an RCT including 511 subjects. *Infect. Dis. (Auckl).* **4235,** 823–828 (2016).

225. The World Health Organisation (WHO). *World Health Statistics 2016: Monitoring health for the SDGs*. (2016).

226. Horcajada, J. P. *et al.* Quinolone-Resistant Uropathogenic Escherichia coli Strains from Phylogenetic Group B2 Have Fewer Virulence Factors than Their Susceptible Counterparts Quinolone-Resistant Uropathogenic Escherichia coli Strains from Phylogenetic Group B2 Have Fewer Virulen. *J. Clin. Microbiol.* **43,** 2962–2964 (2005).

227. Arisoy, M., Rad, A. Y., Akin, A. & Akar, N. Relationship between susceptibility to antimicrobials and virulence factors in paediatric Escherichia coli isolates. *Int. J. Antimicrob. Agents* **31,** 4–8 (2008).

228. Manges, A. R. *et al.* Widespread Distribution of Urinary Tract Infections Caused by a Multidrug-Resistant Escherichia coli Clonal Group. *N. Engl. J. Med.* **345,** 1007–1013 (2001).

229. Jung, M. & Pfeifer, G. P. Aging and DNA methylation. *BMC Biol.* **13,** 7 (2015).

230. López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M. & Kroemer, G. The Hallmarks of Aging. *Cell* **153,** (2013).

231. Olorunmola, F. O., Kolawole, D. O. & Lamikanra, A. Antibiotic resistance and virulence properties in escherichia coli strains from cases of urinary tract infections. *African J. Infect. Dis.* **7,** 1–7 (2013).

232. Hawn, T. R. *et al.* Toll-like receptor polymorphisms and susceptibility to urinary tract infections in adult women. *PLoS One* **4,** (2009).

233. Ferwerda, B. *et al.* TLR4 polymorphisms, infectious diseases, and evolutionary pressure during migration of modern humans. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 16645–16650 (2007).