

Missed urinary tract infection in patients with chronic recalcitrant LUTS and recurrent cystitis

Thesis submitted by

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Declaration

'I, Sheela Swamy confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the text.'

Abstract

Background

MSU culture and Urinary dipsticks as a diagnostic method for urinary infection (UTI) are discredited despite commonly used to exclude UTI in patients with lower urinary tract symptoms (LUTS). The phenotype of painful LUTS has been recast as Interstitial Cystitis (IC) or Bladder Pains Syndrome (BPS) because infection has been excluded on the evidence of these methods. Given that these all-important tests have been found insensitive and misleading, there is justification in re-examining IC/BPS to ascertain whether we have been mistaken. I studied patients with "Chronic recalcitrant bladder pain and recurrent cystitis" (abbreviated "painful LUTS") who had been diagnosed with IC/PBS in order to re-assess their pathophysiology.

Aim

I characterised these patients using the scientific method of consilience, which scrutinised them from unrelated perspectives. These studies implied that infection was a most probable aetiological factor. Therefore, I moved on to test infection as a causal factor using Pearl's three rungs of causation: Correlation, intervention and the counterfactual.

Methods

Data on quality of life and disease experience were obtained. Symptoms and pathophysiological variables in 146 patients presenting with painful LUTS were studied. To achieve Pearl's specifications, an observational study studied intervention and a cross-over study analysed the counterfactual of arbitrary treatment cessation. The evolution of treatment of these patients, using first generation, narrow spectrum urinary agents in protracted courses is reported. Since protracted antibiotic exposure is feared as a cause of antimicrobial resistance (AMR), I measured this in order to round off my findings

Results

The consilience studies incriminated UTI in the aetiology of painful LUTS. It is also clear that the patients suffer terribly, and this is aggravated by professional scepticism catalysed by a misinterpretation of urinalysis data. Antibiotic intervention demonstrated a regression in all disease indicators but there was resurgence of symptoms and signs during trials without treatment. The data on AMR demonstrated a rise in resistance in response to a first prescription without this increasing with persistence of the antibiotic regimen.

Conclusion

These data imply that IC/BPS (painful LUTS) is caused by a treatable urinary tract infection and are sufficient to merit a RCT. Whilst, treatment requires protracted exposure to antibiotics, my data on AMR amongst these patients is surprisingly reassuring. This requires further exploration. Contemporaneous to

this thesis, other have published definitive data that refute urine culture and dipstick analysis.

Impact Statement

Interstitial Cystitis (IC) or painful bladder syndrome (PBS) is a nasty chronic disease primarily affecting women and resulting in life changing symptoms. Until now it was assumed to be unrelated to infection and largely incurable. This thesis has falsified those beliefs and demonstrated clear evidence of an infective aetiology. The accumulated evidence from the studies reported here allows a hypothesis for a straightforward treatment of this condition to be formulated. Whilst treatment involves protracted antibiotic exposure, our fears about AMR from such methods seem to be unfounded. A grant application for £2.5M has been submitted to NIHR to fund a multicentre-RCT to test the validity of a protocol that uses protracted antibiotic treatment for these patients.

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Dedication



Late Emeritus Professor James Malone-Lee was my supervisor, teacher, mentor and friend. I am eternally grateful to Prof for taking me on as his student and for his unconditional support and guidance throughout my PhD and clinical career. The lessons I have learnt and the legacy he has left behind is unmatched and he has made me a better Doctor. I would not be at this point in my Clinical Career if it was not for Prof's constant wisdom and advice.

I would like to dedicate my thesis to Prof Malone-Lee and I will do my very best to carry the work forward and care for our patients the way he did.

Thank you and I miss you very much Prof!

Sheela

Abbreviations, Acronyms, Key words

AE	A&E or Accident and Emergency
AMR	Antimicrobial resistance
ATP	Adenosine triphosphate
BPS	Bladder pain syndrome
CBA	Columbia Blood Agar
cfu	Colony forming units
CI	Confidence intervals
CPS3	Chromogenic agar
CSU	Catheter specimen of urine
CUTI	Chronic urinary tract infection
CXCL8	Cytokines – CXCL8 (IL-8) as well as ICAM-1, CXCL-1, CXCL-5 and CXCL-6. CXCR1 and CXCR2
EPC	Epithelial cells
HRQOL	Health related quality of life
IC	Interstitial cystitis
ICIQ FLUTS	International consultation of incontinence modular questionnaire – Female lower urinary tract symptoms
ICIQ-LUTSqol	International consultation of incontinence modular questionnaire- Lower urinary tract symptoms-quality of life
IFN	Interferon
IL-6	Interleukin 6
LPS	Lipopolysaccharide
LUTS	Lower urinary tract symptoms
ml ⁻¹	per millilitre
MSU	Midstream urine
MYD88	Myeloid differentiation primary response 88
N, n	Number of study participants or controls
NDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHS	National Health Service
p value	Statistical significance
PBS	Painful bladder syndrome
PAF	Population attributable fraction
P2X3	Knockout mice
QOL	Quality of life
RBC, rbc	Red blood cells
RCT	Randomised Controlled Trial
rUTI	Recurrent urinary tract infection
SAE	Serious adverse event
sd	Standard deviation
TLR	Toll-like receptors
TRIF	TIR-domain-containing adapter-inducing interferon-β

UEC	Uroepithelial cells
UTI	Urinary tract infection
UTIs	Urinary tract infections
WBC, wbc	White blood cells
μl^{-1}	per microliter

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Chapter 1 Introduction – Setting the scene

1.1 Lower Urinary Tract Symptoms

Five unpleasant conditions dominate adult bladder pathology causing lower urinary tract symptoms (LUTS); stress urinary incontinence, bladder outflow obstruction, the overactive bladder (OAB), interstitial cystitis (IC) / painful bladder syndrome (PBS) and urinary tract infection (UTI).

These blight the lives of many persons particularly in late life. Epidemiologists have found that across cultural and national boundaries the distribution of LUTS is similar. The prevalence of at least one LUTS at least 'sometimes' was 72.3% for men and 76.3% for women, and 47.9% and 52.5% for at least 'often' for men and women (1). The prevalence of all LUTS increases with advancing age in men, but only certain LUTS increase with age in women (urgency, urgency with fear of leaking, weak stream, urgency incontinence, and nocturnal enuresis) (2).

The estimated prevalence of OAB is between 8 -11% (1-3) making it more commonplace than asthma and heart disease. The occurrence of this condition increases with age and is similar in both women and men. It is independently associated with falls and fractures in the elderly, urinary tract and skin infections, sleep disturbances and depression. In one study it was estimated that OAB cost five EEC countries in excess of €4.2 billion in the year 2000(4). In 1998 the Continence Foundation calculated the cost of incontinence to the NHS as £354m annually.

It is reported that 1 in 3 women will have at least one UTI by 24 years and 40% to 50% of women will experience one UTI during a lifetime and 44% will experience recurrences. There are several well-recognised risk factors that include advancing age.

This thesis focuses on Interstitial cystitis or painful bladder syndrome (IC or PBS) which has a prevalence from 1.2/100,000 in Japan (5) and 18/100,000 in Finland (6). In the United States, data from US Nurses' Health Study found a prevalence 52–67/100,000, (7). The available studies have shown that PBS/IC disproportionately affects women by a ratio of 10:1. The mean age of those affected is in the 40's. Data from a managed care population showed an even higher prevalence of 158 per 100,000 women and 28 per 100,000 men on a diagnostic code (8).

A key step in the diagnosis of IC/PBS and all LUTS is the exclusion of UTI. It is therefore a great concern that the methods used to screen for UTI have been discredited in recent time (9, 10). This has considerable implications for our understanding of this phenotype and provides the motivation for this thesis.

The LUTS syndromes are not truly distinct entities and exhibit a considerable overlap, sharing of common symptoms. This is particularly the case with IC/PBS, overactive bladder, acute UTI with UTI being closely associated with voiding symptoms (11).

1.1.1 The Overactive Bladder

The Overactive Bladder (OAB) syndrome has been defined by the International Continence Society as a spectrum of symptoms in which incontinence may or may not overlap with urgency, frequency, and nocturia (12). Urgency, the hallmark of OAB, is defined as the sudden compelling desire to urinate, a sensation that is difficult to defer. Urinary frequency is denoted as voiding eight or more times in a 24-hour period. Nocturia is the need to wake one or more times per night to void. OAB is an unpleasant condition with high morbidity, patients consistently reporting a negative effect on the quality of life, particularly, less self-assurance and reduced social activity (1, 13, 14).

OAB is a predominating LUTS symptom that does not feature acute frequency or dysuria and features as a part of non-dysuric LUTS. It is therefore a most important target in the study of the pathophysiology of non-dysuric LUTS.

Not everyone is happy with the term “OAB”. It is a symptomatic diagnosis in contrast to “detrusor over-activity” which is diagnosed by the use of urodynamic studies. Despite the best efforts of the ICS standardisation committee, the two terms have become synonymous in the literature, because the treatment strategies are the same. The aetiology and natural history are not yet fully described and varying hypotheses have been proposed (15). It is assumed by many that a number of different pathophysiological factors contribute to the development and progress of OAB symptom complex (16). Much of our current data are gleaned from animal experiments, which play such an important role in the study of biological mechanisms. The preferred models use guinea-pig and rat. There are species differences, so it is important to bear this in mind when extrapolating these data to the human experience. There are three pathophysiological hypotheses for OAB in the literature, unreasonably they are given the status of theory: The “myogenic theory”, the “neurogenic theory” which applies particularly to MS and the “autonomous bladder theory” (17). In truth there are sparse human data, only sufficient to assist in formulating hypotheses which have yet to be tested thoroughly. It is important to note that the OAB diagnosis is as compromised by the necessity of excluding UTI as IC/PBS and others have studied this conundrum (Kiren Gill thesis) and this work is apposite to this thesis because of symptoms overlap.

1.1.2 Excluding urinary tract infection

The reference, gold standard test for diagnosing urinary tract infection during the last sixty years has been to culture a midstream urine specimen and identify a pure growth of a known urinary pathogen (18).

The threshold counts may be adopted, ranging from 10^2 to 10^6 but these do not escape the facts. The quantitative urinary culture thresholds rest on assumptions that were not properly checked:

(1) *The normal bladder is sterile* – It is not sterile - several groups have refuted this (19-26).

(2) *There is a quantitative relationship between the culture results and the probability of infection* - Culture numbers are more likely to depend on the ease of growth (10, 19, 20, 21 , 25-33).

(3) *The infection should be caused by a single species* – Modern published data imply that with mixed organisms is more likely (19, 20, 23, 25, 26, 34).

(4) *Cultures of mixed organisms imply contamination* - Modern data refute the notion of mixed growth of doubtful significance – they are significant (19, 20, 23 , 25, 26, 34).

(5) *If epithelial cells are seen in the midstream urine specimen then it indicates contamination (35)* – This is not true, most of these cells come from the bladder and reflect bacterial cystitis (25, 36-42).

(6) *If the culture is negative then there is no infection* – This is not true, the cultures are incapable of this property (18, 19, 25-27, 34, 43-48).

(8) *Systemic markers of inflammation such as the ESR and C-reactive protein can exclude a significant infection if negative*: This is not true as numerous researchers have found (49-55, 56).

The wrong gold standard

It is common for a patient with appropriate symptoms to be undiagnosed on negative culture. That confuses absence of evidence of disease with evidence of absence of disease, but there is the more egregious error of ignoring the base-rate probability of UTI conditioned on the symptoms. Recently Heytens et al (2017) have claimed that “The woman that is visiting you with typical urinary complaints has an infection. There is nothing more to explore.” The culture misleads (57). The influence of guidelines and the imposition of three-day UTI treatment regimens followed by culture-based treatment seems to be generating a surge in patients with chronic recalcitrant bladder pain and recurrent cystitis who get diagnosed with IC/BPS/PBS.

Screening tests deficiencies

The popular screening test for UTI, used throughout the health services, involving dipstick analysis of the urine has been calibrated to quantitative urine culture assuming it to be an accurate gold standard. By doing this the errors of the culture method are added to the errors of the dipstick making matters worse (10, 25, 33). It should be no surprise therefore that there is a substantial literature that criticises the sensitivity and performance of this test. Nevertheless, numerous symptomatic people are dismissed as normal on the grounds of a negative dipstick test. There is no scientific justification for this (26).

These tests cannot exclude acute or chronic UTI and do not take into account differences in bacterial strain virulence, host genetic variability, intracellular bacterial reservoirs, or even the dilution of the urine specimen due to high liquid intake before the test. Therefore, it is conceivable that legitimate infections will be missed when relying on a rigid numerical threshold to distinguish between 'infected' and 'not infected' (24).

The frequentist Yes/No categorisations are a hallmark of diagnostic test interpretation. Biology is not suited to distinct classifications with most phenomena distributed to along continua. The dichotomised approach to diagnosis imposes a crude, arbitrary threshold on a notoriously complex situation and it is bound to force error into the system (58).

1.1.2.1 Causation and assumption in UTI

Historically, the predominant causative organism of acute UTI in women is reported to be *E. coli*. The prevalence of *E. coli* in symptomatic patients with positive urine cultures is between 50-77% in the literature (59, 60). Other pathogens of importance are *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Proteus mirabilis*.

Khasriya et al (2010)(61) using a culture method that studied the uroepithelial cells in the urinary sediment found that in patients with LUTS the dominant isolates were *Streptococcus spp* (18%), *Enterococcus spp* (10%) *Lactobacillus spp* (15%) , *E. Coli* (7%). This paper refuted the belief in the sterile normal bladder and a single species microbial ecology.

Sathianathamoorthy et al (62) using molecular methods found a rich polymicrobial mixture in the UTI patients and controls and defied between group discrimination. *E.coli* and the other microbes described above were identified in equal measure between both groups. History of the diagnosis of UTI shows a signal absence of definitive work on causation with much depending on assumption that only makes sense given a sterile normal bladder.

1.1.3 Urothelial cells

It is well recognised that urothelial perturbed by infection will increase turnover and exfoliate of large numbers of cells (36, 38-40, 63) and mast cells appear to play an important part in this (64). It would seem to be the principle method for clearing the urinary tract of the parasitised urothelial cells. This phenomenon consistently seen in association with UTI. There is increased exfoliation of uroepithelial cells in normal human pregnancy (65). Perhaps this is an evolved mechanism, deployed to protect against UTI. It should be appreciated that mast cells have been attributed a causative role in IC/BPS and even proposed for diagnostic criteria, but these propositions have not considered the exfoliative function of these cells.

The urothelial cell surface is coated with a layer of glycosaminoglycans (GAGs), which inhibit bacterial adherence. These are mucopolysaccharides that also contribute to the make-up of bacterial biofilms that will always form on

water/surface interfaces. A hypothesis has been floated, that GAG layers deficiencies are causative in the development of IC/BPS. This overlooks the point that the GAG layer will be shed along with the urothelial cells being shed by the innate immune response. The hypothesis has gripped peoples' imagination such that there are numerous papers correlating GAG layer deficiencies with IC/BPS diagnosis. Correlation is not the same as causation (41).

1.1.4 ATP as a marker of disease

ATP, the all-important energy provider for life, has been shown to rise in association with inflammation (66). ATP is an abundant component of cells and should be expected to rise in response to increases in cell numbers in the tissues. Dividing bacteria would be an excellent source as would be an inflammatory infiltrate. The bacterial source has led to ATP measurement being used to detect bacterial contamination in the catering industry.

We now know that epithelial cells also secrete increased ATP (67) and the innate immune response of the bladder stimulates urothelial metaplasia and an increase in abundance of urothelial cells..

1.1.5 Painful bladder syndrome – Interstitial cystitis

In 1987, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (68) described a set of criteria for research purposes, which became accepted as the diagnostic criteria for IC without a foundation in evidence. These required findings of bladder pain, urgency, in the bladder wall glomerulations after Cystodistension, or a Hunner's ulcer, as well as the absence of 18 specific clinical conditions; it is a diagnosis of exclusion without a pathophysiological explanation. These criteria were found to be too restrictive in clinical practice, excluding 60% of cases where painful bladder was evident (68). In 2002, the International Continence Society (ICS) published new recommendations and proposed that IC should be renamed painful bladder syndrome (PBS). The ICS diagnosis of PBS is based on suprapubic pain related to bladder filling, day or night time frequency, and the absence of other obvious pathology (69), thus invoking exclusions again. The term IC was retained to describe those who had cystoscopic findings of glomerulations and/or Hunner's ulcer. Diagnoses of exclusion presume that all possible aetiologies have been ruled out reliably by tests capable of achieving this (70).

There is an important consideration that must be brought to bear. These patients describe pain and this tends to manifest neuropathic qualities. There is an important network of pain transmitting C-fibres located beneath the trigonal urothelium and it would be unusual for inflammatory pathology of the trigone not to be seen amongst these patients. Chronic inflammatory states are known to be associated with painful C-fibre neuropathy. Studies have shown that chronic psychological stress can exacerbate LUTS and enhance chronic pain (71-73). Chronic stress can alter sensory pathways in the bladder, leading to reduction in sensory thresholds and amplification of sensation of pain and the authors conclude that stress-induced bladder dysfunction may be a result of increased sensitivity of C-fibres and mechanoreceptors (73).

There are no published data that are helpful in describing the pathophysiology of this condition. Work on PBS has been piecemeal, sporadic and lacking a coherent narrative. There are numerous published ideas on the aetiology, given the status of theory despite the scarcity of hypothesis-driven critical research. There is a pressing need for a systematic, critical scrutiny of this condition, eschewing all evidence-free assumptions about infection, inflammation or immunity

1.1.6 Measuring lower urinary tract symptoms

A key element in the study of this problem is the measurement of symptoms. The International Consultation on Incontinence Questionnaires (ICIQ) are strong candidates (74). Since 1999 these symptom scores have been developed under supervision of a board of international experts on incontinence. The questionnaires selected for this study have been validated. They measure male and female lower urinary tract symptoms and crucially, a clinically significant difference in score change has been established.

The symptoms measures (ICIQ) (75) depending on gender:

- ICIQ-Male Lower Urinary Tract Symptoms (ICIQ-MLUTS)
- ICIQ-Female Lower Urinary Tract Symptoms (ICIQ-FLUTS) (Appendix 11).
- The ICIQ-FLUTS OR ICIQ-MLUTS (Long forms) were used to measure the patients' symptoms.

1.1.6.1 Measuring symptoms of urgency

Our centre (LUTS Clinic at Whittington Hospital) developed a simple scale to measure urinary urgency. It was noted that patients spontaneously volunteered information about the circumstances in which urgency was most troublesome. A number of common themes were found to recur, and particular situations were associated with different disease experience severity. A formal analysis of these observations resulted in the discovery that the circumstances in which individuals noted the exacerbation of urgency could be ranked linearly in relation to frequency and incontinence. The linear qualities of these relationships inevitably suggested a simple summed scale (76).

The 10-item scale was validated on data from experiments using a single group repeated measure design (77). A total of 475 patients, including 411 females and 64 males, with a mean age of 57.3 years who had been diagnosed with overactive bladder were treated with a bladder retraining regimen and antimuscarinic agent (10 mg oxybutynin - controlled release per night or 4 mg tolterodine slow release per night). At each visit patient urge symptoms were recorded by the scale. Reported average daily frequency and incontinence episodes were also recorded. Of patients who were not satisfied with the symptoms 130 had 25 mg imipramine per night added to their prescription and in 130 the treatment was changed to 10 mg solifenacin per night.

Construct validity was tested by comparing the urgency scale to frequency and to incontinence (Spearman's rank correlation coefficient $r = 0.38$, $p < 0.001$ and $r = 0.15$, $p < 0.001$, respectively). Internal consistency showed Cronbach's $\alpha = 0.83$. Test-retest reliability was determined in 30 patients and interobserver

reliability was determined in 58 (Pearson’s $r = 0.99$, $p < 0.001$ and $r = 0.99$, $p < 0.001$, respectively). Internal responsiveness in the imipramine add-on study in 130 patients showed a standardized response mean of 0.6 ($p < 0.001$) and in the solifenacin swap study in 130 it showed a standardized response mean of 0.69, while external responsiveness showed a standardized response mean of 0.69 (each $p < 0.001$).

This “Whittington urgency score” succeeded in all validation studies and is a suitable means of measuring the degree of urgency symptoms in patients with OAB (78) (Appendix 10).

1.1.6.2 Measuring symptoms of PBS

There was a problem with the measurements of bladder pain encompassing the syndromes of acute and chronic cystitis, IC/PBS that has since been resolved (11). The difficulty was that the measures used for PBS/IC relied on tautologically validating against a symptom-based diagnosis so that they were self-referential. In 2009 Chaliha, C. et al (79) published data on a set of symptoms that had been abstracted from a self-reported set from a cohort of patients with painless and painful LUTS (Table 1.1).

There were eight dysaesthetic / pain symptoms that were analysed in 768 women as follows: (i) Internal consistency was examined using Cronbach’s alpha. (ii) Construct validity was assessed using comparisons of symptom counts between groups: (a) pyuria/no pyuria & (b) bacteriuria/no bacteria. (iii) Responsiveness was assessed by comparing symptom count before and after clearance of pyuria; and clearance of bacteriuria. (iv) External responsiveness was assessed by correlating the summed pain symptoms against the urgency score; 24-hour frequency; and 24-hour incontinence.

Thus, this inventory was well-validated. Later the same set were integrated into a wider scale that was published following further validation (11). I used the eight pain questions as described by Chaliha, C. *et al* (2009) (Appendix 9) (79) to assess the patients on this study Table 1.1.

Table 1.1 Symptoms set from Chaliha, C. et al (2009)

Symptom
Discomfort with filling
Bladder pain
Loin pain
Dysuria
Genital pain
Iliac fossa pain
Abdominal pain
Leg pain

1.1.7 Recalcitrant Lower Urinary Tract Symptoms

The dictionary meaning of recalcitrant is:

- obstinately defiant of authority or restraint
- difficult to manage or operate
- not responsive to treatment

I have used the term recalcitrant LUTS because it fits well with the patient symptoms not responding and persisting despite all known guideline driven treatments.

1.2 Paucity of screening and diagnostic tests for UTI

1.2.1 Introduction

"Lower urinary tract symptoms" (LUTS) is a collective term that includes storage symptoms, such as frequency, urgency, urge incontinence; symptoms of stress urinary incontinence; voiding symptoms such as hesitancy, reduced stream and intermittency; and finally sensory symptoms that include various degrees and expressions of pain. The prevalence of LUTS increases with age and is reported in up to 40% of men and 28% of women aged 70-79 years (3, 80, 81). Nowadays UTI is increasingly implicated in the aetiology of LUTS, most notably in patients presenting with voiding and overactive bladder symptoms ((25). The importance of infection to these symptoms has become more significant since the recognition of the shortcomings in the tests used to exclude UTI (82-84) ((85, 86).

1.2.2 Problems with diagnostic tests

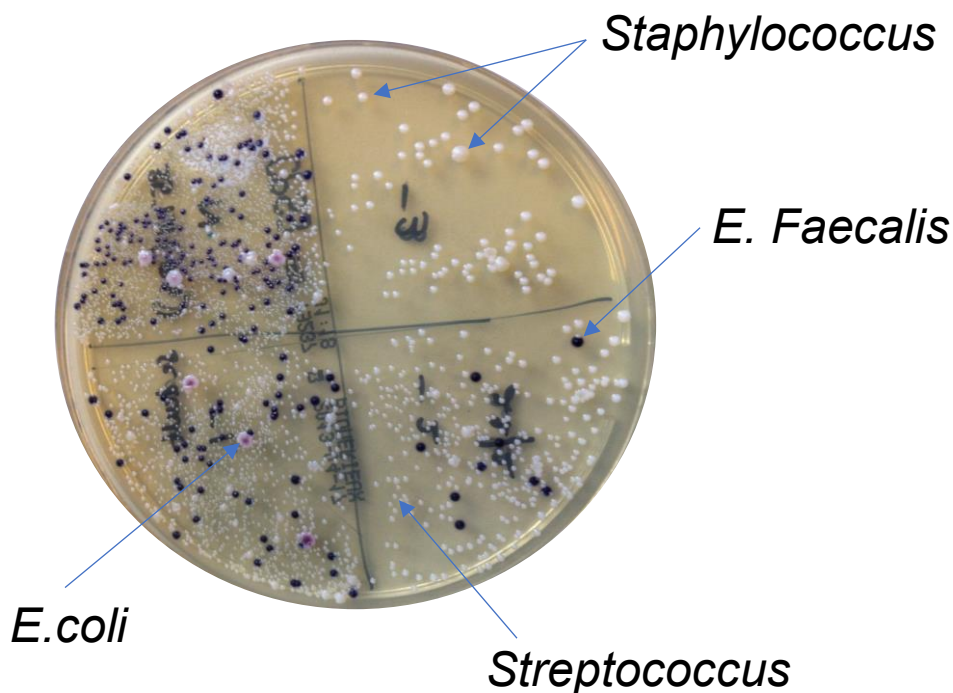
1.2.2.1 MSU Culture

Throughout the world, UTI diagnosis is subordinate to quantitative microbial culture applied to a clean catch midstream urine sample (MSU). The diagnostic threshold adopted varies, unaccountably, between 10^2 and 10^6 colony forming units (cfu) ml^{-1} , of a single species of a known urinary pathogen. These criteria are derived from work by Kass of 1957(18) who claimed that the distinguished genuine UTI from contamination of a specimen, given the assumption that the normal bladder was sterile. This was based on a study of 74 women, with acute pyelonephritis, and 335 asymptomatic controls (18). Subsequently he conducted a study on pregnant women with pyelonephritis to represent severe infection in a further confirmatory analysis of his threshold which of 10^5 cfu ml^{-1} (87). Why a threshold to discriminate causative pathogens from contaminants should have come to be deployed as the diagnostic arbiter of UTI is a mystery. It was criticised by some authors in the 1970's but their warnings went unheeded (88, 89).

Previous assumptions of a sterile normal urinary tract implied that a microbe isolated from an uncontaminated specimen must be pathological, have been refuted (19, 26, 90). Normal urine is not sterile, as many have confirmed (61, 88, 91). The insistence of a single organism reflected Koch's postulates ((92, 93) but nowadays we find that polymicrobial infection is the norm (Figure 1.1). Kass (18) was not correct in assuming that abundance of a single microbe was a characteristic of causation or that mixed growth were evidence of contamination (94, 95). In fact, properly collected clean catch MSU is remarkably free of contamination; its contents coming from the bladder (96, 97). The clinical situation is aggravated because the surrogate dipstick tests for UTI are calibrated the erroneous routine MSU culture.

Figure 1.1 Clean catch specimen showing a mixed growth culture in a symptomatic patient with Chronic UTI using the spun sediment culture technique.

Routine MSU culture was reported as no growth. The picture shows 5 different organisms: wet white small and medium colonies – 2 different types of *Staphylococcus*; Purple colonies - *Enterococcus faecalis*; Pin-point white colonies – *Streptococcus*; white with mauve centre – *E. coli*.



1.2.2.2 Urine Microscopy

The measurement of pyuria has replaced bacterial culture in many clinical services. Evaluated by microscopy or urinary dipstick, or automated methods, it is often used as a stand-alone surrogate, to triage samples submitted for bacteriological culture. The absence of 'significant' pyuria is frequently considered definitive evidence of the absence of UTI. The validity of this assumption has been refuted.

None of the tests for pyuria have the sensitivity to claim such power over the diagnostic process. "No evidence of disease" should never be confused with "Evidence of no disease"

The identification of urinary leucocytes using light microscopy was first described in 1893. Early pioneers studied the centrifuged deposits of large volumes of collected urine (98), although doubts about the veracity of this approach were expressed by some (99, 100). Duker (100, 101) reported data from a method using a cell counting chamber and fresh, uncentrifuged urine. His study of 300 midstream urine (MSU) samples from asymptomatic controls produced estimates for normal mean leucocyte counts of $1.6 \text{ wbc } \mu\text{l}^{-1}$ and $5.4 \text{ wbc } \mu\text{l}^{-1}$ for males and females respectively. These data showed wide dispersion and positive skew in the range $0\text{-}50 \text{ wbc } \mu\text{l}^{-1}$. His use of the mean to summarise his data was wrong. Had he used the median, the appropriate measure of centrality, he would have

had to conclude that any pyuria was potentially pathological. Regrettably he arbitrarily set a threshold between normal and abnormal of ≥ 10 wbc μl^{-1} .

Dukes experiments were not replicated until the 1950's, when several groups, making the same statistical errors of analysing non-Gaussian data with parametric methods, and making similar assumptions, reported results, that ultimately bound us to the ≤ 10 wbc μl^{-1} threshold in clinical practice as insignificant. The veracity of those conclusions have now been refuted (88).

From recent studies it is clear that urine needs to be evaluated for pyuria immediately after collection, as rapid leucocyte lysis occurs in the hours following sampling (102). This cell destruction appears to be retarded by boric acid, although significant cell loss appears inevitable (103). Urinary centrifugation affects cell salvage so that it is inappropriate for use in clinical practice. Vital staining techniques appear to confer no significant influence on leucocyte detection (104).

1.2.2.3 Urinary Dipstick

Meta-analyses of the use of urinary dipsticks in adults (28, 31) and in children (31) have been reported. Hurlbut and Littenberg (28) concluded that dipsticks do not exclude infection reliably in most clinical settings. Deville et al (31) referencing the MSU Kass criterion of 10^5 cfu/ml, reported a leukocyte esterase sensitivity of 0.76 [95% CI 0.6–0.98] and a specificity of 0.46 [95% CI 0.32–0.68], and a nitrite sensitivity of 0.49 [95% CI 0.38–0.62] and specificity of 0.85 [95% CI 0.73–1.0] in the primary care setting (30). The considerable variance in these measures is not reassuring.

A study by Khasriya et al (10) examined the performance of dipsticks in patients with chronic lower urinary tract symptoms without dysuria. A total of 508 midstream urine samples were used to compare leukocyte esterase, nitrite dipstick and urine microscopy with cultures seeking 10^5 cfu/ml. Similarly, 470 catheter urine samples were used to compare the same surrogates with 10^5 cfu/ml and with an enhanced culture method seeking 10^2 cfu/ml. A comparison of leukocyte esterase on dipstick against microscopic pyuria on fresh urine microscopy was made using the 508 midstream and 470 catheter specimens of urine (CSU). Midstream urine specimens were provided by 42 normal volunteers (controls) for comparison.

In patients with positive midstream urine culture ($\geq 10^5$ cfu/ml), 56% were positive for leukocyte esterase [95%CI 46–66], 10% for nitrite [95% CI 6–18] and 56% for microscopic pyuria [95% CI 46–66] with specificities of 66% [95% CI 61–70], 99% [95% CI 98–100] and 72% [95% CI 67–76], respectively (Table 1.2).

Table 1.2 MSU samples: Comparison of surrogate markers (Dipstick) to Culture gold standard $>10^5$ cfu/ml (10).

MSU Samples	Leucocyte Esterase	Nitrite	Microscopic Pyuria
Positive	56%	10%	56%
Specificity	66%	99%	72%

In CSU samples the sensitivity for the gold standard was 59% for leukocyte esterase [95% CI 47–70], 20% for nitrite [95% CI 12–31] and 66% for microscopic pyuria [95% CI 54–77] with specificities of 84% [95% CI 80–87], 97% [95% CI 95–99] and 73% [95% CI 69–78], respectively (Table 1.3).

Table 1.3 CSU samples: Comparison of surrogate markers (Dipstick) to Culture gold standard $>10^5$ cfu/ml (10).

MSU Samples	Leucocyte Esterase	Nitrite	Microscopic Pyuria
Positive	59%	20%	66%
Specificity	84%	97%	73%

The enhanced method of CSU culture [10^2 cfu/ml] proved positive in 137 subjects [29%], inevitably more than the gold standard did in 71 [15%]. The surrogate markers were less sensitive for 10^2 cfu/ml [table 1]. In CSU samples the sensitivity for the enhanced standard was 45% for leukocyte esterase [95% CI 36–53], 13% for nitrite [95% CI 8–20] and 53% for microscopic pyuria [95% CI 45–62] with specificities of 86% [95% CI 82–90], 98% [95% CI 96–99] and 76% [95% CI 71–80], respectively (Table 1.4).

Table 1.4 Enhanced Culture - CSU samples: Comparison of surrogate markers (Dipstick) to Culture gold standard $>10^5$ cfu/ml (10).

MSU Samples	Leucocyte Esterase	Nitrite	Microscopic Pyuria
Positive	45%	13%	53%
Specificity	86%	98%	76%

The sensitivity of leukocyte esterase for microscopic pyuria was 81% [95% CI 75–87] and specificity was 83% [95% CI 78–87].

These exhaustive data demonstrate that our current tests cannot exclude UTI despite their adoption by official guidelines. The tests are insensitive but have a high specificity so that even trace positive results reflect a high probability of infection. Nevertheless, trace result is judged to exclude the diagnosis.

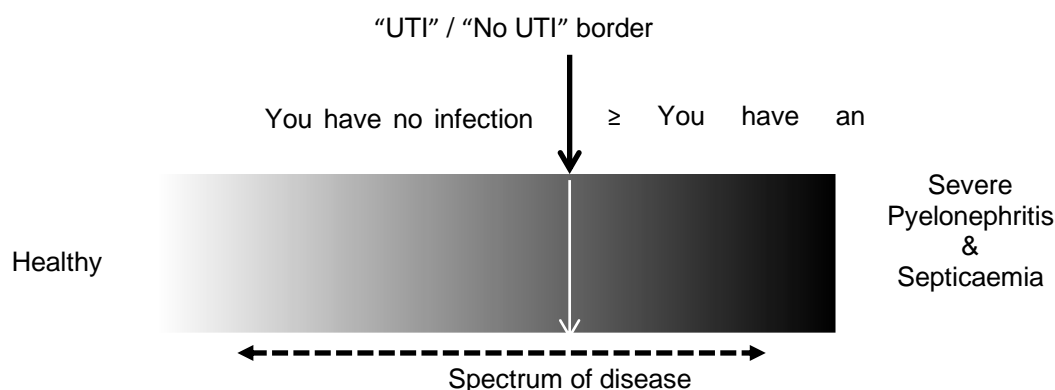
1.2.2.4 Categories

One of the explanations for the muddle that we have got ourselves into may lie with the liberal use of categories. The use of a universal diagnostic threshold of $\geq 10^5$ cfu ml⁻¹ of a single species of a known urinary pathogen imposes a dichotomy: “Urinary tract infection” or “No urinary tract infection” but this makes no sense when applied to biological systems. Medicine has always used categories to help understand the complexity of the data we attempt to assimilate. Unfortunately, we rarely question their true validity. Immanuel Kant warned that categories are inventions of the mind that should not be confused with reality (105). More recently, Karl Popper encouraged us to abandon the absolutism of categorisation on the grounds that they generate ill-advised certitude. So instead of informing a patient that “you do not have an infection” we should instead be

using statements of probability, drawing on the whole clinical picture and which takes a full account of our uncertainty (106).

Figure 1.2 illustrates the problem that we must confront. A spectrum is drawn between two extremes of no urinary tract infection (negative MSU culture result) and urinary infection sufficient to threaten life (positive MSU culture with pyelonephritis and or sepsis). A single diagnostic threshold places an arbitrary boundary on this biological continuum and declares all below this boundary as “No UTI” and all above as “UTI”. On reflection this seems crass.

Figure 1.2 Categorisation imposed on the disease spectrum



It is simply wrong to impose categories, least of all dichotomies, on natural spectra. Nature is inimical to categories. Biological phenomena are dispersed across continua. Charles Darwin never tired of emphasising the gradualism in nature (107) and Dawkins wrote a devastating criticism “The tyranny of the discontinuous mind” (58). Slavish adherence to such arbitrariness is bound to generate error as we see today.

1.2.2.5 Systemic markers of infection

There is an expectation that systemic inflammatory markers; leucocytosis, ESR and CRP must be elevated, particularly in more severe lower or upper urinary tract infection. These variables may be affected if the infection spills over into the circulation, but it is possible to have pyelonephritis without these being elevated (51, 56).

1.2.3 Molecular methods used to detect bacteria in the Urinary microbiome

Given the limitations in the culture-based methods to detect causative organisms, a few centres have gone on to characterise the composition of the bladder microbiome in LUTS patients and healthy controls using DNA based approaches DNA-based, which does not require specific conditions that are needed for culture. The microbiome in patients with overactive bladder (21), urgency urinary incontinence (90) stress urinary incontinence(108), uncomplicated UTI (109), neurogenic bladder dysfunction (110), have been described. A few of these studies have also included comparison of the microbiome in the above groups to asymptomatic controls (20) (90) (110) (111) (112) (113).

The studies share a key finding; the outcomes from patients and the controls are polymicrobial and there is extensive overlap to the extent that the data are unable to distinguish between the two groups. That distinction is the first rung on the ladder of causation, so failing that criterion means that the current molecular methods cannot address causation and do not have a diagnostic role.

1.2.4 Validity of Urine Dipstick and MSU culture in Pregnancy

Urine dipsticks in early pregnancy are used to screen for ketonuria in patients with Hyperemesis gravidarum. In the second and third trimesters urine dipstick are used particularly to screen for protein, leucocytes and nitrites, in order to pick up pre-eclampsia (114), UTI or chronic renal disease (115-118). NICE & PHE primary care guidance advise treatment of women with dysuria on the basis of symptoms alone but MSU culture and sensitivity data are often requested and relied upon in women who are not responding to first line treatment(119).

The use of urine analysis in pregnancy was studied exhaustively by Dr Jane Currie in her PhD (65). Her findings indicate that the use of these tests in pregnancy are crying out for scientific scrutiny. A second PhD student has embarked on that project. Currently, honesty admits that we do not know how to interpret these tests in pregnancy.

1.3 Discovery science in the development of new treatments for lower urinary tract symptoms (LUTS).

1.3.1 Introduction

The randomised controlled trial (RCT) and its developments in meta-analysis (120) have gripped the imagination of our professions to the extent that many equate scientific evidence and discovery with randomised trials. An RCT is not a creative activity, so there must be more to clinical science than this and scientific evolutionary epistemology fills that gap. This approach was crystallised by Karl Popper (121) as trial and error elimination and then David Campbell (122) went on to coin the term scientific evolutionary epistemology.

1.3.2 Scientific evolutionary epistemology

Given a rigorous protocol the repetitive cycle can capture the properties of evolution to become powerfully creative.

Evolution requires:

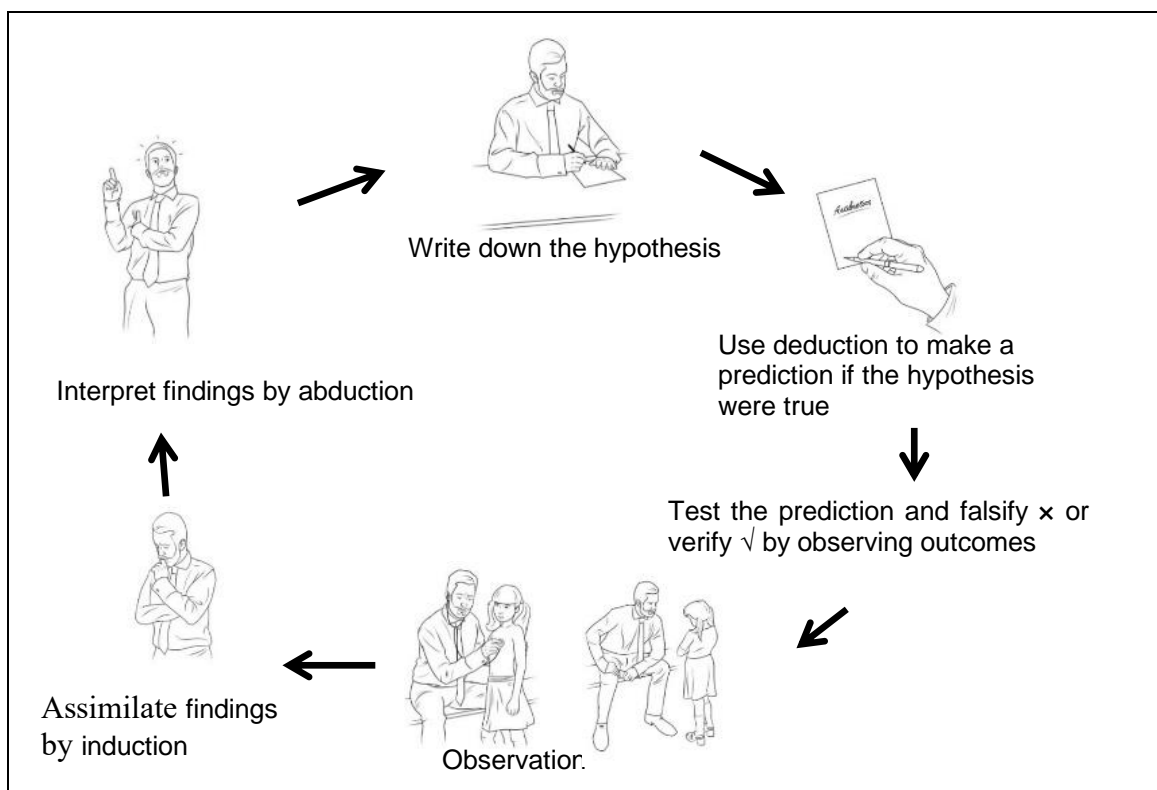
- (1) Variation, provided by the human intellect and imagination during abduction.
- (2) Replication, provided by the recursive cycling through the sequence.
- (3) Selection pressure, provided by the testing of a prediction and the rejection of the falsified and acceptance of verified.

The human imagination and intellect provide an accelerator that speed this process way faster than natural selection. A key component is rigorous collection of clean data in large amounts. The dataset can then be used to identify patterns and clusters that might otherwise be elusive. The RCT can be incorporated to test the prediction but that is the limit of its role.

This process can be applied to the clinical situation, which is illustrated in Figure 1.3, The starting point is observation by application of our senses typified by the clinical history and examination. The data are then assimilated through the process of induction. Whilst induction has served science well over the last 300 years, there is this inherent weakness arising from the fickleness of human perception “Hume’s problem” (123): What we might believe that we observe may not be the case. Scientific epistemology v. *infra* addresses that problem. After the induction we use intellect and imagination in the process of abduction and to formulate a hypothesis which must be put in writing. Typical examples would be a differential diagnosis in a clinical setting. The next step is to deduce a prediction that must be correct if the hypothesis is true: “Given the hypothesis the following prediction must be true”. The prediction is also written down and then tested. In clinical medicine this typically involves a treatment or a test.

The prediction is then verified or falsified by observation of the outcome of the test or the treatment, thus new data are collected and the whole cycle is repeated.

Figure 1.3 Scientific Evolutionary Epistemology in clinical practice



The fact that a prediction, and not a hypothesis, is being tested raises a problem with clinical trials that is too often neglected. Nowadays statisticians have established the principle that RCTs should test for the occurrence of a difference in effect sizes between treatment and control groups. Through the process of sample size calculation, a priori the effect size must be predicted to lie between the boundaries of a confidence interval. Such predictions can only be estimated if the variance (average squared deviation from the mean) can be calculated. Given normal, or quasi normal data, this presents little problem, but there exist data sets where that is impossible.

There are commonly occurring distributions that are heavily skewed and subject to power laws of the form $y = a x^b$ where 'a' and 'b' are parameters. If the distribution is skewed with a long tail the mathematical properties are such that predictions cannot be made from the data. It was the ignoring of this rule in complex economic models that led to the financial crash of 2008 (124, 125). Similarly, the dogmatic beliefs in the supremacy of RCTs and meta-analyses to often ignore this point.

1.3.3 Power laws and Predictions

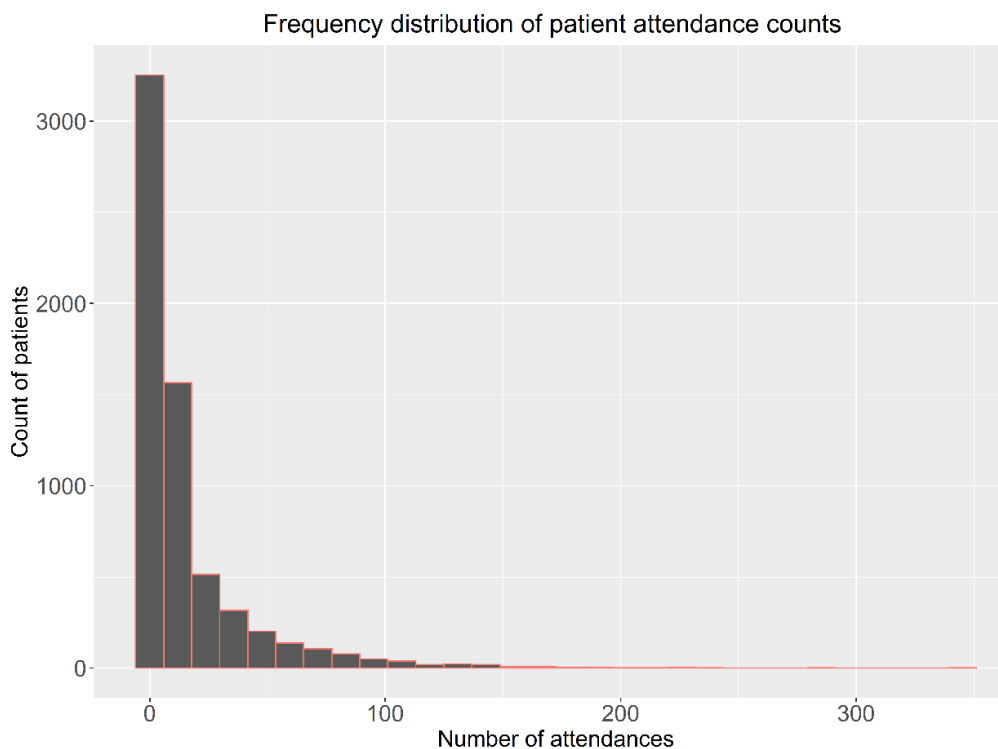
Figure 1.4 is a probability distribution of the attendances that patients might require when treated by our service; it is from 1783 female patients with LUTS. Attendance rate is an informal measure of the difficulty with treatment. It does correlate with biological markers of disease. The distribution is markedly skewed and 30% of the patients are spread over a long tail. The skew means that there is no legitimate mean nor variance. We should not use those variables to estimate the population distribution and we cannot calculate quantifiable predictions from

these data. Unfortunately, some do by averaging over many different samples to achieve an aggregate estimate, which will obey the central limit theory and provide a reassuring mean with a normal distribution. In short, the exceptions get averaged out of consideration. The long tail is important because it is evidence of wide distribution of individually rare events, but the collection means that there is a 30% chance any such event. Exceptions are more common than we might think.

This thesis is about exceptional patients who have failed all guideline managements.

Before we came to understand these principles, we did mount two RCTs to test an aspect of our treatment methods. One was in our usual patients, and another was in people with MS. The interventions were ethical, simple and tightly defined to comply with research governance. The study failed because patients declined the risk of placebo for six-week periods. As it happens, the study design was flawed. We were testing a six-week course of Nitrofurantoin 100 mg twice daily against placebo. We have since found, as power laws predict, that our patients are subject to too many random events influencing treatment decisions. These include idiosyncratic drug intolerances, differences in dose requirement, variations in the treatment duration needed, unpredictable responses to treatment cessation, polymicrobial infections, mixed antibiotics susceptibilities, super-added infections, psychological difficulties and intercurrent illness and many more random perturbations. In short, one size does not fit all. The clinical science must take account of these complexities and be adapted accordingly. Had our clinical trial recruited successfully, we should have reported misleading results despite conscientious adherence to the methods.

Figure 1.4 Frequency distribution of the number of attendances per patient, n=1783



Analysis of the different treatment regimes that have been found to fit individual patients, reveals numerous mutable combinations and permutations. This situation is inimical to a “Clinical Trial of an Investigational Medical Product” (CTIMP) using European Research Governance criteria. We know this because we tried hard to achieve it.

The most telling lesson for us was the point that you should never launch off on a RCT before you have fully understood the subject that you are scrutinising. There is a cultural pressure to solve all our clinical science with an RCT and that results in premature use of this approach. This is not helped by the widespread view that no data are acceptable other than from a RCT. Thankfully our patients’ refusal to cooperate brought us up sharp before we were allowed to bungle the research by accommodating this belief.

We report on a rigorous observational study in 3, reassured by the modern evidence that the data compare, to some degree, with RCT data (126, 127) (128, 129).

1.4 Summary of Introduction chapter

The phenotype of patients presenting with chronic painful recalcitrant LUTS with negative dipstick and negative urine culture, who are refractory to mainstream management of LUTS symptoms and recurrent UTIs and are given the diagnosis of IC or PBS. The inherent problem is that the tests used to exclude infection have to be capable of achieving that accurately.

The data described in this chapter provide compelling evidence of major deficiencies in the clinical tests currently used to exclude urinary tract infection (9, 10, 19, 25, 26). Replacement tests are certainly needed but the history narrated here cautions us to invest in careful validation, which will take time. In the meantime, we should face the facts. We have some tests that will raise the probability of a suspected infection but provide no other guidance. This means that given current evidence, our best option is to the patients' symptoms and fresh urine microscopy to plan treatment and tailor that treatment in reaction to the symptom response and changes in pyuria.

Unpalatable though it maybe, we have to accept that there is nowadays sufficient science to refute our past assumptions, and this leaves our beliefs about the nature of painful LUTS in a parlous state.

Thus, I began my thesis by studying the quality of life and the patient journey with recurrent UTIs and their guideline-driven treatment prior to arrival in the unit. I used qualitative methods to study the impact on their lives. I then went on to characterise the chronic painful LUTS phenotype by studying the pathophysiological signals in the urine of new patients referred to this centre. I also undertook two pilot studies looking at a subset of diabetes patients and another sample of patients in whom the symptoms flared while on long-term antibiotic treatment.

I then conducted a study of the effects of treating these patients with antibiotics, given evidence of infection. An important part of that analysis scrutinised the patients when apparently effective treatment was stopped. Because antimicrobial resistance is such a major concern, I also studied the resistance patterns associated with the treatment protocol and compared those data with resistance patterns seen in patients presenting to A&E with uncomplicated acute UTI.

Chapter 2 QOL & Psychological impact of Chronic UTI

2.1 The psychological effects of chronic recalcitrant painful LUTS on quality of life of a patient.

2.1.1 Introduction

At this point it is important to ask the question “So what?”. Many will acknowledge the fickle qualities of our tests but will claim that there are no significant sequelae (130). As will become clear in subsequent chapters we are well able to characterise the patients of interests using the tools described in the first chapter. However, before deploying these it makes sense to seek, from those affected by the symptoms, the burden of the disease. My hypothesis is that because UTI is being excluded from the diagnosis by incompetent tests, despite the symptoms, so that appropriate treatment for infection is being denied prescribed. This leads to a prediction should the hypothesis be true: There will be women who live with untreated chronic urinary infection which should be associated with noxious symptoms and suffering. This is a prediction, conditioned on a hypothesis, so it is testable and such an exercise is worthwhile

I wished to explore these matters more formally and thoroughly amongst the patients who were the subject of this thesis. I was keen to improve on anecdotal reportage which these aspects of disease can become mired in.

I consulted with some patient support organisations in order to identify the most common experiences that patients described when receiving a consultation about their LUTS when the urinalyses were negative. The suggestion that the symptoms were psychosomatic was by far the most common report. I therefore fashioned a set of three questions designed to explore that matter.

2.1.2 Aim

I set out to ask the following questions of my study sample:

1. Has it ever been put to you that your LUTS symptoms are of psychological origin?
2. If so what reason or evidence was given to you by the clinician?
3. What is your view on this theory or proposition?

2.1.3 Methods

400 patients diagnosed at our centre to have a Chronic UTI who were identified to have email contact with the clinic were contacted to provide a response to this survey. The email read as follows (Figure 2.1):

Figure 2.1 Email to patients

LUTS SERVICE- PSYCHOLOGY SURVEY

Dear Patient,

It is quite often quoted to our patients that tests are negative hence the symptoms must be psychological. Please could you email us the answers to the questions in this survey. We know that some of our patients have undergone extensive psychological assessments and counselling and also taken antidepressants, anxiolytics etc. prior to seeing us. We are very keen to collate your opinion and experience so I would be very grateful if you can answer the short survey questions:

SURVEY QUESTIONS:

- 1.Has it ever been put to you that your LUTS symptoms are of psychological origin?
- 2.If so what reason or evidence was given to you by the clinician?
- 3.What is your view on this theory or proposition?

There is no word limit or format to answer these questions. Please note that we are not expecting you to present any medical literature for or against the questions asked. We simply wish to know your opinion and experience regarding this theory.

Dr Sheela Swamy
Senior Research Associate to Professor Malone-Lee
Community Lower Urinary Tract Symptoms Service (LUTS Service)
2nd Floor ,Hornsey Central Health Centre
151 Park Road, London
N8 8JD

2.1.3.1 Collection of Survey Data

All patients voluntarily submitted their responses and consented by email to their anonymised data being used for presentation at Hospital meetings and at conferences. The emails were acknowledged by the researcher and a standard response was sent to the participant (Figure2.2).

Figure.2.2 Response to patient

Dear LUTS clinic patient,

Thank you for your response to the email survey. Please be reassured that all data that you have submitted will be anonymised and will be presented at future MDT meetings and conferences. Your personal identifiers (name, date of birth or hospital number, email address) will not appear on any document being presented at these meetings and your clinical care will not be impacted in any way.

Thank you once again for your timely response and for your opinion.

Dr Sheela Swamy
Senior Research Associate to Professor Malone-Lee
Community Lower Urinary Tract Symptoms Service (LUTS Service)
2nd Floor
Hornsey Central Health Centre
151 Park Road
London
N8 8JD

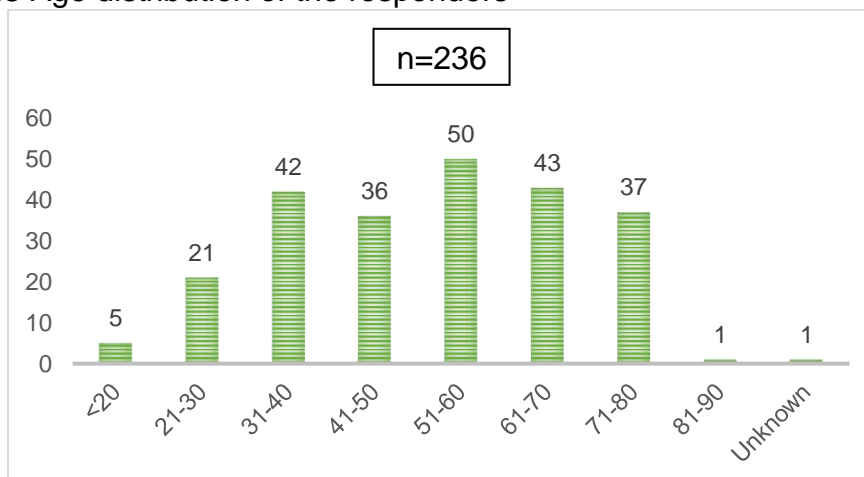
2.1.4 Results

236 (59%) responses were received via email from 400 emails sent to patients who were registered with an email on Artemis Database. This is a very good return rate for an online survey.

2.1.4.1 Age of Responders

The maximum number of responses were received from the 51-60 year group and this fits with the mean age being 53 years in our clinic population. Parents of 3 children answered on their behalf and their ages were 6, 7 and 15 years (Figure 2.3).

Figure 2.3 Age distribution of the responders



2.1.4.2 Gender of responders

9/236 responses were from male patients. It is important to note that >95% of our clinic population is made up of women. 96% were women, 4% were men and we had 1 transgender patient (was Male and now Female).

2.1.4.3 Response to Question 1

Has it ever been put to you that your LUTS symptoms are of psychological origin?

42% responded to the question with a 'Yes' and 57% answered a 'NO' and 1% provided no response to Question 1. Of the 42% patients who answered Yes to Question 1, 51 were told so by their GP, 49 from specialists and 16 patients had both the primary and secondary care physicians suggest that their symptoms were of psychological origin.

2.1.4.4 Response to Question 2

If so what reason or evidence was given to you by the clinician?

In 136 patients (57%) this question was not applicable as they had never been told that their symptoms were of psychological origin. 32% (81 patients) were thought to have symptoms of psychological origin as their urine tests and other investigations were coming back with negative results.

Other reasons for this conclusion were as follows:

- Frequent attendance to the surgery with negative tests
- No response to all known regimes

- Clinician’s belief that LUTS symptoms are of psychological origin.
- Positive responses to antibiotics were attributed to placebo effect by one specialist when challenged by the patient.

2.1.4.5 Response to Question 3

What is your view on this theory or proposition?

A variety of views were expressed by the patients who responded to the survey (Table 2.1). Some of these comments were from personal experience of being told they had no infections, and their symptoms were psychological. Some felt insulted that a clinician would even dare to suggest a psychological theory. A few had researched the condition and concluded that anyone suggesting the psychological theory is not well informed and perhaps should not be practicing as doctors.

Table 2.1 Response to Question 3

Against the theory	200
Unsure	2
Possible	3
Chronic UTI leads to Psychological problems	19
No opinion expressed	12

85% (n=200) of patients did not find the theory acceptable as they felt their symptoms were physical and not psychological. No opinion was expressed by 5% all of whom had answered “No” or “Not applicable” to the survey questions. 19 of the patients felt that they did not agree with the theory but continuing visits to clinic to be told that the tests are negative and having to live with the condition when no one believed their symptoms to be genuine could itself lead to the onset of psychological problems.

One patient wondered whether her loneliness led to the infections and one thought the stress of looking after a disabled child may have resulted in her infection. Three patients said that the theory or proposition could possibly be true but has not been proven so until then their condition feels real.

2.1.5 Discussion

The data collected imply that about 40% of patients experience their symptoms being attributed to psychosomatic disease. There is no evidence anywhere in the literature that have demonstrated these symptoms having a psychosomatic origin. Psychosomatic disease is not known to induce inflammatory exudates in the urine. Where somatisation is contended the symptomatology are exclusively sensory, they are not implicated in the cause of an inflammatory response. The tenacious belief the powers of a placebo effect bear witness to the persistence ideas that attribute physical disease to the mind. Time and again these are shown to be a manifestation of regression to the mean and the effects of the history of the disease process. The domain for the influence of our minds has been reduced to 1% to 2% by modern science and even there we have not proof. A key matter is that the Hawthorne effect (131), which has been so influential, has been

discredited as a fraudulent claim. Thus, if we seek to introduce psychological explanations for this disease, we are stripped back to the more rational view that they reflect the mental distress of the disease experience.

The response rate although only 59%, is considered good as most healthcare related surveys done through our societies (British Society of Urogynaecology - BSUG & United Kingdom Continence Society- UKCS) involving patients and staff generate 10-15 % response rate. I was an Information Technology committee member and now a Clinical Governance committee member of BSUG, hence able to state the patient engagement statistics without referencing.

The data described here seem to imply that over-reliance on tests and a misunderstanding about interpretation are causing doctors to be baffled by the circumstances of their patients. Thrashing around for an explanation, they resort to aetiological conjectures drawn from the psychoanalysis traditions. There is a paradox in this with a reliance on an empirical test, assumed to be evidence-based, siring the use of non-evidence based psychoanalytic surmise.

The patients with IC, PBS, recurrent and Chronic UTIs suffer terribly, their lives are plagued by endless daily symptoms and they are desperately vulnerable (*Personal communication 2020 Chronic UTI Global Support*). The consequences for them are so destructive they will do anything to seek respite. The prevalence is about 1:2000 individuals (68). The paucity of diagnostic tests to detect UTI in these individuals leads to many patients being denied antibiotic treatment despite classic symptoms. Over reliance on the dipstick and MSU culture, which are not validated tools for excluding UTI, are reported by patients to lead to unconvincing, evidence-free, conjectured explanations for their symptoms which do not reassure.

Clinical guidelines to this day provide hygiene advice to women; “wipe from front to back”, “make sure you maintain good personal hygiene” and “pee before sex and after sex”(119, 132) There is no evidence for the efficacy of these measures. In truth they make little sense given what we now know about pathophysiology of the condition. In cases of rUTI that I was studying I noted that the history taking often elicited patients describing how they were told such things as; “it is all in your head as the tests are yet again negative”, “nothing can be done as tests are negative, so learn to live with it”, “why don’t you try some antidepressants?”, “you probably have some sort of issues at work or in your marriage”, “stop thinking so much about your bladder” and the list goes on.

Many chronic UTI sufferers describe exposure to batteries of tests including urodynamics, renal and pelvic USS, repeated STI screens, multiple cystoscopies, biopsies, as well as procedures such as urethral dilations and bladder distensions. Some have experienced extensive psychological assessments, counselling and have been prescribed antidepressants, anxiolytics and potent pain medications. A question hangs over these actions which is: what is their purpose and what is the evidence that justifies their use? A careful study of the literature exposes a marked deficiency of such information other than evidence for the drugs which justify their primary purpose but not use for UTI.

Chronic illnesses are defined as health conditions that either have symptoms on a constant basis or as episodic flares such as Diabetes, Heart disease, COPD,

Hypertension, mental disorders, stroke, cancer, obesity, cystic fibrosis, SLE & chronic UTI. Chronic illness is the cause of 7/10 deaths according to CDC 2009 figures and account for >50% of all deaths. We know that chronic illness may cause significant psychological changes affecting, lifestyle, emotional life, education, self - esteem and social relationships, with depression reported as the most complication (133). It is estimated that 1/3rd of individuals with a chronic medical condition experience symptom of depression. The evidence that we have implies that the psychological anguish is a result of the disease and not a cause of the symptoms of the disease.

The lived experience of this chronic condition and the detailed effect on the quality of life of these patients may be difficult to capture on a Likert scale and open-ended questions are likely to generate more data for Qualitative analysis.

I have gone on to measure the quality-of-life impact of a Chronic UTI diagnosed at our centre using validated questionnaires and also obtained qualitative data using patient reported stories on their journey with this chronic disease in later parts of this chapter.

2.2 ICIQ-LUTSqol in the measurement of Quality of life – A comparison of CUTI patients and controls

2.2.1 Introduction

The symptoms of this disease affect many different aspects of a patient's life (4, 134). However, despite this it was noted that treatment assessments failed to include appropriate measures of QOL in the outcome surveillance. So, some groups working in this field, developed tools that could be used to respond to this deficiency. One of the products of these efforts was the ICIQ-LUTSqol is a psychometrically robust patient-completed questionnaire evaluating quality of life (QoL) in urinary incontinent patients for use in research and clinical practice across the world (135, 136) (Appendix 12).

UTIs also impose a substantial economic burden on healthcare systems. Despite the clinical and economic impact of UTIs, there is a surprising lack of data on their effect on quality of life (QoL). This grabbed the attention of physicians attention while searching for health-related QoL data to inform a cost–utility analysis developed as part of the National Institute for Health and Clinical Excellence (NICE) guideline on Infection Prevention and Control (137). They have reported on a systematic review of 864 papers that explored the impact of urinary tract infections on health-related quality of life (137). This review demonstrated the deficiency of QOL data in the literature and paucity of a specific UTI questionnaire was evident.

Patients with lower urinary tract symptom have been an interesting subject for study for the past 3 decades as a result of Prof Malone-Lee's research group questioning the validity of diagnostic tests resulting in other academic units participating in understanding concept that bladder is not sterile and intracellular colonisation being demonstrated on confocal and electron micrography.. In addition, to medical management and performing procedures, such as urodynamics to seek measurements and answers, an alternative method was sought. Validated questionnaires became a supplementary method to measure patient's symptoms and response to treatment. The international continence society (ICS) and the International consultation on incontinence (ICIQ) working group (138) recommend the ICIQ-LUTSqol which captures the Quality of life impact of the LUTS on quality of life of a patient.

The ICIQ-LUTSqol is the King's Health Questionnaire (KHQ) (139) adapted for use within the ICIQ structure and provides a measure to assess the impact of urinary incontinence on quality of life with particular reference to social effects. It is an ideal research tool as it explores in detail the impact on patients' lives of urinary incontinence and can be used as an outcome measure to assess impact of different treatment modalities.

Level of validation according to ICI grades of recommendation: Grade A, validity, reliability and responsiveness established with rigor in several data sets. The average Completion time is 10-15 minutes. Scoring: 19-76 overall score with greater values indicating increased impact on quality of life. Bother scales are not incorporated in the overall score and indicate impact of individual symptoms for the patient (Table 2.2).

Table 2.2 ICIQ-LUTSqol domains

Domain 1 Role Limitation	Domain 2 Physical / Social Limitation	Domain 3 Effect on Personal relationships	Domain 4 Emotions	Domain 5 Sleep and Energy
<ul style="list-style-type: none"> Household tasks Job 	<ul style="list-style-type: none"> Physical activities Travel Social life Friends 	<ul style="list-style-type: none"> Partner Sex life Family 	<ul style="list-style-type: none"> Depression Anxiety/nervousness Bad feeling about self 	<ul style="list-style-type: none"> Sleep/rest Tired
Domain 6 Adaptations	Domain 7 Self Esteem	Overall impact on QOL	Total Symptom Score	Total Bother Score
<ul style="list-style-type: none"> Pad use Fluid restriction Clothing Issues 	<ul style="list-style-type: none"> Smell Embarrassment 	Point Score/10	Point Score/76	Point Score/190

Despite the chronicity of CUTI, we have no formal guidance to managing these complex patients and the QOL impact is not routinely assessed in clinical practice and there are no validated questionnaires to assess QOL in a patient with CUTI. Because of this deficiency I elected to use this method which was designed for patients with LUTS and incontinence because it was so well validated and explored a similar symptoms frame (7).

2.2.2 Methods

The data was gathered from new female patients attending the LUTS clinic and diagnosed to have a CUTI based on symptoms, microscopic pyuria. These measures were compared to a group of volunteering controls with differences validating the pathophysiological state of the patients. Adult patients and controls provided written consent and completed the ICIQ-LUTSqol questionnaire (135). Any patient or control on antibiotic treatment for CUTI or other illnesses were excluded from participation. Bother scores were analysed as a total score without subclassification based on recommendations by the ICIQ committee.

To analyse the data without bias, the data was blinded to age, occupation and menopausal status of the patients.

Statistical analysis was carried out using nonparametric analysis of the data. I am graphing the data and included mean and 95% confidence intervals because they provide some clarity to the differences that were observed.

2.2.3 Ethics

This study had ethical approval from East London & City REC: Ref 11/LO/0109 (Appendix 3). An amendment was requested and granted to collect quantitative and qualitative data using validated questionnaires including patient biographies.

2.2.4 Results

Completed datasets were obtained for 42 controls and 176 patients. The average age of patients was 53 years and controls were 41 years. The symptom sets were

further graded according to the 7 domains and overall impact on QOL was documented.

Further subdivision of data between the 7 domains with the questions: Role limitations, physical/social limitations, effect on personal relationships, emotions, sleep and energy, self-esteem and adaptations showing the difference and quantified impact on various aspects of a person's life is shown in the data below (Table 2.2 & Table 2.3).

Table 2.3 Questions outlined in the questionnaire

Domain 1	Role Limitations
Question 1a	To what extent does your urinary problem affect your household tasks (e.g. cleaning, shopping, etc.)?
Question 2a	Does your urinary problem affect your job, or your normal daily activities outside the home?
Domain 2	Physical and Social limitations
Question 3a	Does your urinary problem affect your physical activities (e.g. going for a walk, run, sport, gym, etc.)?
Question 4a	Does your urinary problem affect your ability to travel?
Question 5a	Does your urinary problem limit your social life?
Question 6a	Does your urinary problem limit your ability to see/visit friends?
Domain 3	Effect on Personal relationships
Question 7a	Does your urinary problem affect your relationship with your partner?
Question 8a	Does your urinary problem affect your sex life?
Question 9a	Does your urinary problem affect your family life?
Domain 4	Emotions
Question 10a	Does your urinary problem make you feel depressed?
Question 11a	Does your urinary problem make you feel anxious or nervous?
Question 12a	Does your urinary problem make you feel bad about yourself?
Domain 5	Domain 5: Sleep and Energy
Question 13a	Does your urinary problem affect your sleep?
Question 14a	Do you feel worn out/tired?
Domain 6	Adaptations
Question 15a	Wear pads to keep dry?
Question 16a	Be careful how much fluid you drink?
Question 17a	Change your underclothes when they get wet?
Domain 7	Self Esteem
Question 18a	Worry in case you smell?
Question 19a	Get embarrassed because of your urinary problem?
QOL impact	Overall impact on QOL
Question 20	Overall, how much do urinary symptoms interfere with your everyday life?

Table 2.4 The responses to individual domains

Response	Score
Not Applicable	0
Not at all	1
Slightly	2
Moderately	3
A lot	4

The sum of scores for the one to three questions exploring each domain ranges between 1 = not at all and 4 = a lot and they scored a 0 if not applicable (Table 2.4). The significant difference between patients and controls across all domains and overall impact on QOL are depicted in Table 2.5 and Figure 2.4.

There was a significant difference in total ICIQ-LUTSqol symptom scores across all 7 domains including the overall impact on everyday life between controls and patients as shown in Table 2.5.

Table 2.5 ICIQ LUTS qol Symptom and Bother scores

ICIQ LUTS qol - Symptom and Bother Scores	Group	Mean	SD	SE	CI	CI (Lower)	CI (Upper)	Kruskal-Wallis Chi-squared	df	p value
Role Limitations	Controls	2.52	1.42	0.22	0.44	2.08	2.97	45.731	1	<.001
	Patients	5.22	2.26	0.17	0.34	4.88	5.56			
Physical and Social Limitations	Controls	4.93	2.67	0.41	0.83	4.1	5.76	44.952	1	<.001
	Patients	9.78	4.54	0.34	0.68	9.11	10.46			
Personal Relationships	Controls	3.29	1.2	0.18	0.37	2.91	3.66	29.778	1	<.001
	Patients	5.8	3.23	0.25	0.48	5.31	6.28			
Emotions	Controls	3.55	1.79	0.27	0.54	3	4.09	54.859	1	<.001
	Patients	7.54	3.39	0.26	0.5	7.03	8.04			
Sleep and Energy	Controls	2.83	1.5	0.23	0.47	2.37	3.3	49.873	1	<.001
	Patients	5.51	1.99	0.15	0.3	5.21	5.8			
Adaptations	Controls	4.05	1.71	0.26	0.53	3.51	4.58	36.07	1	<.001
	Patients	6.88	2.89	0.22	0.43	6.45	7.31			
Self Esteem	Controls	2.14	1.46	0.22	0.45	1.69	2.6	53.172	1	<.001
	Patients	4.75	2.28	0.17	0.34	4.41	5.09			
Overall impact on everyday life	Controls	1.12	2.31	0.36	0.72	0.4	1.84	55.426	1	<.001
	Patients	6.67	3.51	0.27	0.53	6.14	7.19			
Total Symptom Score	Controls	24.43	12.49	1.93	3.89	20.54	28.32	38.076	1	<.001
	Patients	47.8	23.91	1.73	3.41	44.38	51.21			
Total Bother Score	Controls	9.6	27.42	4.34	8.77	0.83	18.37	80.205	1	<.001
	Patients	113.3	51.65	4.26	8.42	104.87	121.71			

Not applicable was not included in the analysis and analysed separately for question 7 and 8 where effect on personal relationships was explored. 32 Patients and 16 controls answered not applicable for all three questions in this domain 3. 69, 67 and 36 patients; 19, 19 and 16 controls marked not applicable to Questions 7a, 8a and 9a respectively.

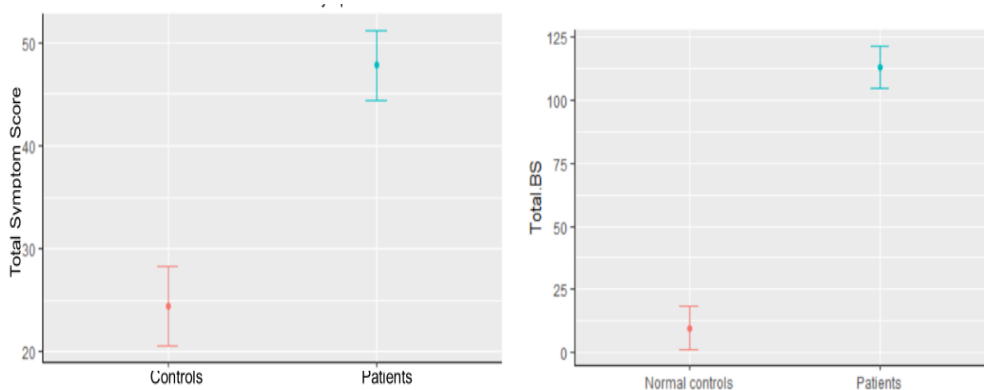
The final question in this questionnaire assessed the Overall impact of urinary symptoms on everyday life. The scoring scale was a Likert scale with a score between 0 (not at all) and 10 (a great deal).

There was a significant difference in the overall impact of LUTS symptoms on everyday life between patients and controls as shown in Table 2.5.

The total symptom score adds the score across all domains and ranges between 19-76. Study participants grade their symptoms based on the scale as detailed in Table 2.4. An individual who is not at all affected by LUTS symptoms would score 19 and the highest score is 76. There was a significant difference between patients and controls as shown in and Table 2.5 and Figure 2.4.

Both scores were not analysed for 19 patients and 2 controls in the study as they had not completed them for the individual domains. The scale of bother for each question was marked by the study participant using the Likert scale shown in Table 2.5 with 0 being not at all a bother and 10 means a great deal of bother. The sum of all bother scores for the 19 questions is 190. Mean bother scores from urinary symptoms for controls was 9.60 and for patients was 113.29 showing a significant difference as depicted in Table 2.5 and Figure 2.4.

Figure 2.4 Total Symptom Score and Bother score (Total BS) between controls and patients



2.2.5 Discussion

It is evident that the LUTS symptoms have a significant effect on the quality of life of a CUTI patient and the questionnaire data is evidence for this. The data obtained from the validated questionnaires provides quantifiable data on the influence of the disease on quality of life. A subgroup analysis was not sought as the numbers would be too small to provide any meaningful data

Whilst it may be that we should use such data as part of our measures of outcome in clinical practice it would be wise to validate such a claim carefully because the collection and analysis of the data are time-consuming and primary goal of the clinical service is to treat the patients in a timely manner. For example, the time taken to answer these questions is 10-15 minutes and evaluation of these symptom questionnaires even with a electronic computerised data collection system is not practical to use in in daily NHS practice.

A shorter version of validated symptom set known as Artemis questionnaire (Chapter 3, Table 3.1) is thus used in this clinic to guide treatment success and the total symptom score is plotted against the microscopic pyuria count at each visit and I have alluded to this in the chapter 6. This has proven to be a most useful tool for the clinician to guide the management of patients and track their progress in this centre.

These numerical assessments are good research tools and provide insight into the patient experience. However, they can be complemented by deploying qualitative methods. Thus, in the next section I describe a qualitative study from 68 patient biographies which were provided by these participants.

2.3 Biographies in the QOL impact of a CUTI - An analysis of 68 stories

2.3.1 Introduction

Qualitative research is a type of social science research that collects and works with non-numerical data and that seeks to interpret meaning from these data that help understand social life through the study of targeted populations or places. People often frame it in opposition to quantitative research, which uses numerical data to identify large-scale trends and employs statistical operations to determine causal and correlative relationships between variables.

While quantitative research is useful for identifying relationships between variables, it is qualitative research that can explore why this connection exists by going directly to the source- the patients themselves. So qualitative researchers investigate meanings, interpretations, symbols, and the processes and relations of social life.

The focus of this type of research is everyday life and people's experiences, qualitative research lends itself well to understanding themes, using the inductive method, which can then be tested with further research.

Methods of qualitative research include:

- Observation and immersion
- Interviews
- Open-ended surveys
- Focus groups
- Content analysis of visual and textual materials
- Oral history

Much of the data collated in the unit has been through meticulous history taking, repetitive capture of symptoms on the unit's database (Artemis) for every patient attendance including email and telephone consultation and careful documentation of patient's progress. My research had focused on the use of quantitative assessment of patient symptoms using validated question sets on the clinic database (Artemis questions - Table 6.1) and use of ICIQ-LUTSqol (Appendix12).

Our patient group presented with years of unrelenting symptoms and were often quite meticulous in bringing in their case files and sometimes a summary which offered a detailed summary of the patient's journey and treatment with a CUTI. This was an invaluable resource providing in depth knowledge which would not be routinely captured even with focused history taking, given the time constraints in a busy NHS service. These patients reported accounts also provided a wealth of information of the impact of the condition on their entire life.

This prompted me to ask a group of patients whether they would be interested in participating in a structured interview process or whether they would prefer to explain their UTI journey in their own words without the pressure of an interview. They requested unanimously to be allowed to submit their experience in their own words for the following reasons:

- It would allow them to look back and accurately describe their journey.
- Looking back at several years of unrelenting symptoms would be quite traumatic so they would like to do it in their own time.
- A self-reported format would place no time constraint or pressure on their account and recollection.
- This would allow them to express themselves without fear of their care being affected.

What this type of research produces is descriptive data that the researcher must then interpret using rigorous and systematic methods of transcribing, coding, and analysis of trends and themes.

2.3.2 Methods

The patients who were diagnosed to have chronic UTI at our centre and had ongoing treatment in the clinic were invited to submit their biographies for qualitative data analysis and written consent was obtained. We had asked them to submit their biographies via email and only 1 patient submitted the biography in paper format.

2.3.2.1 Statistical analysis

I have used NVivo pro12 software to analyse the content of the UTI stories and patient biographies. NVivo helps to organise, analyse and identify themes in unstructured large volume data that these biographies offered.

To begin with, I reviewed the entire content of the anonymised biographies by running a word frequency query which provided counts of word occurrences. I used these to identify recurring themes which I were logged into a project map, which is described by NVivo as a mind map. These themes were then coded as “Nodes” on NVivo. A Node is a collection of data source reference to occurrences of words that map into a recurrent idea in a data set that belong to a theme. This enables to capture the occurrences of words invoking concepts, ideas, opinions or experiences recorded in the patient biographies. The nodes covered broad themes, so I went onto create subfolders under each “Node” to enable more focused analysis the reported data.

To report common themes under each Node or subfolder, tables and graphs were created where appropriate to catalogue the reference occurrences. Some data could not be categorised using their methods because of their wide dispersion and multiplicity so that tabulation and frequency distributions were uninformative. To catalogue recurring themes and to quantify their contribution to the overall aggregate of patient reports, I selected out the most frequently used words in the dataset. I performed word frequency analyses across nodes, sub nodes and data files. This process generated a weighted analysis of occurrence of words expressed as the frequency of the word relative to the total words counted. This describes the words frequency as a portion of the words in each group, so that the overall total does not exceed 100%. Names of clinicians, specific hospitals, dates, age of the patient and any censored language was categorised as a “stop” word which then was excluded from analysis.

The 68 biographies included long pages of text in varying styles. When these were loaded into NVivo they were analysed to create a large database of weighted frequencies. To enable visualisation of the weighting of the word frequency under each node or subfolder, I created “word clouds” with the software within the NVivo programme. Word clouds (also known as text clouds or tag clouds) are simple to view and help communicate important information at a glance especially when the data set is text based rather than numerical. A word cloud is a collection, or cluster, of words depicted in different sizes. The more a specific word appears in a source of textual data, the bigger, bolder and more centred it appears in the word cloud. The bigger and bolder the word appears, the more often it’s mentioned within a given text and the more important it is. The colour of these words and shape of world cloud has no specific meaning or relevance and chosen to make the data visible.

2.3.2.2 Ethics

This study had ethical approval from East London & City REC: Ref 11/LO/0109 (Appendix 3). An amendment was requested and granted to collect quantitative and qualitative data using validated questionnaires including patient biographies.

2.3.3 Results

A total of 68 (67 females and 1 male) adult patients submitted their UTI Story/UTI biographies. 67 were sent via email and 1 was a handwritten which was transcribed into word format. The mean age of the patients was 47 years (18-85 years). The 85year old’s daughter and carer had sent the UTI story on behalf of her mother but the rest were self-reported. The mean duration that these patients experienced symptoms was 12.7 years with a minimum of 1 year and maximum of 42 years.

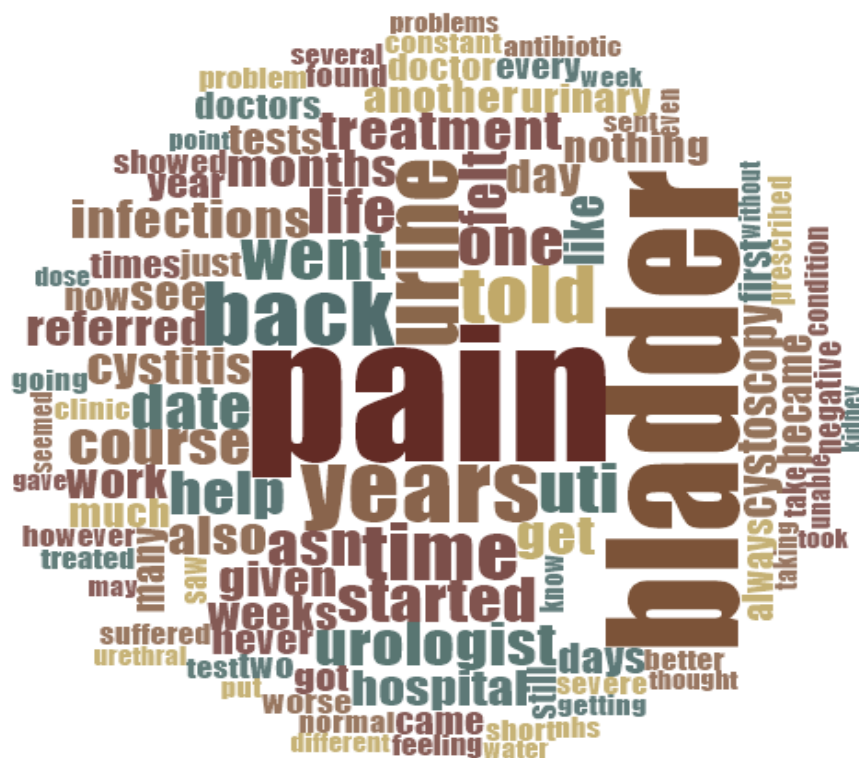
The patients used a variety of formats, fonts, styles and the stories were collated on a word document maintaining all originality and deleting patient identifiers and assigning them research study numbers (ASN-Artemis study number). The description of the document is summarised in Table 2.6.

Table 2.6 Word document content

N	68
Words	47,582
Pages	64
Characters	216,398
Paragraphs	739
Lines	3107

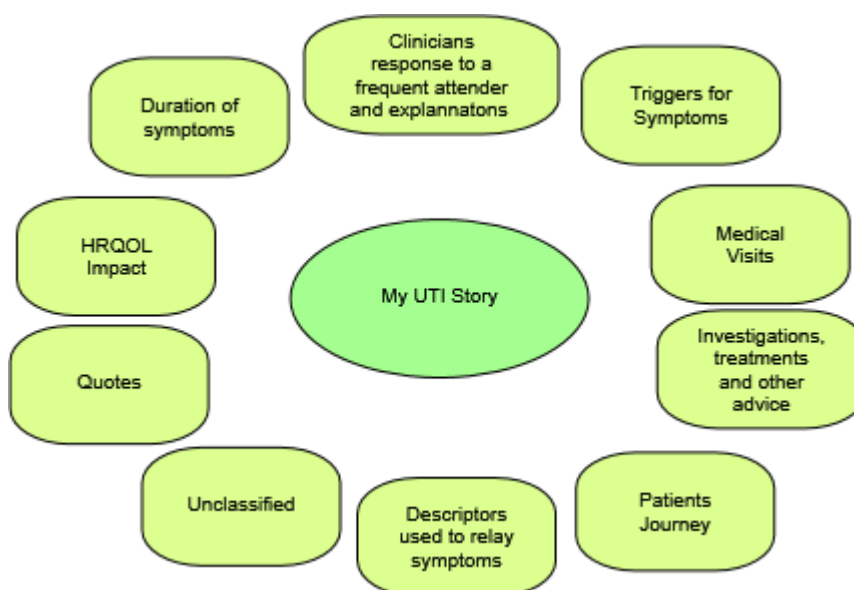
A word cloud was created and the frequency of the words is depicted in Figure 2.5 below. Infection, antibiotics, bladder, pain, symptoms were recurring words.

Figure 2.5 Word Cloud of frequently used words in the 68 patient biographies



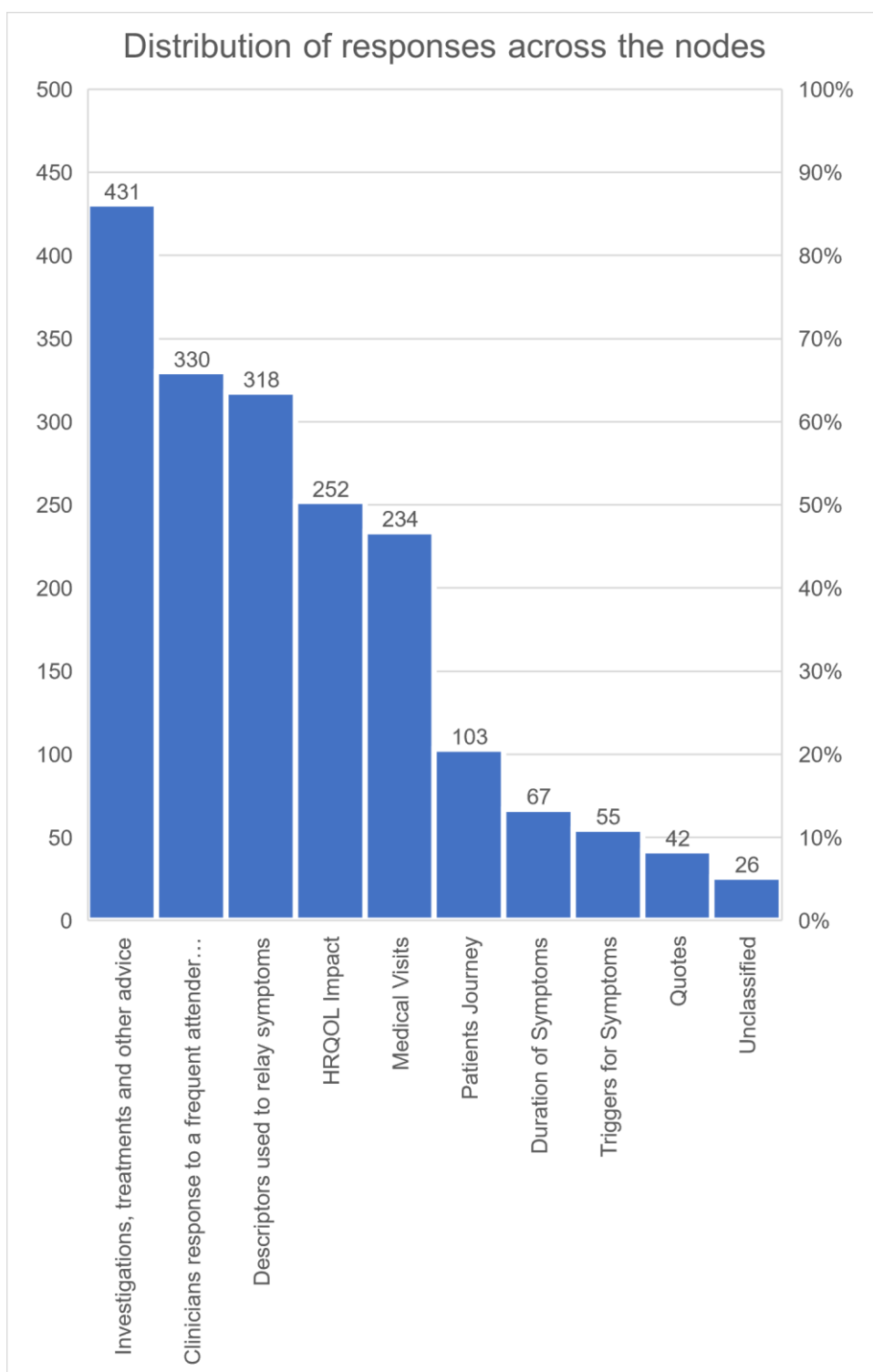
After an initial read through of the collated anonymised data, a mind map was created using the information and this allowed the 68 patient biographies to be classified under 10 nodes which were chosen based on the recurrence of themes as shown in Figure 2.6.

Figure 2.6 Mind map of the data



The individual nodes were then examined and each reported. The bar chart in Figure 2.7 shows the distribution of responses and percentages in descending order of response frequency.

Figure 2.7 Distribution of nodes and percentage of total labelled in descending order of frequency of responses



2.3.3.1 Node 1: Descriptors used to describe symptoms

The descriptors used to relate the symptoms and the adjectives used by patients are summarised in the Word map below. A total of 318 words and adjectives were used in total. The symptoms of pain were the most common.

2.3.3.2 Node 2: Triggers for Symptoms

55 responses were retrieved from the dataset where patients described a trigger for their symptoms. The main trigger for the initial symptoms was commencement of sexual activity. Childbirth, catheterisation, vaginal surgery, hospitalisation for other conditions, travel to destinations, sexual and physical activity were commonly stated as triggers for initial and recurrent infections.

2.3.3.3 Node 3: Duration of symptoms

The mean age that these patients experienced symptoms was 12.7 years with a minimum of 1 year and maximum of 42 years). 14 of these patients being treated infrequently when younger for cystitis episodes before developing recurrent and constant symptoms.

2.3.3.4 Node 4: Clinicians response to a frequent attender and explanations offered

I noted 330 responses to this node, and I have subclassified this into themes stated in the Table 2.7. 64 patients said that their symptoms are dismissed at multiple consultations both under primary and specialist care and most often they were told that the tests are negative hence a urinary infection is unlikely.

Table 2.7 Clinicians response to frequent attenders and explanations offered

Clinicians response to a frequent attender and explanations offered		330
1	Negative diagnostic tests	73
2	Dismissal of symptoms	64
3	Caused by other medical conditions	53
4	Nothing can be done	50
5	Stress or psychological cause for symptoms	30
6	Nothing wrong with me	27
7	IC PBS diagnosis	16
8	Blame the patient	11
9	Likely family, personal or marital problems	3
10	Guideline, funding and rules	3

Thirty patients report being told that the symptoms were all in their head or of psychological nature. Three women were told that this is most likely a result of problems in their relationship.

2.3.3.5 Node 5: Investigations, treatments and other advice

This node had 431 descriptives coded with antibiotics being the most common treatment Table 2.8. The remaining subdivisions are reported in subsequent sections of this node.

Table 2.8 Distribution of responses on investigations, treatments and other advice

Investigations, treatments and other advice		431
1	Antibiotic treatment	145
2	Invasive tests and treatments	131
3	Investigations	56
4	Non-evidence-based treatments	55
5	Dietary and fluid advice	27
6	Hygiene chat and advice on sexual practices	9
7	Non-evidence-based advice	5
8	Personal and relationship advice	2
9	Hiprex	1

2.3.3.5.1 Antibiotic treatments and Hiprex (Methenamine Hippurate)

A recurrent theme noted on the biographies was that clinicians were prescribing numerous short courses of antibiotics between 3-7 days in the presence of symptoms. The most common treatments were Trimethoprim and Nitrofurantoin. Only 1 patient in this cohort was prescribed Hiprex prior to coming to our clinic. Low dose prophylaxis was commenced mostly by specialists. Several patients received cyclical antibiotics. Hospital admissions were usually resulted in IV antibiotics however the patients were unable to name the antibiotics used in hospital. Patients were treated empirically only when a symptomatic patient had a positive dipstick. While the MSU cultures were sometimes positive at the first episode, subsequent analyses rarely yielded a positive result, hence no therapy was offered. The Word map Figure 2.8 depicts the frequency of antibiotic related responses from the biographies. Antibiotic prophylaxis included use of low dose single daily dose of Trimethoprim 100mg, Nitrofurantoin 50mg or cefalexin 250mg once at night. Some patients were asked to take one of two doses of a low dose of the above antibiotics post sex. Cyclical regimes often had the patient rotating between 3 antibiotics in full or low dose with changes 2 or 4 weeks and often maintained for 3-6 months and <40% of women found this beneficial who then reported that the antibiotics stopped working after some time. Hospital admissions where known were associated with use of higher generation of IV antibiotics like Tazocin, Gentamicin, Ciprofloxacin and Augmentin.

2.3.3.5.2 Dietary and fluid advice

27 women were offered dietary and fluid advice. Most common advice was to drink more water “copious, gallons, drink load and loads, flush out the bugs, are you drinking enough?”. Patients were often told to avoid citrus, caffeine, alcohol and other bladder stimulants. A few were asked to go on an IC diet herbs, and cranberry juice were recommended. Specialists recommended excessive fluid consumption some up to 3-4 litres and one patient had been recorded to consume 7.5 litres of fluid daily and despite which she was not relieved of her urinary symptoms.

2.3.3.5.3 Hygiene chat and advice on sexual practices

All our patients were given the usual “wipe from front to back”, a few quizzed the patients on toileting practice. A few were advised to pass urine before and after sex or to avoid sex. Only 9 of the 68 patients reported the advice given under this node.

2.3.3.5.4 Invasive tests and treatments

131 responses were received under this subclassification of the above Node (Figure 2.8). Cystoscopy was the most common test and out of these patients 67 of these patients underwent at least one cystoscopy and 42 of them reported more than one and the maximum being 6 cystoscopies. Some were told that they had a bladder stretch before and others were informed after and some did not know. Majority of the patients reported that their symptoms often worsened shortly after a cystoscopy. Urodynamics were performed by specialists when the tests were negative, and the patient had persistent symptoms. Cystodistension was only reported by 2 women. Only 5 women recollect a bladder biopsy although 16 of them were given a diagnosis of IC.

2.3.3.5.5 Investigations reported

56 of the biographies reported further investigations (Figure 2.8) aside from urine dipstick and MSU. An ultrasound scan of the kidneys was the most frequent investigation undertaken following a specialist review. Other investigations included STI screens, Vaginal swabs, Cytology, MRI, CT, micturating cystourethrogram, transvaginal USS and blood tests for infection markers. Some patients report repetition of investigations on moving from one specialist or hospital to another.

2.3.3.5.6 Non evidence-based advice

Five women were offered the following advice:

- “Pregnancy and childbirth often cured cases of recurrent infections such as mine, so told to have a baby”
- Rest
- Relaxation
- Heat treatment
- Change of partner

2.3.3.5.7 Non evidence- based treatments

The 55 non-evidence-based treatments (Figure 2.8) that these patients reported are depicted on the word map with acupuncture reported most frequently. Many women reported taking over the counter remedies, OAB medications, NSAID's, neuropathic painkillers (pregabalin, gabapentin, amitriptyline, opioids), anti-depressants, cinnamon oil, hormonal treatment, gas and air, D mannose, per urethral antibiotics, bladder instillations and seen by herbalists, Chinese medicine

2.3.3.6 Node 6: Medical visits and complications related to a UTI

Medical attendances were captured under separate node and patient visits into their GP surgery could not be captured with numerical accuracy as the patients summarised this as a frequent occurrence. Patients in this dataset often requested a specialist referral as they were unwilling to accept the GP's dismissal. A 112 mentions of specialist referrals were found in the dataset and most commonly these women saw a urologist. Other specialist referrals include- Gynaecology, sexual health, Urogynaecology, Pain team, Hypnotherapist, Psychologist, Psychiatrist, Renal clinic, Endocrinologist and physiotherapist.

12 women reported attending A&E with symptoms and being given oral antibiotics, 13 women reported to have been admitted via A&E and treated with IV antibiotics. Of these most patients stayed in hospital for 1-4 days with diagnosis of pyelonephritis +/- urosepsis and one patient was admitted for 2 months and was diagnosed to have renal failure and underwent a renal and pancreatic transplant within 6 months of the initial admission.

29 women reported these complications relating to a CUTI. These have been reported in Table 2.9.

Table 2.9 Complications relating to CUTI

Complications relating to CUTI	Number of Patients
Renal failure requiring renal and pancreatic transplant	1
Haematuria	4
Recurrent thrush	1
Pyelonephritis	9
Diarrhoea following treatment	1
C Diff	1
Renal stents	1
Premature labour	1
Miscarriage	1 x 6*
Urinary retention needing catheters short term	6
Indwelling catheter for 4 weeks	1
CISC	1
Fall	1

*1 person reported 6 recurrent miscarriages

2.3.3.7 Node 7: HRQOL- Health related quality of life impact

I have retrieved 252 comments related to impact of their condition on HRQOL were subdivided into the themes stated in Table 2.10 and Figure 2.9.

Table 2.10 Reporting impact on HRQOL

HRQOL Impact		252
1	Overall QOL impact -Work, ADL	96
2	Impact on patient's psychology	86
3	Patient's perception of her condition	38
4	Impact on other aspects -personal, physical and family	18
5	Mistrust in medical profession	11
6	Acceptance	3

2.3.3.7.1 Overall QOL impact

There are 96 entries describing the negative impact of CUTI on their activities of daily living, ability to look after their family, children, ability to travel or go on holidays and performance at work. The word cloud demonstrates the frequency of descriptors and words “life, work, family, unable”, have been used more frequently in the patient reported impact.

2.3.3.7.2 Impact on patient's psychology

86 women commented on the impact of the condition on their psychological and mental wellbeing. Some of the notable quotes are listed in Table 2.11 and Figure 2.9. “*Suicide, pain, depression*” were frequently used words. They also expressed anxiety, despair, embarrassed and being a burden and two women expressed desperation and guilt for not being able to be a good mother.

Table 2.11 Some quotes from patients about how they felt with a CUTI

<p>“regressing into being a child”</p> <p>"I was almost suicidal at having to live with this level of pain for the rest of my life"</p> <p>"I felt suicidal"</p> <p>"feeling down and having suicidal thoughts"</p> <p>"kill myself because I was in so much pain"</p> <p>"suicide at times as I just cannot live on with the pain"</p> <p>"My world fell apart again. I felt I was being a nuisance and a time waster"</p> <p>"Humiliated, unable to cope, at my wit's end"</p> <p>"At one specialist pain clinic I came out feeling suicidal and had to call a close friend"</p> <p>"I have had suicidal thoughts"</p> <p>"This was a dark time for me I thought my life was over"</p> <p>"I was suicidal, felt like a death sentence, I felt suicidal"</p> <p>"I was verging suicidal"</p>

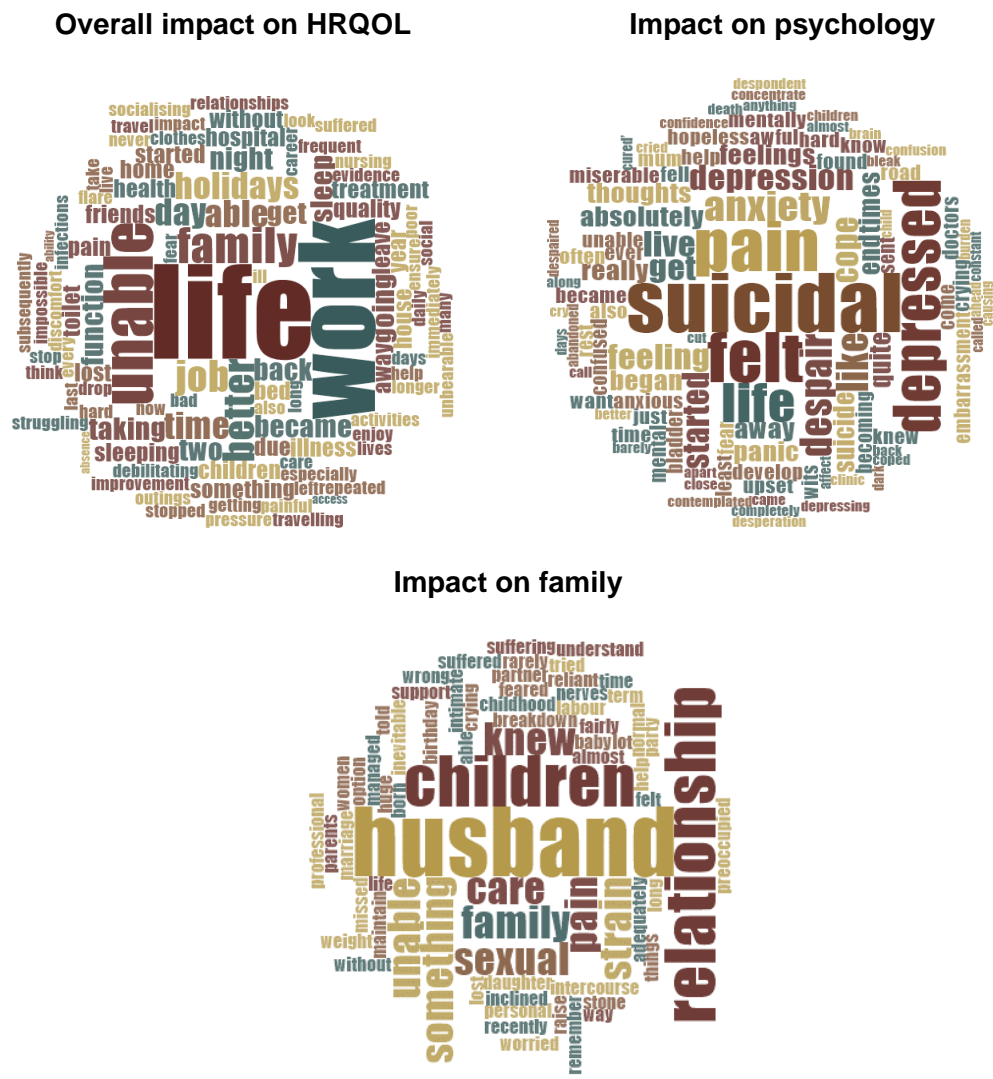
2.3.3.7.3 Patient's perception of her condition

These patients have stated 38 times that they knew they had a UTI all along at multiple attendances despite the doctors saying otherwise.

2.3.3.7.4 Impact on other aspects - personal, physical and family

18 women expressed the impact of their condition on their personal relationship and their children. They were unable to have sexual activity as these triggered worsening symptoms and felt guilty about this. They also knew the strain that this placed their marriage, and two women reported a separation. One woman reported that she was taken seriously only when her husband accompanied and corroborated her symptoms (Figure 2.9).

Figure 2.9 Word cloud depicting the frequency distribution of responses related to Overall impact on HRQOL, Impact on Psychology and impact on Family



2.3.3.7.4 Mistrust in medical profession

11 women commented on mistrust in medical professionals due to repeated dismissal of their severe symptoms. Some patients reported anxiety facing a clinician and avoided seeking help until they became very ill. They were also afraid of being labelled as a troublemaker or overthinker. Many sought referral to

a specialist due to the primary care physician unable to help any longer. Some feared for their life and in despair that no medical professional is able to diagnose or treat them.

2.3.3.7.5 Acceptance

Of the 68 women, three women reported that they felt nothing could be done hence accepted their condition and had to manage on their own and even stopped antibiotics as they were told even though they were helping. However, these women went on to write that they made several dietary and lifestyle modifications to cope but eventually sought help when the symptoms became unbearable.

2.3.3.8 Node 8: Patient journey

2.3.3.8.1 Positives experiences that the patients reported

68 women described things that they had found helpful and which they appreciated.

“I was listened to”, “I had an infection in my urine”, “I have to take long term antibiotic”, “the treatment is working”, “I can email the clinic and get a quick response”, “I feel my life is back”, “I am lucky”, “I have my life back”, the team is wonderful”, “research into UTI”, “I fear for my life if the clinic closes”, “I have gone back to work”, I can be intimate with my husband” were some of the recurring reports of patient experience.

2.3.3.8.2 Comments on exposure to treatments outside of guidelines

13 patients in this cohort acknowledge that the treatments protocols in the unit are outside guidelines and are willing to take long term antibiotics and they reported a life-changing improvement in their HRQOL.

2.3.3.8.3 Support Group Access

22 of these women sought a referral following their own research and sites they found useful have are OBBI, COB, google search of “embedded UTI” or “Biofilm infections”, or heard about the clinic from allied health specialists like MS nurses and Physiotherapists.

Nearly half the patients who sought a referral to this unit reported resistance from clinicians to make the referral or hearing negative opinions and comments regarding the service.

Only one positive comment was noted among the quotes when a patient sought a referral. “My best description of his clinic to those seeking treatment is that it is like coming into a safe harbour after being battered on stormy seas.”

2.3.3.9 Node: 9 Quotes

A word cloud of the 42 quotes bore similar themes to that described in the explanations given for recurring symptoms by clinicians and the despair expressed by patients at not having a diagnosis. The language borne out in these quotes is shown here with “Told” being the most frequent followed by “like, pain, infection, symptoms, antibiotics, help, suffering, condition, help and others”.

These quotes address repeated themes, in which patients have symptoms, they go to the clinician, they are told they have no infection due to negative tests despite the continued suffering from recurring and constant symptoms and reports the frustrations expressed by the clinician and patient.

2.3.3.10 Node 10: Unclassified

This node had 26 references and the theme in these references mainly bore out the repeated attendances and frustration expressed by the patients on not getting better. Some clinicians were reported as understanding but unable to help. Some of the language was emotional and even questioned the clinician’s competence to practice medicine.

2.3.4 Limitations

The main limitation of this study was that these patients were grateful that they had met a specialist clinic and finally had a diagnosis hence may have been more willing to write a detailed biography.

This is a group of patients who have improved after failing most known regimes hence more likely to have provided positive feedback for our clinic.

2.3.5 Discussion

The qualitative data in the patient biographies has clearly provided a different side to my understanding of these patients and their journey prior to treatment in the unit. The word clouds although traditionally not used in Medicine has highlighted very poignant descriptors for the symptoms we measure in medicine and has drawn out what is important from the patient’s point of view. Given the recent review “First Do no Harm” released in July 2020 (140), the Biographies have drawn out important themes that are important to the patient and becomes important step in understanding a patients journey and helps shape treatments specific to this unique group involving the patient in the shared decision making process.

The mean duration of symptoms in this cohort is 12.7 years while in subsequent chapters with larger sample population, I have reported this as 6.5 years. The mean age of the patients was 47 while the mean age of the clinic population is 53 years which is not relevant.

While I am a good clinician and pride in being objective and taking a thorough history, I would never have had the time to explore in detail the impact that the

condition had on these patients prior to referral to our unit in a routine consultation.

The paucity of the diagnostic tests is what has mainly led to the condition being dismissed as being of psychological origin. The overreliance of diagnostic tests and clinicians often telling patients that there is nothing wrong with them led to the patients feeling anxious, stressed, afraid that there is no help, or they have not been believed and made them start to doubt their own feelings.

The recurrent visits to primary care, A&E and hospital admissions are often not captured in detail during routine clinical evaluation and patients often coped with their illness due to fear of not being believed or classified a hypochondriac until they became more unwell.

Pain was the most frequent symptom described closely followed by the remaining LUTS symptoms. As clinicians we are often used to charting pain on a Likert scale with "0" being no pain and "10" being maximum pain (141). This analysis provided us with numerous descriptors for the pain symptoms that we do not capture during a routine consultation.

The biographies also detailed the impact of this condition on a patients work, personal and family relationships and the word clouds brought out the recurrent themes and the frequency of impact. Studying psychological impact in a traditional way using validated questionnaires, provide very little meaningful data as the numerical data does not explore individual experiences and struggles. The Biographies brought out the psychological impact of the condition in detail with the real-life descriptors which are often not explored or understood in a routine clinical consultation. This is much needed for the clinician to begin to understand the impact and certainly helped transform the experience of a patient as stated by the patient during subsequent visits to the centre.

These patient biographies brought in a more personal and humane perspective on the patients journey with this condition and I have gone on to study this population further in subsequent chapters in my thesis.

2.4 Medical language used for explanations to patients for their symptoms

2.4.1 Introduction

The doctor-patient relationship has evolved over history in reaction to scientific and culture changes. For a long time, relationship was predominantly pedagogic with a patient seeking help from a doctor who issued instructions that the patient passively obeyed. This was advocated by Parsons (142) who described well a paternalistic approach to medicine. Mead and Bower (143) contradicted Parsons by championing a democratic structure of equality in the doctor-patient relationship. Our contemporary culture has tended to favour a shift in the doctor-patient relationship from the 'guidance-co-operation' model to 'mutual participation' (144), whereby power and responsibility are shared with the patient, although this is not universally supported by the patient caucus. Byrne and Long (1976) (145) suggested that patient-centered consultations reflect recognition of patients' needs and preferences, characterized by behaviours such as encouraging the patient to voice ideas, listening, reflecting, and offering collaboration. Over the last 2 decades, open access journals and information easily available in the public domain has meant that the patients with chronic illnesses are able to become better informed, and where a niche interest is concerned, they may be more knowledgeable than many health care providers. Clearly a patient's level of education and commitment to learning is no constant. Patient advocacy organisations can do great work through appropriate communication and information structure for a lay audience. However, it should be kept in mind that ideology and anti-science attitudes can infiltrate these sources. Like all organisations they are susceptible to conflicts of interest, which have been such a major concern in our professions.

2.4.2 Aim

The aim of this study was to explore some of the rationale and advice that clinicians have offered to patients who present to a surgery with symptoms of recurrent or chronic UTI and negative urine tests.

2.4.3 Methods

I contacted the patient support groups and asked them to provide me with vignettes describing what clinicians had said to them about their symptoms, the explanations and advice on treatment. The responses to these enquiries were provided by emails and I collated the texts of these submissions into an anonymised database.

I used the NVivo pro12 software to analyse the texts in order to identify recurring themes and to quantify their contribution to the overall aggregate of patient reportage. Details of NVivo system are reported in Chapter 2.3.2.1.

2.4.4 Results

115 patients submitted a total of 146 quotations that they used to describe their witness of the dialogues they experienced during their contacts with health carers in relation to their LUTS. These consultations were with primary care physicians

Urologists or Urogynaecologists, specialist nurses, physiotherapists, and psychologists whilst they sought relief from their suffering. I have collated the comments into themed nodes which are described below.

2.4.4.1 Word frequency map and mind map of analysis

Word Frequency query was used to list the 1000 most frequently occurring words or concepts in the data to identify possible themes and presented as a word cloud. These generated a word cloud of the data as shown in Figure 2.11. Other than “patient”, “doctor”, and “bladder” which we should see recurring, words like “just”, “stop”, “negative”, “told”, “need”, “sure”, “nothing” recurred with considerable frequency.

Figure 2.10 Word frequency cloud of the data



The data were divided into nodes based on recurring themes that I identified from the NVivo analysis. I have presented the frequencies of occurrence of these themes in the table, and I have embellished this with a frequency distribution. The theme of attributing blame to the patient was the modal node and this was detected by presage phrases such as “you are not/you do not have”. A mind map of common themes was created (Figure 2.12) at the start of the analysis and this formed the basis for the nodes with frequency depiction in Figure 2.12.

Figure 2.11 Mind map of responses

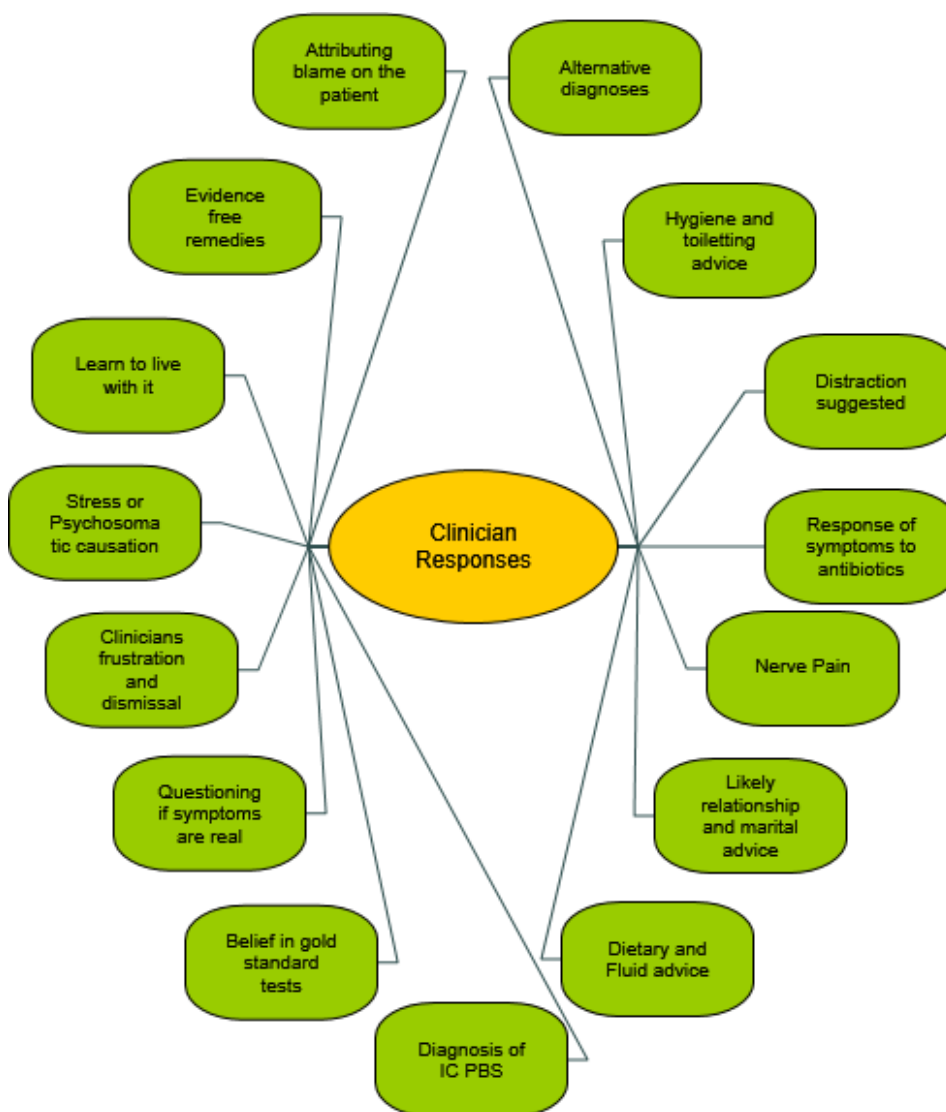


Table 2.12 Distribution of frequency of responses per node

No.	Nodes	Responses
1	Attributing blame on the patient	24
2	Evidence free remedies	17
3	Learn to live with it	15
4	Stress or psychosomatic causation	15
5	Clinician's frustration and dismissal of patient	13
6	Questioning if symptoms are real	13
7	Belief in gold standard tests	13
8	Dietary and fluid advice	13
9	Diagnosis of IC PBS	12
10	Likely relationship and marital reasons	9
11	Alternative diagnoses	8
12	Hygiene advice	8
13	Distractions suggested	5
14	Response of symptoms to antibiotics	4
15	Nerve pain	1

As the data under the notes is limited in content, I have attempted to report the top 5 nodes and summarised the rest.

2.4.4.2 Node 1: Attributing blame to the patient

This node was coded with 24 responses with a coverage of 8.24% with references to individual responses are shown in Table 2.13.

Table 2.13 Frequency distribution of descriptors attributing blame to the patient

Alternative diagnoses	Coverage %	Response
Reference 1	0.64	"You just need to retrain your bladder. Hold on to your wee for longer until your bladder fills properly."
Reference 2	0.56	"It's not your bladder, it's your vagina that's hurting. It's easy to get the two confused."
Reference 3	0.53	"You're a person who holds your emotions all in, that's why your bladder is playing up."
Reference 4	0.51	"You've only just had a baby, go easy on yourself, your pelvic floor is probably weak."
Reference 5	0.50	"Do you think maybe it's just become a bad habit you can't break? In your head?"
Reference 6	0.45	"You need to lose a lot of weight, then all your problems will go away."
Reference 7	0.41	"You need to learn the difference between thirst and a dry mouth."
Reference 8	0.38	"You need to focus more on your life and less on your bladder."
Reference 9	0.38	"You're making yourself like this by giving in to the urges."
Reference 10	0.37	I was "researching [my] symptoms extensively on the internet"
Reference 11	0.34	"You're drinking too much water—you need to cut back."
Reference 12	0.34	"You really need to learn how to provide a clean sample."
Reference 13	0.31	"You just need to stop thinking about your bladder."
Reference 14	0.28	"You should stop going to the toilet so often."
Reference 15	0.28	"You have to train your bladder and hold it in."
Reference 16	0.27	"Stop thinking about your bladder so much."
Reference 17	0.26	"Some women are just prone to getting UTIs."
Reference 18	0.25	"Why aren't you taking daily laxatives?"
Reference 19	0.24	"Your bladder doesn't empty all the way."
Reference 20	0.24	"You just need to retrain your bladder."
Reference 21	0.23	"You need to reset your bladder clock."
Reference 22	0.22	"Stop focusing on it [<i>your bladder</i>]."
Reference 23	0.15	"It's just your anatomy."
Reference 24	0.09	"Try harder!"

2.4.4.3 Node 2: Evidence free remedies

This node was coded with 17 responses with a coverage of 7.56 % with references to individual responses are shown in Table 2.14.

Table 2.14 Frequency distribution of frequency evidence free remedies offered to patients

Alternative diagnoses	Coverage %	Response
Reference 1	1.32	"Surely a beautiful young woman like yourself has lots of partners. The secret is to insert yoghurt into your vagina, I guarantee you won't be back to see me."
Reference 2	1.08	"Just more than you are." She ended up drinking 7 litres a day with a bladder capacity of 1.7litres, only to be told after hydro-distension that I was now 'drinking too much'.
Reference 3	0.84	"My UTI only ever got embedded because a doctor decided to think that my partner was abusing me and didn't want to test or treat me for it."
Reference 4	0.64	"I can guarantee you do not have an infection. Once you stop breastfeeding this will all sort itself out."
Reference 5	0.61	"You're getting repeat UTIs because you're not having lubricated sex. You need to be more lubricated."
Reference 6	0.53	"Here, try some steroid cream and if there's no improvement we'll increase the strength."
Reference 7	0.38	"You can take a paracetamol and here is some Bepanthen crème."
Reference 8	0.28	"Just do some yoga and have a glass of wine."
Reference 9	0.27	"Stop breastfeeding, it'll probably resolve."
Reference 10	0.25	"Take a couple Nurofen, you'll be right."
Reference 11	0.24	"It will stop when you have children."
Reference 12	0.24	"It might help if you went cycling."
Reference 13	0.21	"Are you sure you're not pregnant?"
Reference 14	0.21	"Have you tried anal sex instead?"
Reference 15	0.18	"You need to drink more water."
Reference 16	0.15	"Find yourself a hobby."
Reference 17	0.12	"Get distracted."

2.4.4.4 Node 3: Learn to live with it

This node was coded with responses with a coverage of 7.04% with references to individual responses are shown in Table 2.15.

Table 2.15 Frequency distribution of frequency of comments relating to learn to live with the condition

Alternative diagnoses	Coverage %	Response
Reference 1	0.87	"I've been in this job for 25 years—they all say the same things as you. There's no cure, no treatment, no tests worth carrying out, no point."
Reference 2	0.79	"We can't give you painkillers because you're pregnant. Pregnancy is supposed to be painful so just try and deal with it better."
Reference 3	0.73	This is how your life is going to be now. You need to accept it and practice mindfulness as the pain is in your head."
Reference 4	0.72	"You're like an 83-year-old granny. You should be lucky if you only get 3 UTIs a year. You'll have to live with it."
Reference 5	0.62	"I don't want to give you the wrong idea. I need to tell you straight up I will never be able to fix this"
Reference 6	0.42	"It is all in your head. Get it out of your head and get out of here!"
Reference 7	0.40	"It just happens to some women. You have to learn to live with it."
Reference 8	0.40	"You should grow out of it. Make sure to eat more vegetables."
Reference 9	0.38	"You're only 23. It's common for people your age to have UTI."
Reference 10	0.38	"You're just one of the unlucky ones that suffer with cystitis."
Reference 11	0.34	"This is just the way you are. We will see how you go."
Reference 12	0.29	"Hahaha, every pregnant woman pees all the time."
Reference 13	0.27	"Some women are just prone to getting UTIs."
Reference 14	0.27	"Well, if you are worried you can wear pads."
Reference 15	0.16	"The body will resolve it"

2.4.4.5 Node 4: Stress & psychosomatic illness

This node was coded with 15 responses with a coverage of 7.37% with references to individual responses are shown in Table 2.16.

Table 2.16 Frequency distribution of frequency of comments relating to condition relating to stress or a psychological condition

Alternative diagnoses	Coverage %	Response
Reference 1	1.34	"Some women hold their pain in their pelvis and vagina, that's what vulvodynia is. You have oversensitive nerves. You need to do some relaxation exercises."— <i>she was later diagnosed with a heavy candida glabrata infection</i>
Reference 2	1.02	"In his letter to my GP that I seemed a rather depressed and anxious young woman. He sent me to a psychiatrist who put me on antidepressants for 8 weeks (without any effect)."
Reference 3	0.61	"I think it's stress. You're a busy wife and mother. You could look into some ways to reduce stress."
Reference 4	0.60	"Your MSU results are back and it's negative. You need to stop doing this—this UTI is in your head."
Reference 5	0.50	"I was told it was all in my head because it's on my notes I was sexually assaulted."
Reference 6	0.50	"Do you think maybe it's just become a bad habit you can't break? In your head?"
Reference 7	0.44	"The other doctors here think this is either in her head or in yours."
Reference 8	0.38	"Go for a walk and burn some candles, I think you are stressed."
Reference 9	0.37	"He made me feel like a hysterical woman if that makes sense."
Reference 10	0.31	"Are you sure it's not stress causing your issues?"
Reference 11	0.30	"Oh yeah, that is going around a lot these days."
Reference 12	0.30	"You don't need antibiotics, you need counselling."
Reference 13	0.27	"Pain is a negative word. Call it a feeling."
Reference 14	0.25	"I think you should go to a psychiatrist."
Reference 15	0.19	"You need CBT for your anxiety."

2.4.4.6 Node 5: Clinician frustration and dismissal

This node was coded with 13 responses with a coverage of 7.48% with references to individual responses are shown in Table 2.17.

Table 2.17 Frequency distribution of frequency of comments suggestive of a Clinicians frustration and dismissal of patient's symptoms.

Alternative diagnoses	Coverage %	Response
Reference 1	1.29	"I went to see a local urologist and he never even allowed me to ask the questions I had on my paper! Instead, he just threw prescriptions at me. He did say 'if you don't take Elmiron, you are going to get worse'."
Reference 2	1.13	"My previous urologist didn't like me doing research myself and wouldn't answer any questions I had about

		articles I brought him. He literally waved it away which I felt really undermined me”
Reference 3	1.10	Patient: "I don't understand though. Why would my immune system decide to randomly attack my bladder on 23 October when this all started?" Doctor: "I wish I could give you an answer."
Reference 4	0.66	"I don't know, maybe we should bring you out back and kick in your legs." (kinda like an old yeller movie)."
Reference 5	0.59	"Do you expect me to sit here and read these for you when I could be spending time with my family?"
Reference 6	0.55	"She walked out the room and said she didn't want to see me again and she was discharging me."
Reference 7	0.42	"It is all in your head. Get it out of your head and get out of here!"
Reference 8	0.37	"That isn't a term we recognise as General Practitioners".
Reference 9	0.35	"That's bullshit, I suppose you got that from Dr Google?"
Reference 10	0.27	"Oh, but you have probably already seen many."
Reference 11	0.27	"I don't know what else we can do from here."
Reference 12	0.27	"How many more tests do you want us to do?"
Reference 13	0.22	"I've given you something, now go!"

2.4.4.7 Nodes 6-14

The patient report being questioned if their symptoms were real and often the symptoms were attributed to something else that patient may be upset about. Some interesting phases were reported:

- "Well, I don't really believe in that bacterial stuff, and anyway I treat mostly men. They just scheduled you to see me because I was the first available. You need a female urologist."
- "I think you're pissed off about something. That's why you're feeling pain in your bladder. That's where the term 'pissed off' comes from."
- "I think you are making the pain bigger than it actually is, you should just forget about it and do something useful."
- "You can't need the toilet that much as you've been sitting here talking to me for the past 10 minutes."

Belief in gold standard tests led to reports of clinicians stating the following:

- "I looked inside your bladder myself and everything looks fine, and your last culture came back negative. Now go away, there's nothing wrong with you."
- "You have sterile pyuria—there's nothing for me to treat. It says your test results were negative—no further action required"
- "Your MSU results are back and it's negative. You need to stop doing this—this UTI is in your head."

- “The test shows the infection has gone. It can take the bladder a while to fully recover.”
- "The culture is negative [*despite growth of multiple organisms*] so you don't have a UTI."

The patients were offered evidence free dietary and fluid remedie and main emphasis was to drink a lot of water (suggested between 3-7.5 litres were quoted) and to avoid variety of foods and caffeinated products including alcohol to alleviate symptoms.

12 of these women recall being told that they had a condition known as IC for which there is no cure for the disease and main aim should be to manage symptoms and learn to live with it.

9 responses were received where the clinicians suspected that marital or relationship problems were the main reason for the patients recurrent attendance and a variety of advice was offered:

- “One GP told me that the reason I had the pain was because I must be having doubts about my recent engagement! But my health problems with my bladder started many years before I even met my future husband!”
- "So, you think you get a UTI every time you have sex. Maybe you should stop having sex [*with husband of 20 years*], you already have children so it's not like you need to."
- “Many years ago, I once had a GP tell me my pain was because I must not have achieved orgasm during sex.”
- “I was told it was all in my head” because it’s on my notes I was sexually assaulted.; “Surely a beautiful young woman like yourself has lots of partners.” “Maybe you’re just not as into him as you think.”; “We don’t need intimacy anyway. Could you get a pet of some kind?”

Alternative diagnosis such as endometriosis, Pelvic inflammatory disease pelvic dysfunction, STD, Pregnancy, round ligament pain and urethritis /vaginitis were reported. Advice on perineal hygiene and correct toileting practice was offered by clinicians. Techniques for distraction suggested include:

- “Don’t focus on your urethra, try to go to the hair salon, nail salon, meet your boss. If you can’t start work, you will lose money.”
- "Go for a walk and burn some candles, I think you are stressed."; “You need to focus more on your life and less on your bladder."
- “Go out in the sun to help your stressed-out bladder.”
- “You just need a vacation.”

When the patient reported symptom response to antibiotics, some clinicians stated that this was either a a placebo or anti-inflammatory effect of the antibiotic as the tests did not reveal a urine infection.

2.4.5 Limitations

The quotes are provided by patients who attend this service and have since been diagnosed to have a CUTI. Their relief on receiving a diagnosis will have an impact on the quotes they have chosen to share and cannot be generalised. While the numbers are small and the language used is interesting, some of the quotes may not necessarily represent a doctor wishing to cause harm, it may suggest the frustration on the part of the clinician not having a confirmatory test.

2.4.6 Discussion

“The good physician treats the disease; the great physician treats the patient who has the disease” – William Osler (Canadian Physician, 1849-1910).

Chronic Urinary tract infection (CUTI) is a debilitating condition. There are no reliable tests to diagnose the condition, no guidelines to treat and often these patients are told they have interstitial cystitis (IC), a chronic condition which cannot be treated, and they should learn to live with it. The paucity of diagnostic tests and the big gaps in the knowledge regarding CUTI often resulted in paternalistic exchanges between a doctor and a CUTI sufferer as reported in this survey.

The data presented here shows that patients are often offered alternative diagnoses like STI, Vulvodynia, BPS, IC, IBS and a variety of interventions like psychological and pain team assessment, further diagnostics, invasive surgery and some even undergo a cystectomy and diversion on the advice of their specialists.

The feeling of their symptoms being dismissed and the fear that the doctor does not know what to do or cannot help often leads to a loss of confidence in the clinician and makes consultations much more difficult. It is often devastating to receive a diagnosis with a poor prognosis but some of our patient's state: “they would rather get a diagnosis of cancer, as there is an end to the disease, but with this, we seen no cure or end and living with it is simply not possible”.

Chronic illnesses lead to a huge impact on quality of life, daily tasks, ability to work and may even lead to relationships and marital breakdown and asking the patient to “get a life, a hobby, a pet, find a new man” are certainly terms not befitting a medical consultation.

A ‘patient-centred’ approach is increasingly regarded as crucial for the delivery of high-quality care by doctors. However, there is considerable ambiguity concerning the exact meaning of the term and the optimum method of measuring the process and outcomes of patient-centred care. When a doctor patient relationship involves competence and communication, typically there is better adherence to treatment, improved health and better QOL.

It is imperative that as clinicians we keep ourselves apprised of literature. Mutual participation, respect, and shared decision-making must replace passivity. Shared decision making between the doctor and patient will determine the most appropriate and best course of action for an individual patient and result in better outcomes.

There is no data to compare this patient reported experiences as Chronic UTI itself was not accepted as a condition so my attempt here was to gather enough pilot data to identify recurring symptoms, themes and patient experiences which will in future help us generate a condition specific validated questionnaire. The questionnaires should aid in alerting a clinician about possibility of a CUTI thus giving us prospective data on symptoms and QOL impact on patients.

Chapter 3 A blinded cross-sectional survey of the pathophysiological signals detectable in the urine of patients with chronic recalcitrant bladder pain and recurrent cystitis

3.1 Introduction, hypothesis, objective, study groups and recruitment

3.1.1 Introduction

The aim of this study was to survey patients with chronic recalcitrant LUTS, bladder pain and recurrent cystitis symptoms compared to normal controls with the aim of exploring the differences in the symptoms and a set of urinary pathophysiological markers.

3.1.2 Research question

What is the evidence for bacterial infection in the aetiology of these symptoms in women?

3.1.3 Hypothesis

Patients with chronic recalcitrant LUTS, bladder pain and recurrent cystitis show differences compared to normal controls in symptoms and urinary pathophysiological markers of inflammation that correlate with commensurate differences in the observed urinary microbiome.

3.1.4 Ethics

This study had ethical approval from East London & City REC: Ref 11/LO/0109 (Appendix 3)

3.1.5 Study groups

- Normal adult women with no symptoms; “Asymptomatic controls”
- Women presenting for first time with chronic recalcitrant painful LUTS and symptoms of recurrent cystitis

3.1.5.1 Study Group Characteristics

Normal adult subjects with no symptoms; “Asymptomatic control patients”

- Urinary frequency, voiding <8 in a 24-hour period.
- Nocturia ≤ 1 episode per night,
- No urge or stress incontinence
- No Bladder pain on filling, voiding, or after voiding
- No Pyuria (≥ 10 wbc μL^{-1})
- No current or past treatments for recurrent UTIs

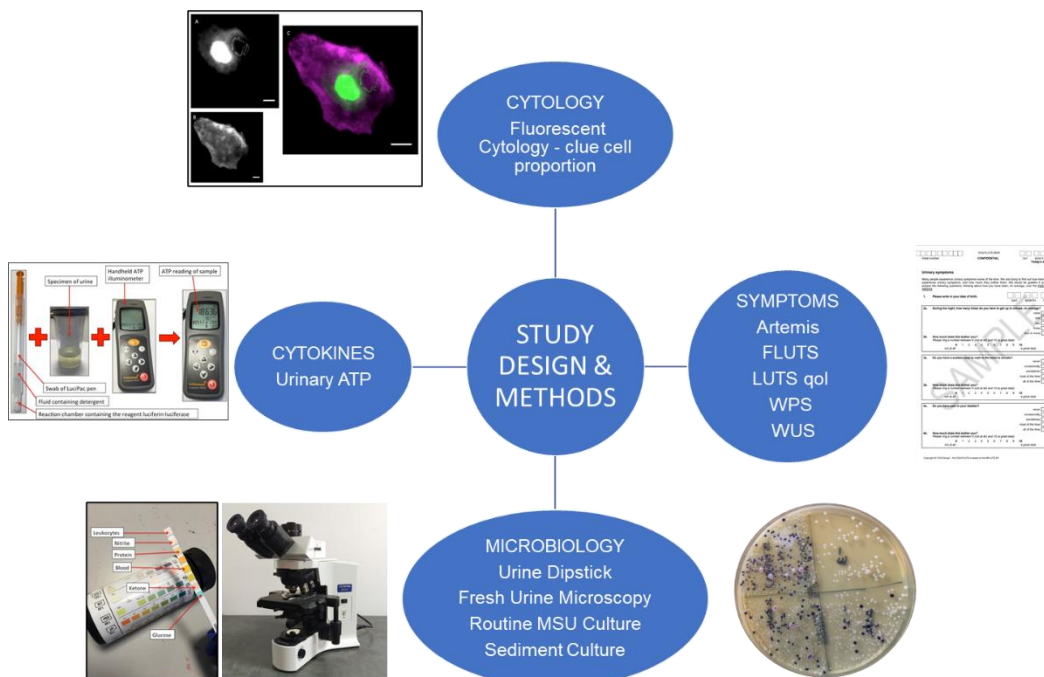
Adult patients with chronic recalcitrant painful LUTS, recurrent cystitis. Patients with any combination of the listed symptoms and not on antibiotic therapy for at least 4 weeks.

- Urinary frequency, voiding >8 in a 24-hour period.
- Nocturia ≥ 1 episode per night,
- Urgency with or without urgency incontinence
- Stress incontinence
- Bladder pain on filling, voiding, or after voiding
- Pyuria (≥ 0 wbc μL^{-1})
- Not had antibiotics for the last 4 weeks for any indication

3.1.6 Study design

A blinded cross-sectional comparative survey of the urinary pathophysiological signals detectable in the urine of patients with chronic recalcitrant painful LUTS (overactive bladder, voiding symptoms, stress incontinence, painful bladder syndrome) and normal controls (Figure 3.1).

Figure 3.1 Study design and methods



The approach uses consilience by which evidence from independent sources is collected in order to ascertain whether they converge in support of a particular hypothesis. The greater the concordance the more probable the accuracy of the hypothesis although causation is not necessarily proven.

3.1.7 Outcome Measures

3.1.7.1 Primary Outcome measure

The primary outcome was the count of colony forming units (\log_e cfu ml⁻¹)

- Pyuria count on an unspun specimen in a haemocytometer (61)
- Dipstick analysis (41)
- Routine hospital MSU culture
- Spun sediment culture obtained from a spun urinary sediment (19)

3.1.7.2 Secondary Outcome measures

a. Symptoms (The questionnaires are to be found in Appendix 9-12)

- Artemis
- International Consultation on Incontinence Questionnaires ICIQ-FLUTS and ICIQ-LUTSqol (146)
- Whittington Urgency Score (78)
- Whittington Bladder Pain Score (79)

b. Evidence of urothelial distress signalling & Urothelial cell innate immune response

- Urinary ATP concentration (147)
- Urothelial cell shedding on fresh urine microscopy (104)
- Proportion shed urothelial cells demonstrating bacteria adhesion on DAPI epifluorescent cytology (104)

3.1.8 Blinding Process

New patients booked to attend clinic were sent the patient information sheet prior to the appointment and were approached by the researcher on the day of their appointment. The information sheet contents were discussed again, and all their questions and doubts answered.

When the participant agreed verbally to provide a fresh midstream urine sample for research, they were given the consent form and LUTS questionnaires to complete. A Case Record Form (CRF) was then issued along with a random study number. The only identifying information on the CRF was the study number. All visit data was recorded in the CRF and on the unit bespoke clinical database Artemis.

The participant samples were labelled and identified only by the study number and no other information that could be used to ascertain whether the specimen was obtained in specific clinical circumstances.

Whilst urine microscopy was performed by a blinded microbiology technician, I rechecked these counts independently and recorded the average results. Our laboratory practice complied with the UCL guidelines. Neither I nor the technician had information on the patient's symptoms or clinical history available at the time of performing urine microscopy.

My supervisor, Professor Malone-Lee was solely responsible for the decision on the clinical management of the study patients. Professor Malone-Lee initiated all treatment regimes and his treatment was based on the clinical history, symptoms signs and fresh urine microscopy (148). These patients were followed up routinely according to the centre's management protocol. I was not informed of the clinical outcome of these patients.

3.1.9 Inclusion and Exclusion criteria

3.1.9.1 Subject inclusion criteria

- Adults aged ≥ 18 years
- Able to complete a symptom questionnaire
- Diagnosed with chronic recalcitrant painful LUTS
- Normal control subjects with no LUTS or UTI symptoms
- Able to provide informed written consent

3.1.9.2 Subject exclusion criteria

- Age < 18 years
- Inability to consent
- Patients with concurrent illnesses on antibiotic treatment in the past 4 weeks that in the opinion of the investigator are likely to compromise the validity of the data
- Pregnant women or women actively trying for a pregnancy
- Patients exposed to antibiotic during the previous four weeks for a UTI

3.1.10 Recruitment of participants

3.1.10.1 Recruitment methods

New patients identified from the community LUTS clinic referral letters were written to, with a brief description of the study prior to their first visit to clinic and sent patient information sheets (Appendix 5). They were then approached informally in the clinic during their routine first clinic appointment. They were provided with a means of notifying their willingness to participate in the study. They were reassured that their regular care and consultation according to the clinic protocol would not be compromised in any way whether they took part in the study or declined to do so.

Eligible participants, who could read or write English, were not recruited as the study included patient information sheets, consent forms and questionnaires, which were approved in English only.

Once a patient expressed an interest in participating in the study, they were provided with study specific consent forms (Appendix 4) and questionnaires. The researcher was present to answer further questions whilst they were reading this information.

Following the formal consent, a urine sample was obtained.

3.1.10.2 Informed consent process

Discussion of risks and possible benefits of this study occurred with the patients and their families. Patient Information Sheets describing the study procedures and risks were provided and written consent was obtained prior to obtaining a sample of urine for the study.

Patient Information Sheets and Consent Forms (Appendix 5, 4) were approved by Research Ethics Committee and the patient were given adequate time to read and review the documents. The researcher had the opportunity to explain the research study to the patient again and answer any questions that arose.

Where appropriate the patients were provided the opportunity to discuss the study with their surrogates or take time to think about it prior to agreeing to participate. The patients were also allowed to withdraw consent at any time throughout the course of the study. A copy of the informed consent document was provided to all participants for their records. The rights and welfare of the patients were protected and it was explained to them that the quality of their medical care would not be adversely affected if they declined to participate in this study.

3.1.10.3 Payment of participants

This study was not funded by external grants. There were no plans or budget allocations to offer payment to the participants. They were approached at their first clinic visit and were told that one further visit might be required in a selected few to obtain convalescence data. We offered reimbursement for reasonable travel costs for any unscheduled clinic visit. The travel costs were born by the unit research fund support.

3.1.10.4 Detail of enrolment procedure

Once a patient agreed to participate, they were issued with a unique study number. A new CRF was opened with a unique study number and the patient's initials. The records of all study visits were maintained in the CRF but source data were simultaneously recorded in the center's clinic database, Artemis.

The study had permission to be conducted at six centers:

1. The Whittington Hospital NHS Trust,
2. Centre for Clinical Science and Technology, Wolfson House, UCL
3. University College London Hospitals
4. Royal Veterinary College, London
5. Pfizer Research Laboratories, Sandwich
6. Immunology Laboratories at UCL

We had permission to recruit patients from Centre No:1 and 3 only. The patients for this study have all been recruited from Centre 1. The other centers were mainly for sample processing and laboratory studies.

3.2 Urine sampling

The urine sample was collected in clinical service centre, initially located on the UCL Archway Campus next to the Whittington Hospital. In 2013 this was relocated to Hornsey Central Health Centre. This midstream (MSU) sample was the only sample sent to Whittington Hospital laboratory, this requiring three identifiers i.e., full name, date of birth and hospital number in line with routine hospital laboratory practice. The remaining samples and culture plates were labelled with the unique study identifier. The Aliquots that were frozen within 2 hours of collection at - 80°C were labelled with Laminated Black and Yellow labels which are specifically manufactured for identification of long-term storage of samples. The agar plates and Gram stain slides were disposed of in accordance with standard laboratory protocol. The materials and methods are elaborated under methods in 3.6 and laboratory protocols are referenced in Appendix 6-8. During laboratory work, I was therefore blind to the identity of the source of the sample.

The diagnosis that has been attributed to the patient was recorded by the consultant who saw the patient and entered into the secure clinical database which was maintained in-line with the Data Protection Act provisions.

3.3 Quality control and quality assurance

Appropriate staff were provided direct access to all source data/documents, and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

We maintained an appropriate site file for this study, in compliance with E6 GCP, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects. Reports were submitted to the R&D Director and the R&D Manager on monitoring activities on completion of the study.

3.4 Statistical Considerations

3.4.1 Statistical methods

On completion of the study, the data for the groups were summarised by means, sd, median and 95% CI around median. For data that were found to be normally distributed, the difference between groups in average log colony count were tested by ANOVA at the 5% level of confidence ($\alpha = .05$), and subgroup analyses were achieved by univariate linear regression (GLM). If the data were not normally distributed, the non-parametric, Kruskal Wallis test for the difference between group medians was used.

3.4.2 Sample size calculation

We used a 2-tailed nonparametric test with an effect size (d) = 0.5, which means that an effect in either direction would be interpreted. The criterion for significance (probability of alpha error) was set at 0.05 and power of 80% (1-beta error of probability = 0.8). With the proposed sample size of 170 for the two groups (43 controls and 127 patients), the study had a power of 80% to yield a statistically significant result with an effect size $d = 0.5$.

Cohen's d effect size = 0.5 (Figure 3.2) was selected as this is the smallest effect that would be important to detect, in the sense that any smaller effect would not be of clinical or substantive significance. It was also assumed that this effect size is reasonable, in the sense that an effect of this magnitude could be anticipated in this field of research. Cohen's d is defined as the mean difference expressed in standard deviations. $d = \text{difference between means} \div \text{pooled standard deviations of the two groups}$.

Figure 3.2 Cohen's d as effect size

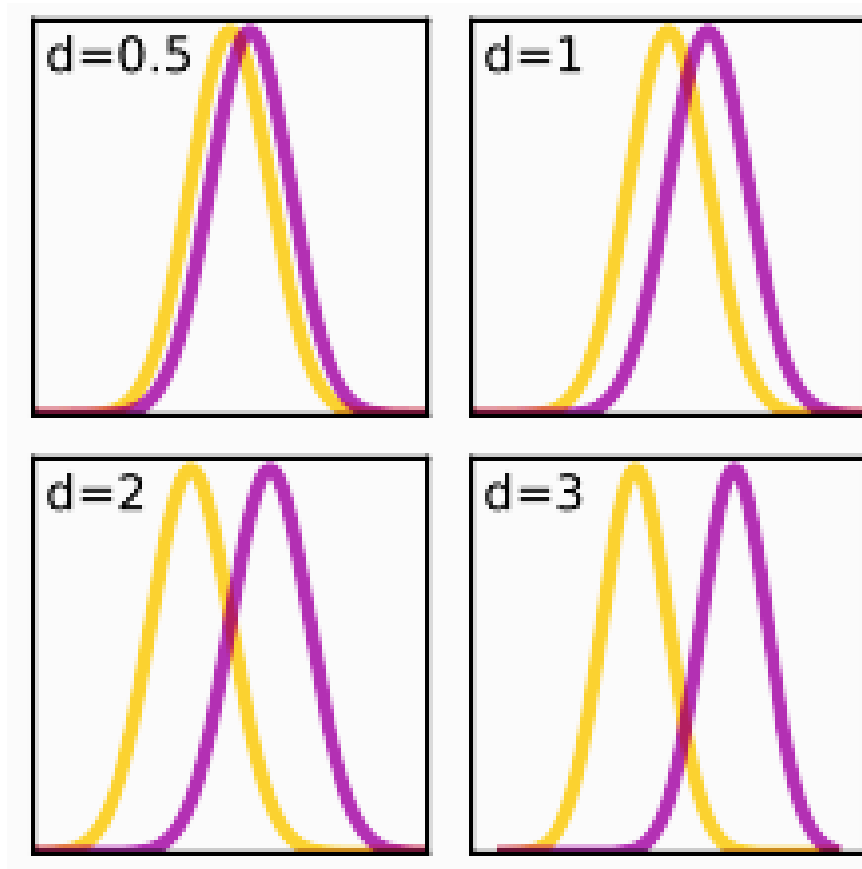
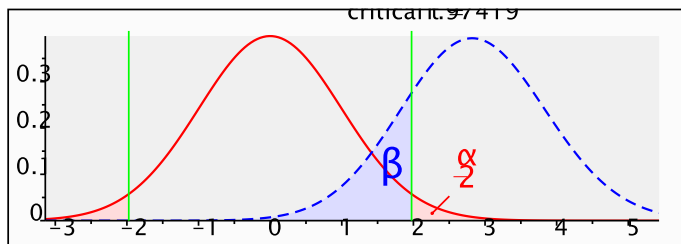


Figure 3.3 Central and non-central distributions



The blue dotted line indicates patient group, and the red solid line indicates the control group. α error or type 1 error is finding of a difference when it doesn't exist, while β error is not finding an error when it actually exists, a classic example is use of dipsticks to diagnose UTI (Figure 3.3).

3.4.3 Grouping variables

Further regression analysis of the subgroups was planned, and the subgroups included:

- Adult normal volunteer controls
- Adult patients with OAB
- Adult patients with PBS
- Adult patients with Stress Urinary incontinence
- Adult patients with Voiding symptoms

For every independent variable included in a regression model, you require 10 subjects or patients. Current clinical presentation does not allow the clear categorization of patients into just one of the groups as the patients present with mixed symptoms of LUTS.

3.5 Methods

3.5.1 Introduction to methods

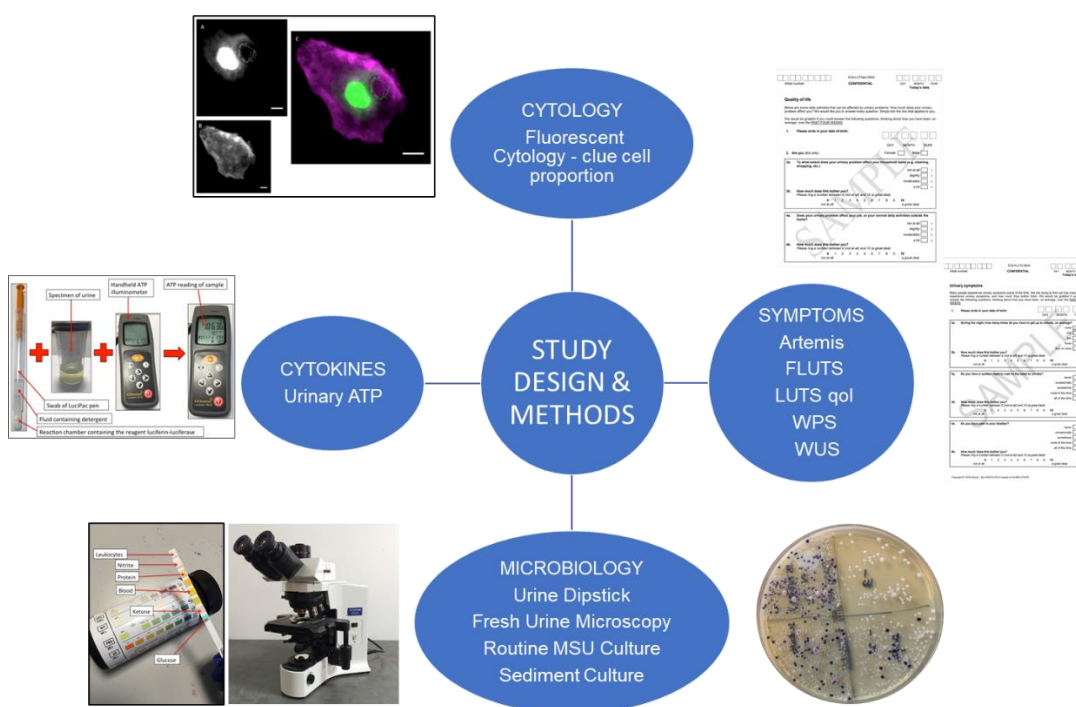
The process of sample collection and methods used in my work are described in the sequence in which they were undertaken during the study process. Once a formal consent was obtained from the patient, she provided a clean catch mid-stream sample of urine and completed a set of validated questionnaires, and the researcher obtained their demography data. They were then seen independently by a consultant for diagnosis and treatment of their symptoms. I was not involved in their clinical diagnosis or treatment in any way. The sample obtained was tested by me using standard dipstick testing and fresh urine microscopy was performed to determine pyuria, epithelial cell count and haematuria. This was also checked by the clinic technician and recorded on the Unit's database. My counts were similar to that recorded by the technician (In the unit we tested the

inter observer variability at 3 monthly intervals for standardisation and Governance purposes). An aliquot of urine was sent to Whittington Hospital laboratory for routine MSU culture and a further aliquot for serum creatinine estimation.

I tested the ATP signal immediately using the automated bioluminescence assay and the reading was recorded.

I transported the samples to the laboratory within 2 hours and processed the remaining laboratory work within 4 hours of the obtaining the sample as per our protocol. Five millilitres of urine was spun to obtain urine sediment for enhanced sediment culture. 80µl of urine was spun down and stained using the DAPI epifluorescence method to obtain clue cell counts. Pathophysiological signals in the urine in patients with chronic recalcitrant LUTS patients compared to controls was studied under these four broad topics namely, cytology, symptoms, cytokines and microbiology (Figure 3.4).

Figure 3.4 Study Design and Methods



3.5.2 Mid-stream urine collection

A meticulous clean catch MSU specimen was obtained using the standard unit guidelines (Appendix 6). Every new patient attending clinic was given written and verbal instructions on how to collect a midstream specimen. They were provided with a foil bowl (labelled with the patient identifiers) rather than a 30 ml boric acid bottle to ensure that the sample collection was easier. A hypoallergenic baby wipe was provided to the patient and they were instructed to part their labia minora and wipe just once to clean the urethra moving the wipe from front to back. Diagrams showing the female genitalia were used when patients were unable to follow verbal instructions. The patients were then asked to begin urinating into

the toilet or urinal. After the urine had flowed for a few seconds, a container (foil bowl) was placed into the stream and a specimen was collected without interruption of flow. They were asked to withdraw the bowl before the end of their void. The sample was handed to the researcher or technician immediately for microscopy, dipstick analysis and samples were divided into aliquots for the various processes required for the study.

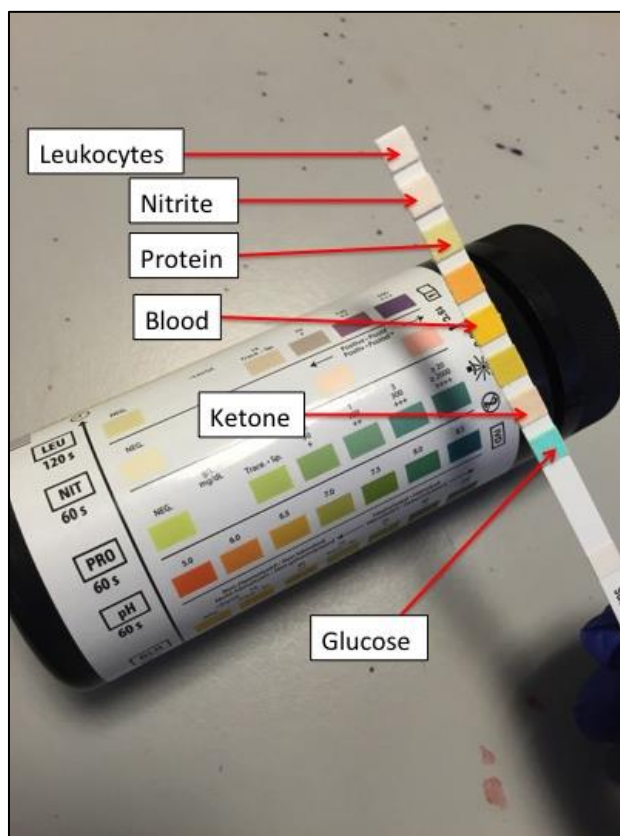
3.5.3 The Urinary Dipstick

A Multistix® 8 SG reagent strip was dipped into each MSU/CSU sample as soon as it was possible after the sample was given, usually within the hour. Method used is as per the manufacturer's instruction:

- Dip the test strip in the urine for no longer than 1 second ensuring that all pads are covered.
- Remove strip from sample running strip edge over the rim of the container.
- Briefly blot the long edge and back of the urine test strip into absorbent paper to remove excess urine.
- Start timer for 120 seconds.
- At 30 – 120 seconds compare results to the colour chart on the side of the strip pot.

The dipstick was then analysed by eye using the or via a Clinitek® Status analyser. Six test results were recorded and recorded at the suggested times: the leukocyte esterase test (2 minutes), the nitrite test (60 seconds), the protein test (60 seconds), the haemoglobin test (60 seconds), the glucose test (30 seconds) and the ketones test (40 seconds) (Figure 3.5).

Figure 3.5 Example of the urinary dipstick bottle and a reagent strip used in this study



The red arrows mark the tests that were recorded for each sample in this study. Leukocyte esterase is an enzyme synthesized by neutrophils (149). Consequently, the test is a surrogate for detecting WBCs in the urine. A positive result suggests pyuria and is an indirect marker of infection, since the most probable explanation for pyuria is infection.

The nitrite test detects the presence of nitrites in the urine. As nitrites are not usually found present in urine, a positive (pink) result suggests the presence of nitrate to nitrite converting bacteria in the urine (149). Known as the Griess Reaction, nitrite reacts with an aromatic amine such as sulphanilamide under acidic conditions to produce a coloured diazonium salt. If urea-splitting bacteria is present, this salt reacts with hydroxybenzoquinone to produce the pink colour (150).

The protein test detects the presence of albumin in the urine (151). As albumin is not usually found in the urine, a positive result suggests proteinuria, which is not associated with UTIs but is a marker of glomerular disease (151). If haematuria were detected the co-existence of proteinuria, is an important red flag.

The haemoglobin test detects the presence of RBCs via haemoglobin (151). A positive test result suggests haematuria. The reasons for a positive test result here are extensive and cannot determine the location of the bleed itself (151). To reduce this limitation, patients were asked if they were menstruating at the time of giving the sample so it could be taken into consideration. Unexplained haematuria would require investigation.

The glucose test and ketone tests detect the presence of glucose and ketones (intermediate products in fat metabolism) in the urine respectively (151). Neither ketones nor glucose are found normally in the urine. Their presence could result from a number of reasons, such as ineffectively managed diabetes (151).

3.5.4 Fresh urine microscopy

Urine microscopy was performed on the fresh specimen first by the technicians and then by the researcher and an average of the two counts was recorded. To prepare the counting chamber the mirror-like polished surface was carefully cleaned with lens paper. The coverslip was also cleaned. Coverslips for counting chambers are specially made and are thicker than those for conventional microscopy, since they must be heavy enough to overcome the surface tension of a drop of liquid. The coverslip was placed over the counting surface prior to putting on the urine sample. A disposable pipette was used to load a 1 μl sample onto one of the V-shaped wells of a clean Neubauer haemocytometer counting chamber (152). The area under the coverslip fills by capillary action. Enough liquid was introduced so that the mirrored surface is just covered. The charged counting chamber was then placed on the microscope stage and the counting grid is brought into focus at low power. The preparation was examined using a x20 objective with a x10 optical (magnification x200). The leukocyte count (WBC μ^{-1}) was enumerated by counting cells in five large squares out of nine and multiplying the result by two because the volume of the whole chamber was 0.9 μl . If a cell overlaps a dividing line, it is counted "in" if the line runs along the top

or right ruling; ignored as "out", if it overlapped the bottom or left ruling (Figure 3.6 and Figure 3.7).

Figure 3.6 A haemocytometer and cover slip
(Copyright of Caprette 2000)

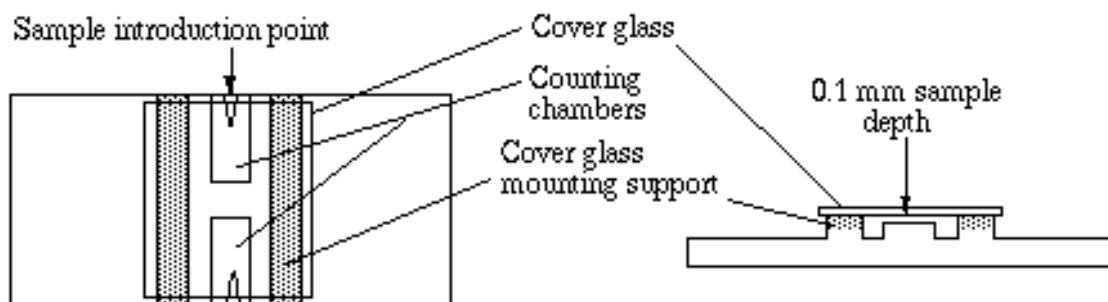
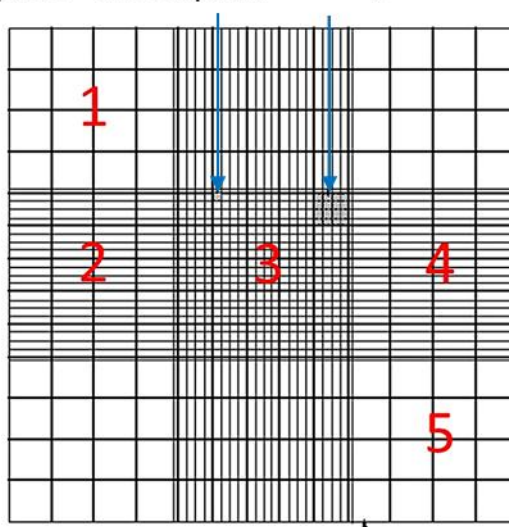


Figure 3.7 A haemocytometer grid

The numbers indicate the sequential order in which the large squares were viewed under the microscope. The arrows indicating the area

Small Square = $1/400 \text{ sq. mm}$ $1/25 \text{ sq. mm}$



Counting Grid (central area)

3.5.5 Routine MSU Culture

For routine hospital laboratory urine culture, 5 to 30 ml of urine in a sterile universal specimen tube were submitted to the Whittington Hospital Microbiology Laboratory, London, UK for routine culture. Once they arrived at the laboratory, the samples were cultured either on the same day, or on the next day following overnight storage at 4°C. The protocol involved inoculating ChromID CPS (now ChromID CPS Elite) chromogenic culture medium (bioMérieux, France) with 1µl of uncentrifuged urine, which was then placed in an ordinary incubator at 37°C. Microbial colonies were identified using the manufacturer's colour criteria. A count of >100 colonies of a single organism was reported as $\geq 10^5$ cfu/ml and was

interpreted as a “significant growth* and subjected to Culture and sensitivity testing. Cultures with a colony count below this threshold were reported as “no significant growth”. MSU cultures with more than one organism were reported as “mixed growth of n types of organisms”.

A significant culture involved the isolation of a pure growth of $\geq 10^5$ cfu/ml of the following urinary pathogens: *Acinetobacter*, *Citrobacter*, *Klebsiella/Enterobacter/Serratia*, *Corynebacterium*, *Diphtheroid*, *Enterobacteriaceae*, *Enterococcus*, *Escherichia coli*, *Morganella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, or *Streptococcus*.

The antibiotics that were tested were Amoxicillin, Co-amoxiclav, Ciprofloxacin, Nitrofurantoin and Trimethoprim.

3.5.5.1 Preparation, inoculation isolation and identification

1 μ l of unspun urine was spread onto a chromogenic agar plate before incubation at $35 \pm 2^\circ\text{C}$ for 24 hours (153). Routine hospital protocols were used to identify the microorganisms (153); inoculation was achieved by streaking the loop across the plate. The culture plate was then incubated aerobically for 24 hrs at 37°C .

Bacterial colonies were identified by colour change and size as below. Rapid reagent testing (‘spot testing’) was employed to supplement colour-based bacterial identification as described previously.

3.5.5.2 Bacterial quantification

Routine culture techniques were semi-quantitative, and bacterial growth was estimated by visual assessment of colony density.

The growth of > 100 colonies was reported as $\geq 10^5$ cfu ml⁻¹ of one single urinary pathogen following 24-hour incubation was interpreted as a positive test result (41). The growth of more than one type of organisms was classified as a mixed growth and the sample was interpreted as contaminated. Any growth that did not exceed $\geq 10^5$ cfu ml⁻¹ was classified as no significant growth and interpreted as a negative test result. The measure of growth was conducted by human eye. The results were then uploaded onto the secure electronic Whittington Anglia ICE database, accessible across all sites that belong to Whittington Hospital.

3.5.6 Spun urinary sediment culture in research laboratory

All spun urinary sediment cultures were undertaken the same day at the laboratories of the Department of Medicine, UCL, initially located at Archway Campus and subsequently relocated to Wolfson House, Stephenson Way. The time taken from the patient producing the sample to processing was a maximum of 4 hours (19) (Appendix 7).

3.5.6.1 Preparation, inoculation isolation and identification

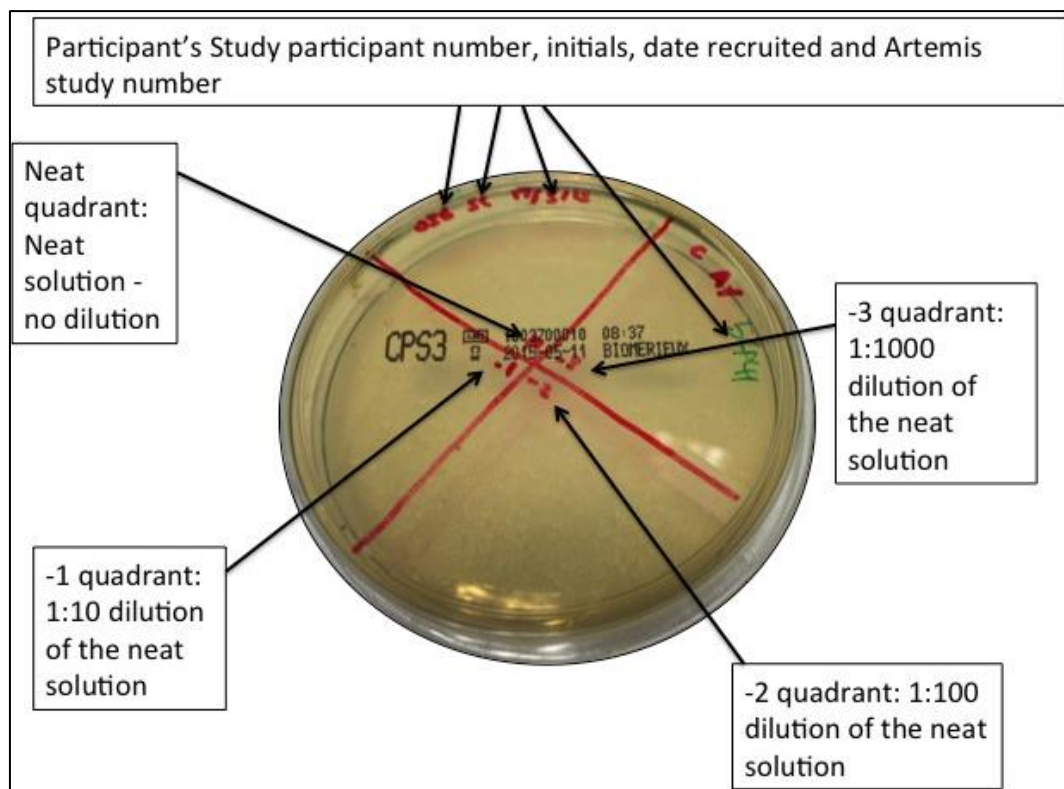
A 5ml sample was centrifuged at 627g (speed of 2000 revolutions per minute) for 5 minutes in a Denley BR401 centrifuge (RMAX=140mm) (Denley, Heckmondwike, UK). at room temperature, resulting in the cell sediment

forming at the base of the container and the supernatant above, which was then removed (Appendix 7). 400µl of sterile Phosphate Buffered Solution (PBS) was mixed with the sediment to form the neat solution (19).

Three serial dilutions of the neat suspension (1:10, 1:100 and 1:1000) were prepared with PBS (104). 50µl of each dilution and 50µl of the neat suspension was then spread onto a chromogenic agar plate (CPS3; bioMérieux, France) in quadrants to enable easy identification and enumeration of any growth obtained (Figure 3.8), The plate was then incubated under atmospheric conditions at 37°C for 24 hours (19).

Following 24-hour incubation, the colour, size and counts of each colony were recorded. Pure growth colonies were sub-cultured onto Columbia Blood Agar (CBA) (Figure 3.9) plates (E&O Laboratories) (19) before incubation under atmospheric conditions at 37°C for a further 24 hours.

Figure 3.8 Example of a sample prepared for cell sediment culture



3.5.6.2 Bacterial sub-culture

For mixed growth cultures, each bacterial isolate identified on chromogenic agar was sub-cultured on a CBA plate (Figure 3.9) prior to supplementary testing. A single colony of the chosen bacteria was streaked on a CBA plate using a sterile 1µl inoculation loop, with care taken to avoid contamination. The inoculated CBA plate was incubated for 24 hours at 37°C and a pure growth of the cultured organism verified before isolate storage. This process was repeated using CPS3 plates until a pure growth was obtained (Figure 3.8).

Figure 3.9 Example of a pure growth obtained (Staphylococcus) on a CBA plate



3.5.6.3 Benchtop tests

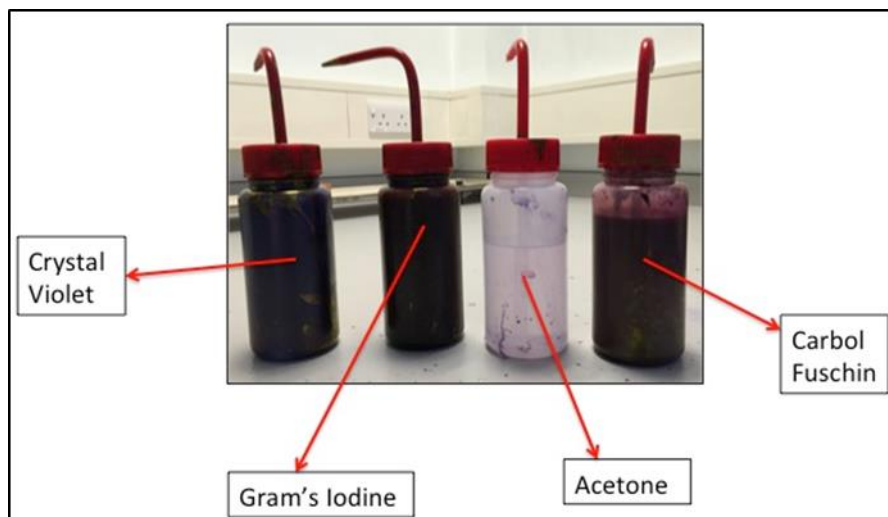
A variety of measures were undertaken to determine the genus of the pure colony growth, including the colony's appearance along with a number of bench top tests (Appendix 7).

3.5.6.3.1 The Gram Stain

All samples underwent Gram staining to determine whether the bacteria was Gram-positive or Gram-negative (Appendix 7) (154). 1µl of the pure colony was suspended in 1µl of PBS on a clean glass slide (154) and heat-fixed. Four staining reagents were used in this procedure (Figure 3.10) Crystal violet was first used to stain the microbes purple by interacting with the intracellular bacterial components after it was taken up by the bacteria's cell wall and cell membrane (154). The slide was subsequently flooded with a mordant; thus Gram's iodine which bound to the crystal violet, trapping it within the cell wall so its staining effect was retained (154).

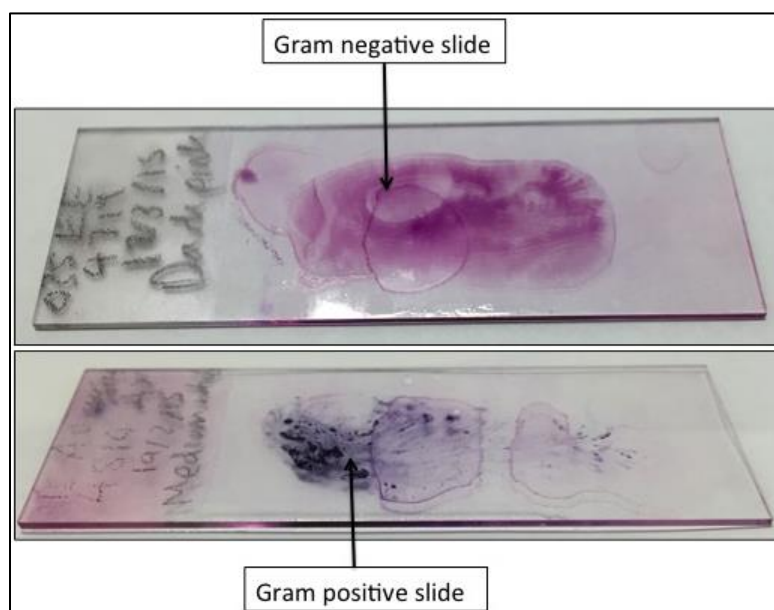
The slide was then washed with acetone to break down the lipid outer membrane of Gram-negative bacteria, washing away the crystal violet and iodine, leaving the Gram-negative cells colourless(154). In Gram-positive bacteria, acetone dehydrates the cell wall, increasing its affinity to the crystal violet and iodine complex. As crystal violet and iodine remain trapped in the cell wall, cells remain purple (154). Finally, the counterstain carbol fuchsin was used. It was taken up by the cell wall in Gram-negative bacteria, staining the cells pink (154). This effect is disguised by crystal violet in Gram-positive bacteria (Figure 3.10).

Figure 3.10 The four reagents used to prepare the gram stain slide



Once dry, the slide (Figure3.11) was then analysed under microscopy to determine the morphology of the bacteria.

Figure 3.11 Comparison of a Gram-negative slide and a Gram-positive slide



The Gram-positive slide has retained crystal violet, so it appears deep blue/purple. The Gram-negative slide has not retained the crystal violet but taken up carbol fuchsin, so it appears pink.

3.5.6.4 Bacterial identification: 'Spot' biochemical testing

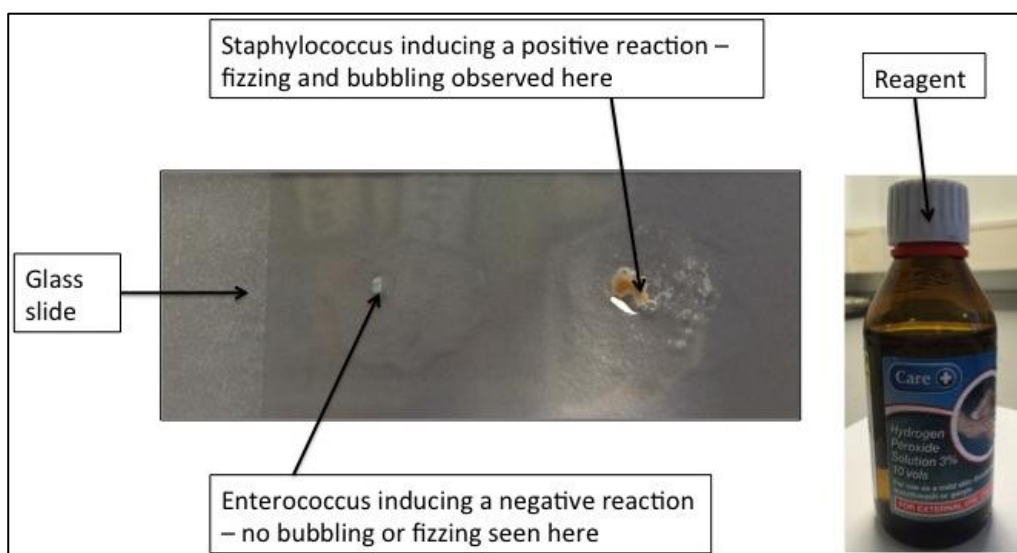
Rapid reagent testing ('spot testing') using indole, oxidase, catalase, and coagulase tests (Remel, Basingstoke, UK) was employed to supplement colour-based bacterial identification. A drop of reagent, selected on the basis of the

suspected bacterial genus, was added to a smear of the isolate. Standard colour changes indicated a positive result.

3.5.6.4.1 The Spot Catalase test

The spot catalase test (Figure 3.12) was used to detect the presence of catalase in Gram-positive bacterial cocci as this could be used to differentiate between the different genera (155). A sample of the colony was dipped into hydrogen peroxide on a glass slide (Appendix 7). A positive test result was recorded if bubbling or fizzing was observed (Figure 3.12). This was due to the catalase breaking down hydrogen peroxide into water and oxygen. The bubbling was produced by the production of oxygen. If catalase was not present, no bubbling or fizzing was produced, resulting in a negative test result.

Figure 3.12 Examples of a positive and a negative Catalase reaction



3.5.6.4.2 The Spot Indole test

The spot indole test was used to confirm the genus of the colony as *E. coli*. A drop of the reagent was placed onto clean blotting paper. A sample of the colony was smeared into the reagent using a 1µl loop (155). CBA plates and CPS3 plates contain the amino acid tryptophan, which is broken down into three end products by tryptophanase (the reagent). The three end products are Indole, Pyruvate and Ammonium (Figure 3.13) (155). Indole reacts with certain aldehydes present in the bacteria thereby colouring (Figure 3.14). The colour formation of pink was interpreted as a negative result whilst blue interpreted as positive, indicating the colony was *E. coli* (155).

Figure 3.13 The chemical reaction explaining how Indole is produced in the test (155)

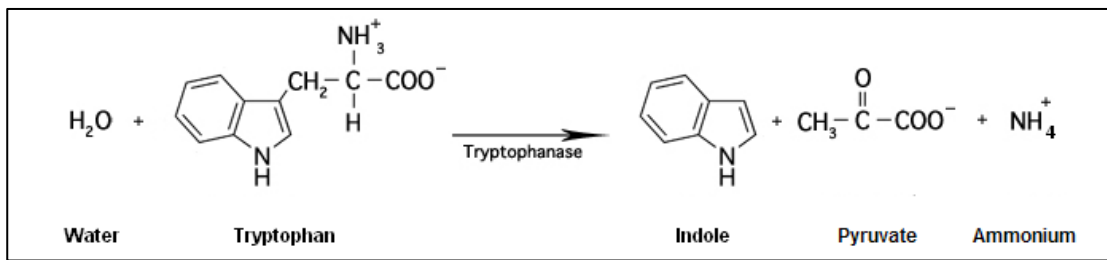
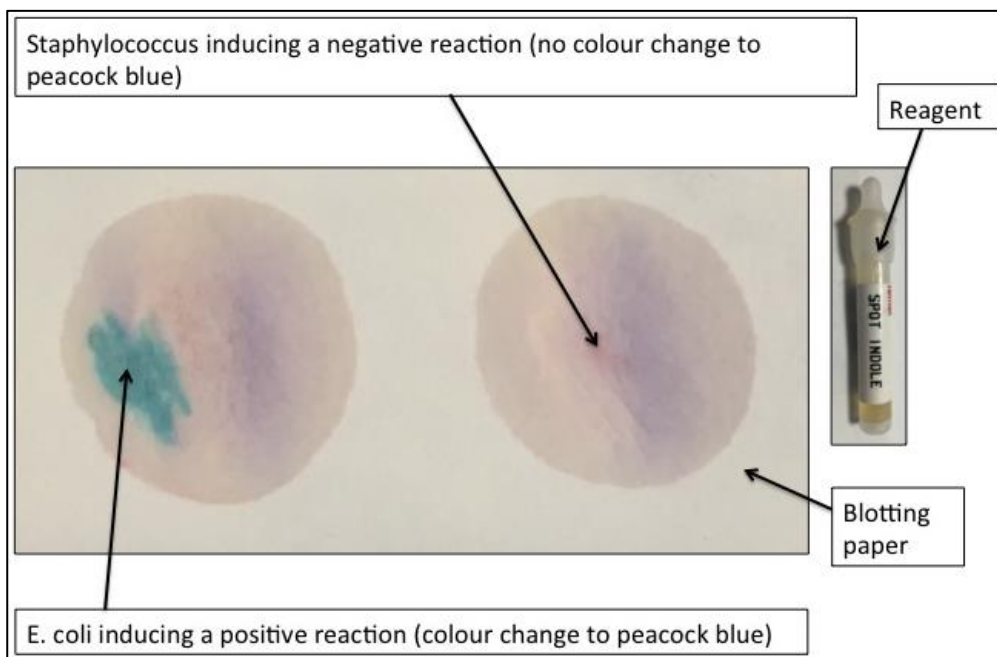


Figure 3.14 A comparison of the positive and negative reactions of the Spot Indole test

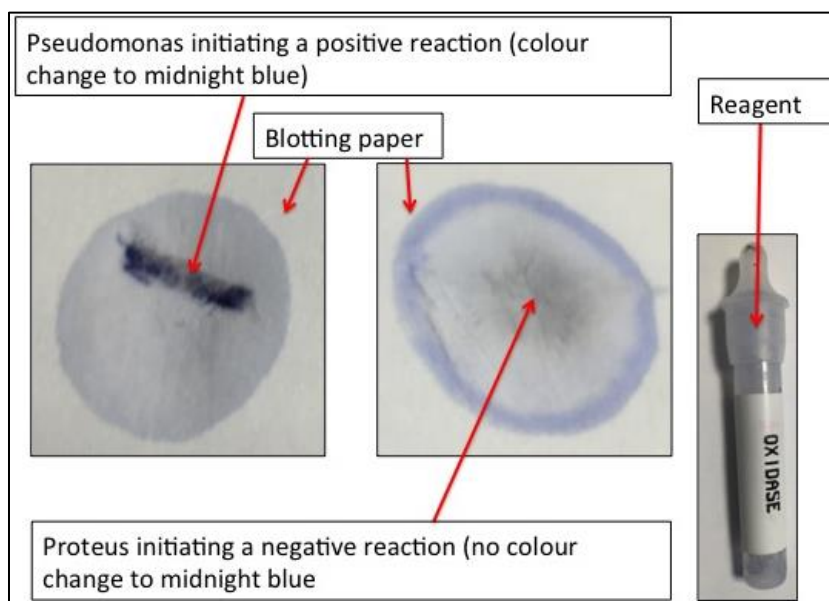


3.5.6.4.3 The Spot Oxidase Test

The Spot Oxidase Test is used to differentiate *Pseudomonas* from other genii such as *E. coli* (155). Oxidase positive bacteria such as *Pseudomonas* contain either cytochrome oxidase or indophenol oxidase. Both enzymes oxidise the reagent to form indophenol blue, a deep purple-blue coloured compound. The reagent here accepts the electrons that are transported from donor compounds (Nicotinamide adenine dinucleotide), a reaction catalysed by oxidase (155) (PHE, 2014. [#134](https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories)).

A drop of the reagent was placed onto clean blotting paper. A sample of the colony was smeared into the reagent using a 1µl hoop (155). The appearance of a deep purple-blue colour was interpreted as a positive test result (Figure 3.15). If no colour change was observed within ten seconds it was interpreted as a negative test result (155).

Figure 3.15 A comparison of the positive and negative reactions of the Spot Oxidase test



3.5.6.4.4 Bacterial quantification on spun sediment culture

Each bacterial isolate was subject to a quantitative count. The number of colonies of each isolate was determined in each sector of the CPS3 plate, corresponding to one of five serial bacterial dilutions.

The mean colony count from all sectors was calculated using the formula:

$$\text{Neat} = (\text{colony count} \times 20 \times 0.4) / 5 = a$$

$$\text{-1 dilution} = (\text{colony count} \times 20 \times 0.4 \times 10) / 5 = b$$

$$\text{-2 dilution} = (\text{colony count} \times 20 \times 0.4 \times 100) / 5 = c$$

$$\text{-3 dilution} = (\text{colony count} \times 20 \times 0.4 \times 1000) / 5 = d$$

$$\text{Total cfu/ml} = (a+b+c+d) / 4$$

This was performed for each isolate and total cfu/ml was then ascertained for all the organisms in each plate

3.5.6.5 Storage of bacterial isolates

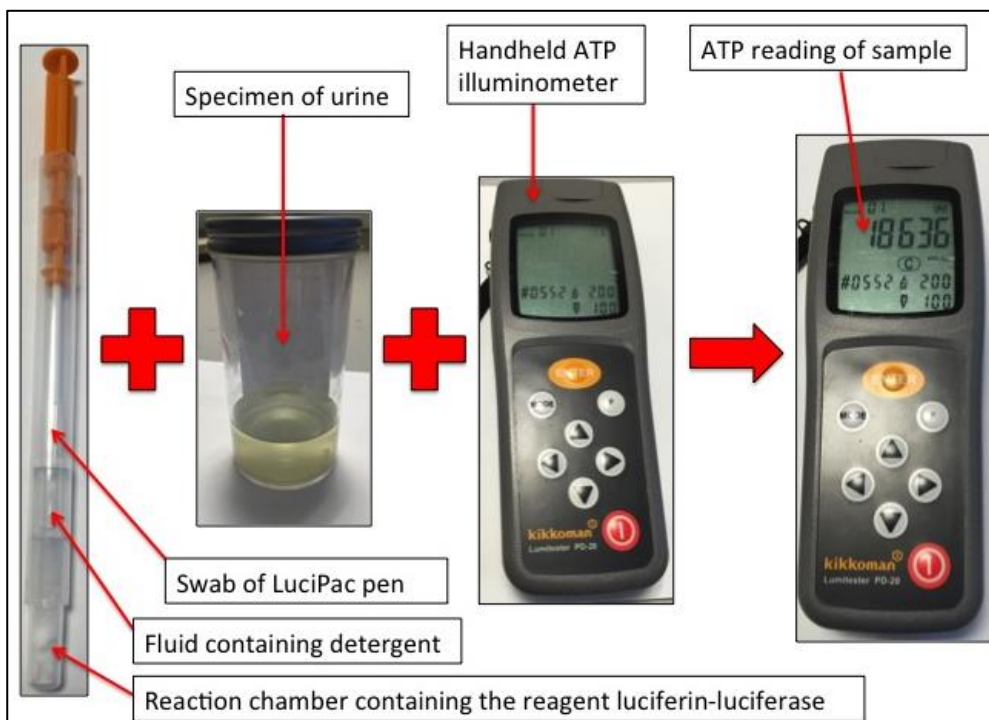
All bacterial isolates were indexed and stored in 2 ml cryopreservation vials (Thermo Scientific) at -80°C in the Department of Medicine, UCL Archway Campus, later transferred to Wolfson Laboratories by qualified laboratory and specimen handling specialist services

3.5.7 Cytokine: Benchtop ATP

The amount of extracellular ATP present in the urine sample was measured using a handheld ATP illuminometer (PD20 Lumitester) (Figure 3.16). The swab of a sterile LuciPac Pen was immersed in the urine, returned to its sheath and pushed into the fluid. The pen was then shaken repeatedly using vertical downwards movements to aid the complete flow of the fluid into the reaction capsule. Once the fluid was inside the reaction capsule, it reacted with the reagent there. The LuciPac Pen was then inserted into the PD20 Lumitester.

The fluid was a detergent solution that releases the ATP and adenosine monophosphate (AMP) in the urine sample when it comes into contact with the luciferin-luciferase present in the reaction chamber (156). The PD20 Lumitester measures the luminescence produced by this enzymatic circulation reaction and expresses it in Relative Light Units (RLU). As the degree of luminescence is dependent on the amount of ATP-AMP present, the more bioluminescence measured indicates there is a higher concentration of extracellular ATP in the urine (156).

Figure 3.16 Diagram outlining how the benchtop ATP test was carried out and what was needed



3.5.8 Cytology: DAPI Immunofluorescence staining

Processing was undertaken within one hour of sample collection and samples were refrigerated at 4°C until assessment. Following mixing of the sample by inversion, 80µl of the specimen sample was pipetted into a Shandon single funnel cuvette assembly for centrifugation (Figure 3.17) (104). The assembly comprised a single channel cuvette and retainer, a Shandon filter card (Fisher Scientific, Loughborough, UK), and a Super frost Ultra Plus glass microscope slide.

The cuvette assembly containing the sample was spun at 75 g for five minutes in a Shandon Cytospin 2 cytocentrifuge (Thermo Scientific, Basingstoke, UK) at a speed of 800 rpm (Appendix 8) (104).

The cellular and particulate components of the sample formed a visible deposit on the slide approximately 5 mm in diameter; excess liquid was absorbed by the filter card. The deposit was circumscribed with an ImmEdge hydrophobic barrier pen (Vector Laboratories, Peterborough, UK) and 100 µl of 4% formaldehyde (Fisher Scientific) was added for 15 minutes at room temperature (≈20°C) as a fixative. The formaldehyde was then aspirated, and the preparation washed three times with 1% PBS (Sigma-Aldrich, Gillingham, UK) at 5-minute intervals.

100 µl of 0.5% Wheat Germ Agglutinin (WGA) bound to Alexa Fluor-488 (Intirogen, Paisley, UK) to label the cell membrane (104) was then added to the deposit. After a 15-minute incubation period at room temperature, the solution was aspirated, and the deposit washed twice at 5-minute intervals with HBSS.

DNA in the deposit was counterstained with 1% DAPI (4',6-diamidino-2-phenylindole) (Sigma-Aldrich). Staining was achieved by the addition of 100 µl of DAPI (1 µg ml⁻¹) to the deposit which was incubated at room temperature for 15 minutes. After incubation, the DAPI solution was aspirated and the deposit washed twice in 1% PBS. After staining, the deposit was immediately mounted with FluorSave reagent (Calbiochem, Darmstadt, Germany). A coverslip was fixed in place with clear nail varnish, and the slide allowed to cure for at least one hour before examination (Figure 3.18). All slides were stored at 4°C in a light-protected environment.

DAPI was used to label the nuclear material of the cells and the bacteria whilst WGA was able to bind to the cell membrane. This was useful in cellular identification and demarcation when counting the cells (104). FluorSave reagent (Calbiochem) was used to directly mount the cells with a coverslip fixed on top (Figure 3.17) (104). Alexa Fluor 488 excites at a wavelength of 495 nm and emits at 519 nm, and cell membrane appears green under fluorescence. DAPI excites at a wavelength of 360 nm and emits at 460 nm giving mammalian nuclei and bacteria a blue appearance under fluorescence. DAPI is capable of penetrating cellular membranes, and intracellular/extracellular pathogens can be labelled without the need for cell permeabilisation.

Microscopic examination of the slide (Figure 3.18) was undertaken using an Olympus CX41 upright epi-fluorescence microscope (Olympus), at the Department of Medicine, UCL Archway Campus. Samples were examined to identify urothelial cell expression, and to determine the proportion of urothelial cells exhibiting associated bacteria. Only whole cells (non lysed) were counted,

and proportion of clue cells vs non-clue cells logged, and proportion was calculated and recorded. Further images using Image J 1.44P and Axiovision Rel. 4.8 software (Carl Zeiss, Cambridge, UK) will be undertaken to capture some images for the thesis (Appendix 2).

Figure 3.17 The Shandon single funnel cuvette assembly before centrifugation

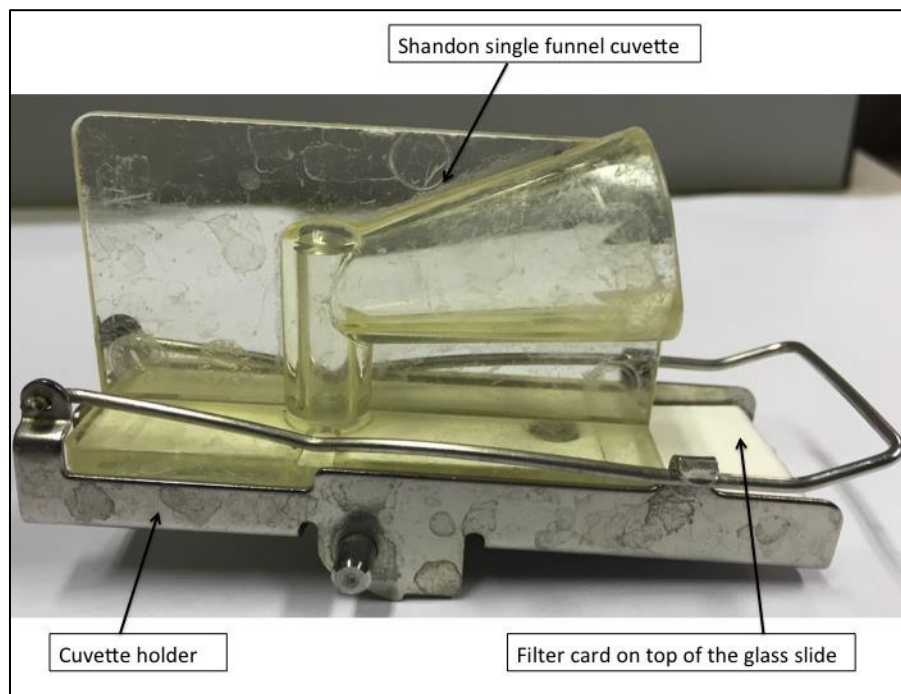
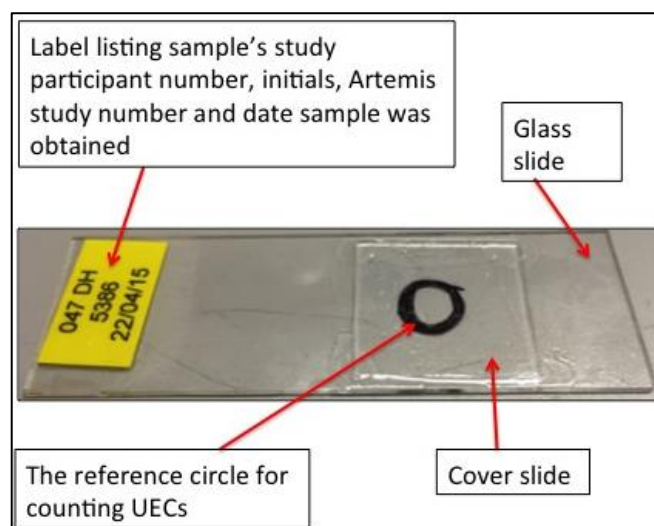


Figure 3.18 An example of a fully prepared slide ready for clue cell analysis



An Olympus CX41 Light Microscope was used to count the number of cells. All samples were blinded beforehand to reduce the risk of bias. All UECs within the reference marker were counted up to a maximum of 100. Clue cells were defined as UECs clearly associated with bacteria. Proportion of clue cells was calculated by the equation $\frac{\text{Number of clue cells counted}}{\text{Total Number of UECs counted}}$.

3.5.9 Urinary creatinine

The measurement of urinary cytokines may be influenced by urinary concentration. Urinary creatinine, a measure of glomerular filtration rate, was used as a correction factor and concentration of cytokines expressed as a ratio of the creatinine concentration. Thirty millilitres of urine in a sterile universal specimen tube was sent to the Whittington Hospital biochemistry laboratory for analysis. Samples were processed immediately upon receipt, or after overnight refrigeration at 4°C. Urinary creatinine was measured using an automated Jaffe technique, in which creatinine and alkaline picric acid produce a red/orange complex measured by spectrophotometry. All analyses were undertaken by trained biomedical scientists.

3.5.10 Symptom Assessment

3.5.10.1 Artemis history database

A complete LUTS history was taken from each participant and their responses uploaded onto the secure onsite database, Artemis. The Artemis symptoms dataset covers a comprehensive range of LUTS detailed in Table 3.1. Additionally, their daytime frequency, nocturia, daytime incontinence and nocturnal incontinence were also recorded. An Artemis score was generated for each of the four LUTS categories: stress urinary incontinence, OAB symptoms (urgency), voiding and pain.

Participants also answered the following validated questionnaires (Appendix 9-12):

- International Consultation on Incontinence Questionnaire – Female Lower Urinary Tract Symptoms (ICIQ-FLUTS)
- International Consultation on Incontinence Questionnaire – Lower Urinary Tract Symptoms Quality of Life (ICIQ-LUTSqol)
- Whittington Urgency Score (WUS)
- Whittington Bladder Pain Score (WPS)

Table 3.1 The range of LUTS that the Artemis history form captures on database

≥1 Stress Urinary Incontinence	≥1 Pain Symptoms	≥1 OAB Symptoms (Urgency)	≥1 Voiding Symptoms
Cough Sneeze incontinence	Suprapubic Pain	Urgency	Hesitancy
Exercise incontinence	Filling Bladder pain	Urgency incontinence	Reduced stream
Laughing incontinence	Voiding bladder pain	Latchkey urgency	Intermittent stream
Passive incontinence	Post void bladder pain	Latchkey urgency incontinence	Straining to void
Bending incontinence	Pain fully relieved by voiding	Waking urgency	Terminal dribbling
Standing incontinence	Pain partially relieved by voiding	Waking incontinence	Post void dribbling
Lifting incontinence	Pain unrelieved by voiding	Running water urgency	Double voiding
Pre cough preparation	Loin pain	Running water incontinence	
	Iliac Fossa pain	Cold urgency	
	Pain radiating to genitals	Anxiety urgency	
	Pain radiating to legs	Premenstrual aggravation	
	Dysuria		

3.5.10.2 The ICIQ-FLUTS Long Form questionnaire

The ICIQ-FLUTS Long Form questionnaire (Appendix 11) measures an expansive spectrum of female LUTS in 18 questions. Developed from the ICS male questionnaire that quantified LUTS symptoms in adult males, it focuses on quantifying the frequency and incontinence symptoms experienced by adult women suffering with LUTS (157). Additionally, for each question, patients rank on a rising scale of 1-10 how much the symptom in question bothers them, which are collectively added to give a Bother Score. The meticulously investigated questionnaire has been demonstrated to have strong levels of psychometric validity and reliability (157).

3.5.10.3 The ICIQ-LUTSqol questionnaire

The ICIQ-LUTSqol questionnaire evaluates the quality of life experienced by patients with urinary problems, with an emphasis on incontinence (75). Consisting of 20 items, the impact on quality of life is measured by how high the score is with a maximum of 76 (154). Patients are also asked to rank each item from 1 to 10 on how much it bothered them, giving a measure of the impact of individual symptoms on the patient's quality of life. It has been thoroughly validated and is regarded as a reliable tool in evaluating different treatments of urinary incontinence in follow-up (75, 135, 158) (Appendix 12).

3.5.10.4 The Whittington Urgency Score

The Whittington Urgency Score consists of ten questions about urgency and incontinence symptoms. Patients are given the choice of "none", "some" or "much" to answer for each question with a total score out of 20. It is based on

the patient's experiences rather than the clinician's judgement on the predicted response of the patient. Al-Buheissi et al. validated this questionnaire in 2008 using a single group repeated measure design for 475 patients suffering from OAB (77). It passed all the validation tests (Appendix 10).

3.5.10.5 The Whittington Bladder Pain Score (WPS)

From a survey of 776 people in the UK conducted in 1997 by the Interstitial Cystitis Association, eight frequency symptoms arose (79). These symptoms formed the basis for the eight questions that make up the WPS (79). Giving a total score out of eight, the WPS aims to describe a symptom phenotype for PBS, enabling it to be distinguished clearly from OAB in patients. It was standardised and validated in 2009 (79) (Appendix 9).

3.6 Results

3.6.1 Demographics

A total of 146 female patients with chronic recalcitrant bladder pain and cystitis and 63 female asymptomatic controls were recruited into the study during the time available. The mean age (Table 3.2) of the patients group was 57 years; standard deviation (sd) = 17; 95% CI = 2.78 (53.93-59.51) and mean age of controls was 46; sd= 14; 95% CI =14 (42.59-49.96) and there is a difference of 11 years. The mean BMI (Table 3.2) was 27; sd = 7, 95% CI = 1.1 (25.63-27.82) for patients and 26; sd = 7; 95% CI = 1.8 (23.77 – 27.37) for controls.

There was no statistically significant difference in the mean Age, BMI and ATP signal between controls and patients.

93% of recruited patients and 57% of recruited controls were White Caucasian (Table 3.3).

Table 3.2 Between group difference in Age and BMI

Age	Mean	SD	SE	CI	CI (Lower)	CI (Upper)
Controls	46.26	14.65	1.84	3.69	42.58	49.96
Patients	56.71	17.01	1.41	2.78	53.93	59.51
BMI	Mean	sd	SE	CI	CI (Lower)	CI (Upper)
Controls	25.57	7.14	0.90	1.8	23.77	27.37
Patients	26.72	6.70	0.55	1.1	25.63	27.82

Table 3.3 Ethnicity (as coded by the NHS digital data on Whittington Anglia ICE Electronic registration portal)

Groups	Controls	%	Patients	%
White Caucasian	36	57	135	93
Asian	13	21	4	2
Black, African/Black Caribbean	14	22	5	2
Mixed Black	0	0	2	1
Other	0	0	0	0
Prefer not to say	0	0	0	0

3.6.2 Comparison of symptoms between groups

The patients showed a significant difference from controls across all symptom measures as demonstrated below on validated Artemis symptom questionnaire which measures frequency, incontinence and LUTS, Whittington Pain and Urgency scores and ICIQ LUTS and QOL symptoms and bother scores (Table 3.4).

Patients had a mean daytime frequency of 9.25 voids while controls had a mean frequency of 6.22. There was a statistically difference in Nighttime frequency, Day and Night-time incontinence, Artemis Urgency, Artemis Voiding, Artemis Stress incontinence symptoms and Artemis Pain scores. The symptom scores also varied significantly on the validated questionnaires namely Whittington Pain score, Whittington Urgency Score, ICIQ FLUTS and ICIQ LUTS – qol. Bother scores for female LUTS were also different on the FLUTS and LUTS-qol questionnaire between the two groups.

Table 3.4 Between group differences in symptom measurements between patients and controls

Symptoms measurements	Group	Mean	SD	SE	CI	CI (Lower)	CI (Upper)	Kruskal-Wallis Chi-squared	df	p value
Daytime frequency	Controls	6.22	2.19	0.28	0.55	5.67	6.77	24.841	1	<.001
	Patients	9.26	4.31	0.36	0.71	8.56	9.97			
Night-time Frequency	Controls	0.92	1.61	0.2	0.41	0.52	1.33	31.953	1	<.001
	Patients	2.19	1.74	0.14	0.29	1.91	2.48			
Daytime Incontinence	Controls	0.03	0.13	0.02	0.04	0.01	0.08	55.618	1	<.001
	Patients	1.9	2.09	0.17	0.34	1.56	2.24			
Night-time incontinence	Controls	0.02	0.13	0.02	0.03	0.02	0.05	8.4301	1	<.003
	Patients	0.32	0.89	0.07	0.15	0.17	0.47			
Urgency	Controls	0.76	1.29	0.16	0.33	0.44	1.09	66.544	1	<.001
	Patients	4.4	4.01	0.33	0.66	3.75	5.06			
Voiding	Controls	0.44	0.95	0.12	0.24	0.21	0.68	84.638	1	<.001
	Patients	3.77	2.59	0.21	0.42	3.35	4.2			
Stress	Controls	0.14	0.4	0.05	0.1	0.04	0.24	35.416	1	<.001
	Patients	1.48	1.85	0.15	0.3	1.18	1.78			
Pain	Controls	0.35	1.09	0.14	0.28	0.07	0.62	63.236	1	<.001
	Patients	3.32	2.99	0.25	0.49	2.83	3.81			
WPS	Controls	0.48	1.08	0.14	0.14	0.21	0.75	63.912	1	<.001
	Patients	4.35	8.97	0.75	1.49	2.86	5.84			
WUS	Controls	0.86	1.89	0.24	0.48	0.38	1.33	88.351	1	<.001
	Patients	8.16	8.49	0.71	1.41	6.75	9.57			
ICIQ FLUTS SS	Controls	3.44	4.37	0.55	1.1	2.35	4.54	109.14	1	<.001
	Patients	25.63	12.86	1.12	2.22	23.41	27.84			
ICIQ FLUTS BS	Controls	6.27	12.94	1.63	3.26	3.01	9.53	106.27	1	<.001
	Patients	68.92	37.09	3.22	6.36	62.56	75.29			
ICIQ LUTS qol-SS	Controls	19.48	5.14	0.65	1.29	18.18	20.77	105.84	1	<.001
	Patients	48.73	16.34	1.42	2.82	45.9	51.55			
ICIQ LUTS qol-BS	Controls	5.03	15.48	1.95	3.9	1.13	8.93	110.01	1	<.001
	Patients	114.66	56.55	4.94	9.78	104.88	124.43			

3.6.3 Comparison of ATP data between groups

There was no statistically significant difference in the mean and log ATP signal between controls and patients. The mean \log_e ATP reading patients was 94.01; $sd=60.05$; $95\%CI = 9.82 (84.19-103.84)$ and in Controls was 86.13; $sd = 54.87$; $95\%CI 13.82 (72.31 - 99.95)$ (Table 3.5).

Table 3.5 Between group difference in log ATP data

Log ATP	Mean	SD	SE	CI	CI (Lower)	CI (Upper)
Controls	86.13	54.87	6.91	13.82	72.31	99.95
Patients	94.01	60.05	4.97	9.82	84.19	103.84

Kruskal-Wallis chi-squared log ATP = 0.8507, $df = 1$, $p\text{-value} = 0.3564$

3.6.4 Urine Dipstick data

3.6.4.1 Leucocyte esterase Dipstick

Standard Leucocyte esterase Dipsticks are calibrated to pick up white cells, where 15 cells = trace, 70 = 1+, 120 = 2+. 3% of our Controls had a pyuria of ≥ 2 ; 18% of patients had a pyuria of ≥ 2 . If Leucocyte dipstick is used as a screening tool, and a patient with zero to + of leucocytes, i.e., 82% of our symptomatic population would be dismissed as having no infection as indicated in the green box in Figure 3.19.

There was a significant difference when the performance of leucocyte esterase dipstick was compared between study patients and controls as shown in Table 3.6. The area within the green box in Figure 3.19 represents the number of samples that would not meet the criteria for MSU Culture as the hospital policy was to only send samples with $\geq 2+$ leucocytes or a positive Nitrite on dipstick to the laboratory for Culture and Sensitivity analysis. These symptomatic patients would also be dismissed as having no UTI.

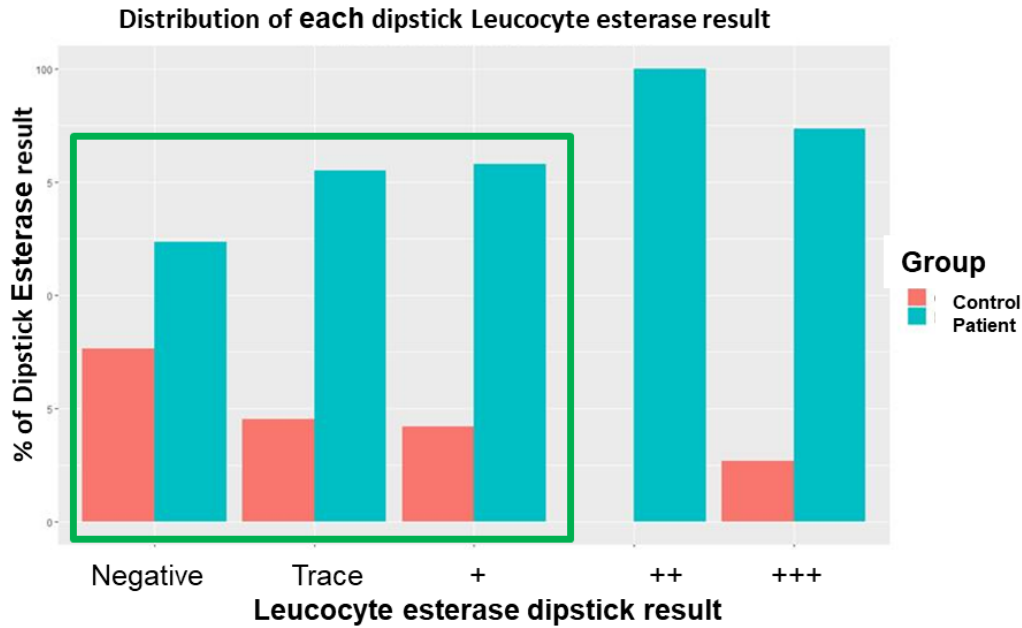
Table 3.6 Comparison of performance of leucocyte esterase dipstick between study patients and controls

Microscopy	Negative	Trace		1+	2+	3+	4+
WBC Controls	50	7		4	0	1	1
WBC Patients	81	24		15	13	13	0
WBC Controls %	79.3	11.1		6.3	0.0	1.6	1.6
WBC Patients %	55.5	16.4		10.3	8.9	8.9	0.0

Pearson's Chi-squared test $X\text{-squared} = 91$, $df = 7$, $p\text{-value} < .001$

Figure 3.19 Comparison of performance of leucocyte esterase dipstick between study patients and controls – Mean proportion from patients vs. proportion from controls.

Each column pairs add to 1 (100%). The green box shows the group of patients (82%) who would not have a culture performed even if symptomatic as dipstick data falls below the hospital guidelines for requesting a MSU culture.



3.6.4.2 Urine Dipstick RBC (red blood cells)

81% of Controls and 48% of patients had negative red cells in the urine. Red cell count of $\geq 2+$ in the absence of menstrual bleeding is a red flag sign warranting further investigation. In this case the five control patients were confirmed to be at the end of a period and hence did not need a urology referral. In patients with CUTI persistent red cells of $\geq 2+$ despite treatment of UTI or persistent red cells ≥ 2 on fresh urine microscopy warranted a cystoscopy. There was a significantly higher dipstick red cell presence in patients compared to controls (Table 3.7).

Table 3.7 Comparison of performance of red cell dipstick between study patients and controls

Dipstick RBC	Negative	Haem Trace	Non-Haem Trace	1+	2+	3+
RBC Controls	51	5	2	0	3	2
RBC Patients	70	21	20	13	13	9
RBC Controls %	80.9	7.9	3.2	0.0	4.8	3.2
RBC Patients %	47.9	14.4	13.7	8.9	8.9	6.2

Pearson's Chi-squared test $X^2 = 90$, $df = 8$, $p\text{-value} < .001$

3.6.4.3 Dipstick Nitrite test

Nitrite Dipstick was only positive in 10% of patients and none of the controls, the difference is statistically significant (Table 3.8).

Table 3.8 Comparison of performance of Nitrite dipstick between study patients and controls

Dipstick Nitrites	Negative	Positive
Nitrite Controls	63	0
Nitrite Patients	132	14
Nitrite Controls %	100	0
Nitrite Patients %	90.4	9.6

Pearson's Chi-squared test X-squared = 87, df = 2, p-value < .001

3.6.4.4 Dipstick Protein test

Overall, 20% of the patient population had a positive dipstick for protein of which 8% had >+ protein. 11% of controls had a positive dipstick, of which 9.5% had a trace and 1.6% (1 control) had +protein in the urine and this is significantly different (Table 3.9). 89% of Controls were negative for Protein on dipstick, with 6 (10%) showing a trace of protein and 1 control showing 1+.

Table 3.9 Comparison of performance of protein dipstick between study patients and controls

Dipstick Protein	Negative	Trace	1+	2+	3+	4+
Protein Controls	56	6	1	0	0	0
Protein Patients	117	17	6	3	1	2
Protein Controls %	88.89	9.53	1.59	0.0	0.0	0.0
Protein Patients %	80.14	11.64	4.11	2.05	0.68	1.37

Pearson's Chi-squared test X-squared = 84, df = 7, p-value <.001

3.6.5 Fresh Urine Microscopy

There was a statistically significant difference between mean pyuria counts between patients and controls. The mean pyuria count in patients was 412, sd = 2997, and in controls was 5.79, sd =8.29 (Table 3.10).

There was a statistically significant difference seen in the mean log epithelial counts between patients and controls. Patient's had a mean epithelial cell count of 28.32, sd =88.75, and in controls was 4.17, sd =6.17 (Table 3.10).

There was a significant difference in fresh urine microscopy red cell counts between patients and controls. Mean RBC in controls was 2 (sd=0.28) and 193 in patients (sd=2118) (Table 3.10).

Table 3.10 Microscopic WBC, EPC and RBC counts

Fresh Urine Microscopy	Group	Mean	SD	SE	CI	CI (Lower)	CI (Upper)	Kruskal-Wallis Chi-squared	df	p value
WBC	Controls	5.79	8.29	1.04	2.08	3.7	7.88	5.644	1	<.017
	Patients	412.78	2998	248.11	490.4	77.61	903.17			
log WBC	Controls	0.49	0.51	0.06	0.13	0.36	0.62	7.07	1	<.007
	Patients	0.9	0.95	0.08	0.16	0.74	1.05			
EPC	Controls	4.17	6.17	0.77	1.55	2.62	5.73	12.146	1	<.00054
	Patients	28.31	88.74	7.34	14.51	13.8	42.83			
log EPC	Controls	0.42	0.42	0.05	0.11	0.32	0.53	11.957	1	<.000492
	Patients	0.8	0.7	0.06	0.11	0.69	0.92			
RBC	Controls	1.26	2.41	0.3	0.6	0.66	1.88	15.66	1	<.001
	Patients	192.89	2118	175.31	146.5	153.61	539.39			
log RBC	Controls	0.17	0.28	0.04	0.07	0.1	0.24	15.166	1	<.001
	Patients	0.53	0.7	0.06	0.12	0.41	0.64			

3.6.6 Hospital MSU culture data between study groups

Routine MSU culture was positive in 5% of controls and 20% of patients (Table 3.11), 60% of cultures in patients and 76% in controls were reported negative (i.e. did not meet the standard quantitative threshold of $\geq 10^5$ cfu ml⁻¹ single species of known urinary pathogen used in our hospital laboratory). On the other hand, 20% of patient samples and 14% of the control samples showed a mixed growth (i.e. $\geq 10^5$ cfu ml⁻¹ of a polymicrobial growth between 2-3 organisms reported as mixed growth of doubtful significance and likely contamination). 3 control samples and 1 patient sample went missing. Of the samples that went missing, they were requested as per Anglia ice (Electronic request portal), packaged in clinic and placed along with the remaining routine clinic samples to be transported from community clinic to hospital laboratory the same day. None of the samples were rejected due to incorrect labelling or spillage. Pearson's Chi-squared test X-squared=13, df=3, p-value=0.005 supports the statistically significant difference between the patients and controls.

Table 3.11 Routine MSU Culture data

Routine MSU Code	Missing Data	No Growth	Mixed Growth	Positive Culture	Total
N Controls	3	48	9	3	63
N Patients	1	88	28	29	146
% Controls	4.76	76.19	14.29	4.76	100%
% Patients	0.68	60.27	19.18	19.86	100%

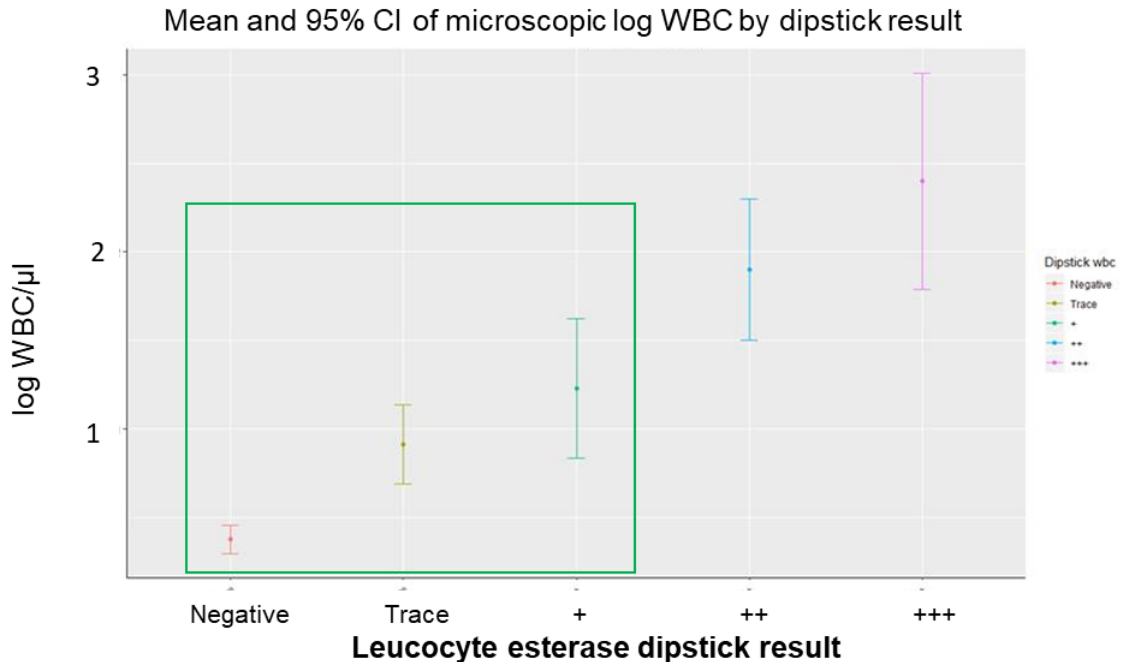
Pearson's Chi-squared test X-squared=13, df=3, p-value = .005

3.6.7 Comparison of microscopic pyuria and leucocyte dipstick

Figure 3.20 shows that there is a quantitative relationship between dipstick and number of white cells (pyuria/pus cell count) on fresh urine microscopy. Dipstick Leucocytes are plotted on the X axis and Mean log microscopic pyuria is plotted on the Y axis. It is a log 10 scale where 1= 10 and 2=100.

In routine clinical practice in the absence of Nitrites, leucocytes <2+ are simply ignored as vaginal contamination in female patients and hence not treated (81% of patients would not be treated even though they had pyuria).

Figure 3.20 Quantitative relationship between microscopic pyuria and leucocyte dipstick in patients.



* The green box in Figure shows the patients who would be dismissed as having no infection.

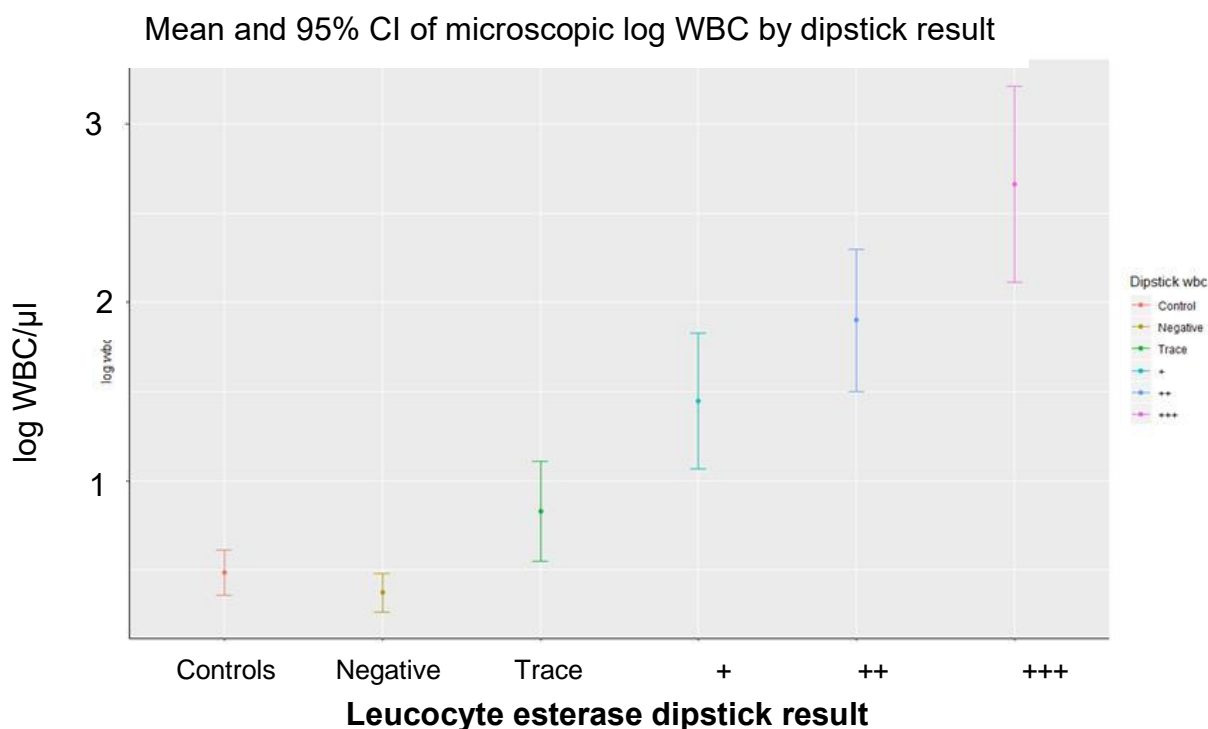
3.6.8 Comparison of microscopic pyuria and leucocyte dipstick in Controls vs Patients

Figure 3.21 shows that there is a quantitative relationship between dipstick and number of white cells (pyuria/pus cell count) on fresh urine microscopy in controls compared to patients. Dipstick Leucocytes are plotted on the X axis and Mean log microscopic pyuria is plotted on the Y axis. It is a log 10 scale where 1= 10 and 2=100.

The higher the white cells in fresh urine microscopy, the higher is the positive result forming a scale trending upwards (11, 25, 26, 33, 41), hence any pyuria signifies inflammation.

On the other hand, pyuria in a control is legitimate and is to be expected. It means that the innate immune system in the individual is functional and working effective against inflammation, hence the patient has no symptoms.

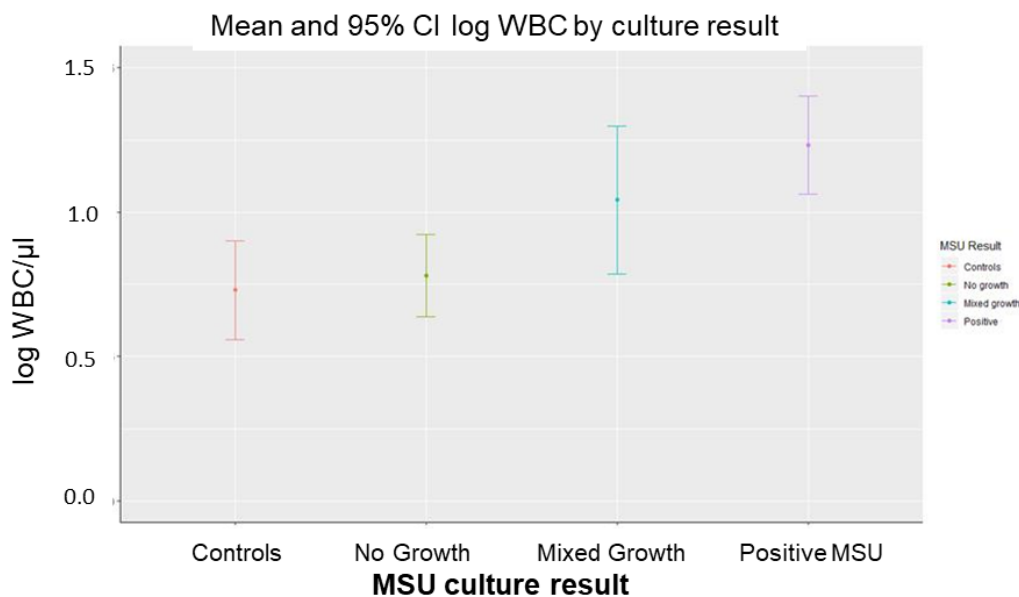
Figure 3.21 Quantitative relationship between microscopic pyuria and leucocyte dipstick in Controls vs Patients



3.6.9 Comparison of microscopic pyuria and MSU culture result in the study population

The Figure 3.22 below shows the relationship between pyuria and log WBC count in the study population (patients and controls). A positive MSU culture is associated with higher pyuria. In other we have seen a relationship between positive cultures and higher pyuria level, suggesting that cultures tend to be productive when the infection is more advanced.

Figure 3.22 Mean and 95% CI log WBC by culture result



The higher the inflammation, there is a higher amount of debris at the base of the bladder and abundance of saprophytes and microbes of multiple types hence not surprising that pyuria if a culture is positive with microscopic pyuria in controls. This is most plausible explanation and needs further testing.

3.6.10 Comparison of Clue cell counts between patients and controls

I have already shown that the epithelial cell counts on fresh urine microscopy were 600% higher in the patients. The mean proportion of these cells showing adherent or intracellular bacteria (clue cells – we may not differentiate intracellular from extracellular) was 78%, sd = 9.72 and in controls these were 72%, sd =23.00 which did not differ (Table 3.12).

Table 3.12 Between group differences in Mean proportion of Clue cells

Group	N	Mean log	SD	SE	CI	CI (Lower)	CI (Upper)
Controls	61	72.32	23.00	2.95	5.89	66.43	78.21
Patients	139	77.77	9.72	0.82	1.63	76.14	79.40

Kruskal-Wallis chi-squared = 0.67708, df = 1, p-value = 0.4106

2 control slides and 7 patient slides did not reveal any intact cells that were eligible for analysis.

3.6.11 Sediment Culture data

3.6.11.1 Total cfu/ml on Sediment Culture

Spun urinary sediment culture results were reported as the number of cfu/ml of each isolate identified to genus level using colour charts, gram stain and bench top biochemical tests. 2 control samples and 1 patient sample did not grow any organisms even after incubation of the plate for seven days, a baffling occurrence.

The total cfu/ml of microbes isolated were calculated using the formula described in the methods and Table 3.13 shows that there is statistically significant difference in the mean log total cfu/ml of bacteria isolated between patients and controls (Table 3.13).

The patients showed greater microbial abundance (Table 3.14): Mean log total colony forming units per ml isolated on culture of spun urinary sediment in patients was 0.94 in controls and was 1.29 in patients.

Table 3.13 Mean log Total CFU/ml on Sediment Culture

Group	N	Mean log	SD	SE	CI	CI (Lower)	CI (Upper)
Controls	62	0.94	0.82	0.10	0.21	0.73	1.14
Patients	144	1.29	0.75	0.06	0.12	1.17	1.41

Kruskal-Wallis chi-squared = 8.0819, df = 1, p-value = .0045

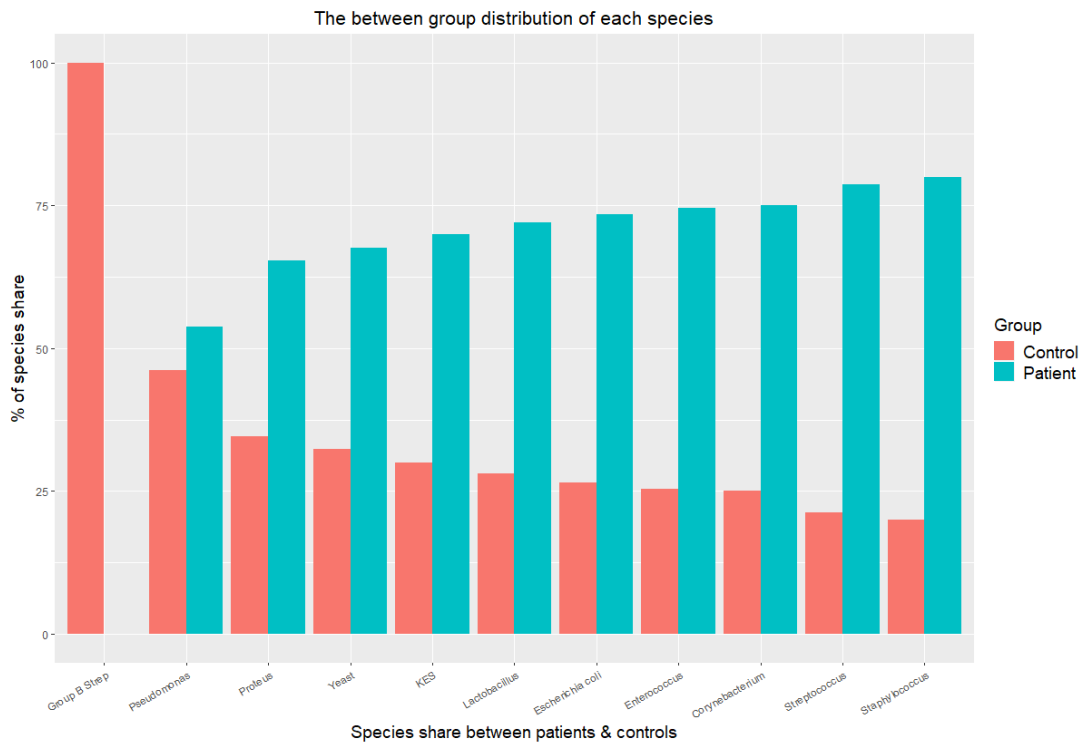
3.6.11.2 Sediment Culture species distribution

There was considerable common ground of cultured organisms between patients and controls as demonstrated in Table 3.14. The graph in Figure 3.23 takes each organism isolated and plots the proportions shared between patients and controls. Controls are marked in red, and patients are marked in blue. The column pairs (one red and one blue) add to 100% in each case. Plot illustrates the overlap but simultaneously demonstrates that fact of qualitative differences concealed in the data.

Table 3.14 Sediment Culture Organisms

	Controls %	Patients %
<i>Corynebacterium</i>	19.67	20.08
<i>E.coli</i>	6.56	5.02
<i>Enterococcus</i>	16.94	18.27
<i>GBS</i>	1.09	0
<i>KES</i>	1.64	2.41
<i>Lactobacillus</i>	4.92	3.41
<i>Proteus</i>	3.28	1.41
<i>Pseudomonas</i>	0.55	0.60
<i>Staphylococcus</i>	28.96	27.31
<i>Streptococcus</i>	14.75	20.08
<i>Yeast</i>	1.64	1.41

Figure 3.23 Mean proportion of each organism isolated: Proportion from patients vs. proportion from controls

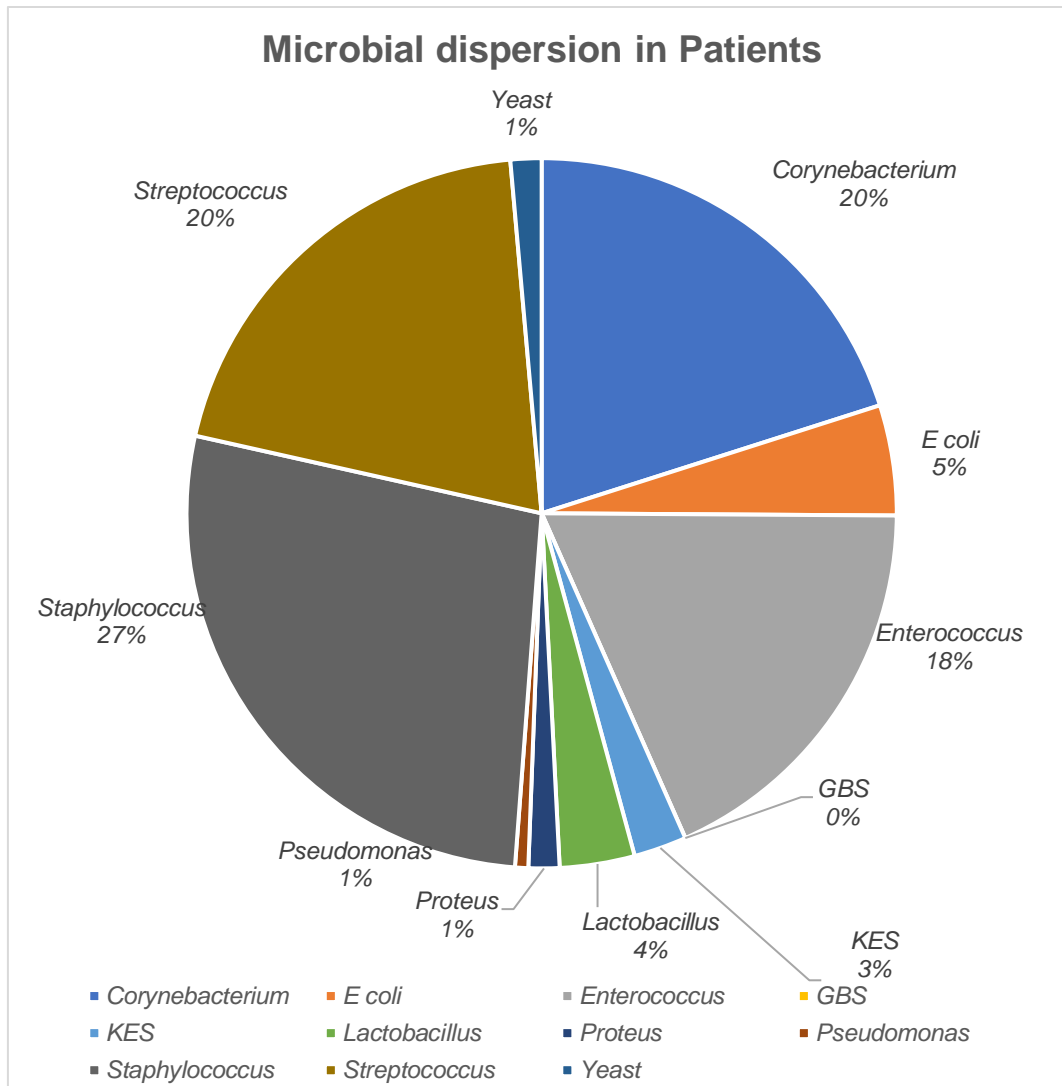


3.6.11.3 Microbial dispersion between groups

3.6.11.3.1 Microbial dispersion in patients

The data are presented as percentages of each isolate obtained. The species distribution is almost similar in the two study groups and no *Group B Streptococcus* was identified in the patient group. *Streptococcus* was more common in patients than controls (Figure 3.24).

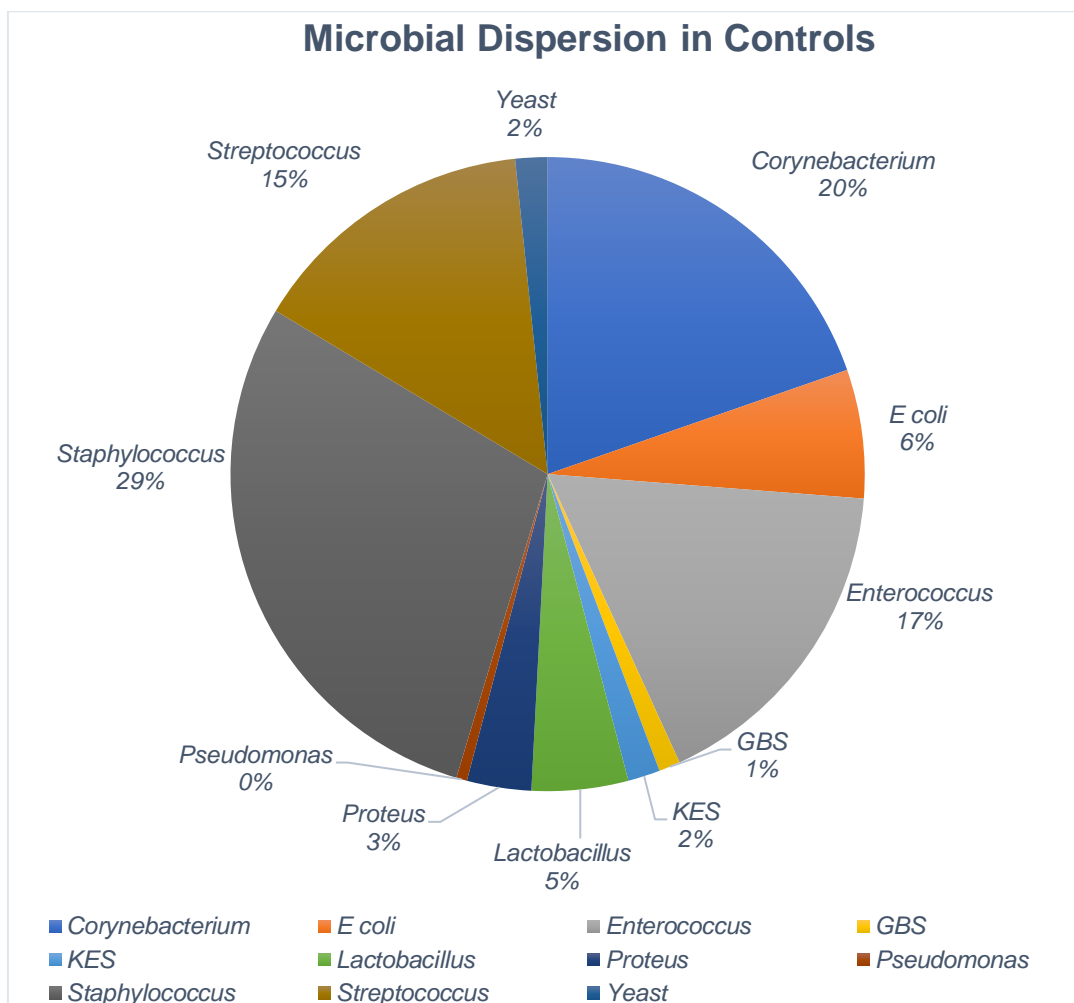
Figure 3.24 Organisms isolated using spun sediment culture technique in patients



3.6.11.3.2 Microbial dispersion in controls

The data are presented as percentages of each isolate obtained. *GBS* was identified in the only in the control group. *Staphylococcus* was more common in controls (Figure 3.25).

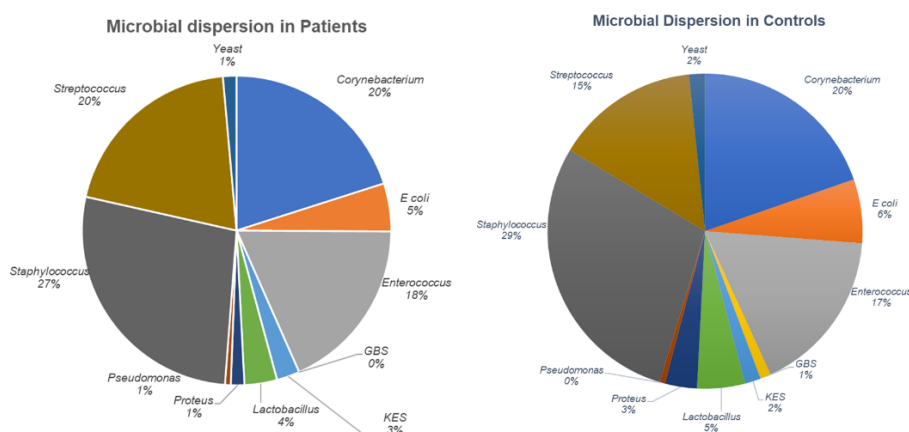
Figure 3.25 Organisms isolated using Spun sediment culture technique in controls



3.6.11.3.3 Microbial dispersion in Patients vs. Control

The patients and controls evince a polymicrobial community that did not differ qualitatively in respect of the microbial genus (Figure 3.26).

Figure 3.26 Microbial dispersion in Patients vs controls on Sediment Cultures using CPS3 & bench top tests – we identified microbes to genus level only.



3.7 Discussion

The patients of focus in this study presented with symptoms of chronic recalcitrant bladder pain and apparent recurrent cystitis, but on multiple occasions a negative dipstick analysis and or urine culture were recorded. They had all been diagnosed with IC or PBS. Their investigation history included negative renal tract scans, at the minimum used ultrasound and negative cystoscopies. They had been exposed to a variety of treatments that are typically used to manage IC/PBS, they had not responded to such interventions.

The diagnosis of IC/PBS hinges on the exclusion of UTI. We now understand that the methods used to exclude UTI are not competent, so there is a risk that infection has been overlooked.

This is the first comparison of patients with IC/PBS, not taking antibiotics, against normal controls. I have found differences in symptoms, notably pain and voiding dysfunction, along with substantial evidence of an innate immune activation in the patient group. These differences appear to be associated with microbial abundance in the patients.

Although there was no statistically significant difference in the age, the controls were 10 years younger. Despite many attempts it was difficult to obtain perfect age-matched controls due to the strict inclusion criteria used to recruit controls. All attempts to obtain older controls were disrupted by the prevalence of LUTS or UTI symptoms.

The ethnicity data obtained using Whittington hospital Digital data showed 93% of recruited patients and 57% of recruited controls were White Caucasian (Table 3.3). This is a true representation of the local patient population. However, the staff used to source control data were known to be a more multicultural group.

55% of patient and 80% of controls had negative dipstick (Table 3.6). Whittington hospital guidelines suggest that MSU cultures are to be requested only in symptomatic patients with $\geq 2+$ leucocytes. Using these criteria, 82% of the patient population who had negative to + of leucocytes would not qualify for MSU culture. There was a significantly higher dipstick red cell presence in patients compared to controls (Table 3.7). Nitrite Dipstick was only positive in 10% of patients and none of the controls, the difference is statistically significant (Table 3.8). These data are commensurate with the performance of dipsticks in other studies (10). Dipstick protein was positive in 20% of patients (Table 3.9). Chronic kidney disease leads to increase in the urinary protein excretion and although this does not measure disease severity, it is compatible with an increase in kidney injury.

There was a significant difference in fresh urine microscopy counts of pus, epithelial and red cells between patients and controls. Treatment of UTI with antibiotics showed clearance of red cells and those that did not respond were referred to cystoscopy to rule out bladder malignancy. The epithelial cell counts on fresh urine microscopy were 600% higher in the patients. Two control slides and seven patient slides did not reveal intact cells suitable for analysis. This could be due to the lysis of cells during the sample preparation process.

Figure 3.20 shows the quantitative relationship between microscopic pyuria and leucocyte dipstick in patients. The significance of this plot is that it demonstrates a scale. The scalar qualities of these tests are not used in clinical practice when the results are assessed by dichotomised categorisation as positive/negative. If we accept the lack of sensitivity and reject the test as capable of ruling out infection, the set of positive results might be useful to plot the progress or regress when treating an infection. The dipsticks are insensitive.

There was no statistically significant difference in the mean and log ATP signal between controls and patients. ATP is used as a surrogate marker of the presence of cells, particularly bacterial and inflammatory. Given the between group differences already demonstrated we should have expected to find a difference. However, in another paper from this centre it was found that the variance in ATP measures mitigated against detecting differences (156).

There was a significant difference between the routine MSU cultures from patients (20% positive) and controls (5% positive). This is an observation of the biology and does not imply a causative relationship between abundance and UTI.

Patients and controls showed polymicrobial abundance in sediment cultures. The total cfu/ml of microbes isolated showed a statistically significant difference in the mean log total cfu/ml of bacteria isolated between patients and controls (Table 3.13), Kruskal-Wallis chi-squared = 8.0819, df = 1, p-value = 0.004471. These findings have been corroborated in other studies by Khasriya et al. from our laboratory in J Clin. Micro and also demonstrated in the thesis by and Dr Gill in patients with OAB (overactive bladder) (19, 25, 26) and in similar studies done in united states (24, 26, 90). It would be folly to assume that the cultured isolates were causative, as the selective agar plates will culture a small proportion of the complex microbiome.

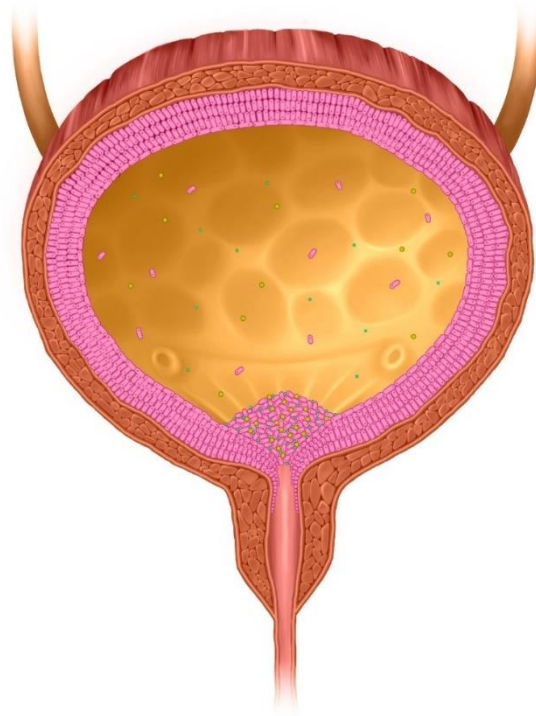
The innate immune reaction will change the ecological environment of the bladder, which in health is far from sterile. We should therefore expect some perturbation of the microbiome from that. Figure 3.27 illustrates a bladder with a sediment forming at the base (42). This will consist of epithelial cells, white cells, and a chemical soup formed from cell lysis containing protein, lipids and sugars which will form a tasty substrate for many different microbes living in a mutualist or commensal relationship with the host. Saprophytes may thrive and enhance bacterial abundance without necessarily having any pathological role.

These data have demonstrated well the fact that the standard methods for excluding UTI in patients with IC/BPS are failing and that when patients with IC/BPS are compared to normal controls we can see that they exhibit microbiological abundance and an innate immune activation that the standard tests should have detected if they were doing their job. Thus, we can say that UTI has not been excluded in patients with IC/BPS who are managed according to published protocols.

These data do not clinch the proposition that IC/BPS is due to infection, but they do fill the first rung of Judea Pearl's ladder of causation (159). The second rung, intervention and the third, counterfactuals will be addressed in subsequent chapters. At this stage I lay claim to correlation alone.

It is so often said that correlation is not the same as causation, but it is the first step on the ladder of causation (159) and so it is crucial because if it is absent, there cannot be a causal relationship.

Figure 3.27 Bladder with sediment forming at the base (reproduced with permission from Professor James Malone-Lee)



3.8 Limitations

The main limitations of the study were as follows:

We were not able to match the patient and control groups for age and menopausal status. There was a 11-year difference between patients and controls and despite widespread attempts to invite older controls, they often screened positive for LUTS symptoms which formed one of our exclusion criteria. In the future we must approach this differently.

The patients that were studied were selected, in the sense that they had already exhausted all the options that are offered for the management of IC/BPS. That did not make them extraordinary, since their clinical presentations were commensurate with others first presenting with IC/BPS to a Urogynaecology service. The reason for this is that published evidence demonstrates that the various treatments that are deployed do not seem to be effective so that the patients of this study had not changed from when they presented to secondary care.

MSU samples did go missing during transport which is a recognised, relevant complication affecting a community clinic. The system is known to be imperfect.

The fact that some sediment culture plates did not grow any organisms was indeed baffling. They were left in the incubator for an extra 5 days (initial 2+5 days). We checked the quality of urine and this was not recorded as dilute, the culture plates had been streaked with a sample and the lab solutions were tested for any contamination and none was found. Other samples processed on the same day did show a growth. One explanation, I can think of is contamination of the plates with alcohol gel which is used a great deal in the laboratory. Some spillage may have occurred so that the substrates were rendered sterile.

Another reason may be that the microbes in the samples did not have affinity for chromogenic agar and hence did not grow in this medium whilst other non-culturable bacteria were thriving. We also acknowledge that we did not test for anaerobes and the samples may have contained VBNC bacteria (viable but non culturable) (160). VBNC bacteria are those which are alive but do not give rise to visible growth under nonselective growth conditions.

Sub categorisation of the patient groups was not an option, given the sample size. However, the patients were homogenous in that they presented with mixed lower urinary tract symptoms of urgency, frequency and incontinence and all patients had pain and voiding symptoms. We did not include patients with genuine stress urinary incontinence symptoms or overactive bladder syndrome patients without pain.

We did not have a sufficiently abundant sample to study the pre and post-menopausal groups separately. I was also affected by the closure of the unit to new patients between Oct 2015 until November 2018 (when a new consultant was appointed) which prevented me from recruiting any further new patients.

Chapter 4 A blinded cross-sectional pilot study of the pathophysiological signals detectable in the urine of patients with diabetes mellitus.

4.1 Study of prevalence of lower urinary tract symptoms in patients attending the routine diabetes clinic appointment.

4.1.1 Introduction

The primary purpose of this thesis was to explore the relationship between the symptoms of IC/BPS and indicators of UTI. The motivation for this work was the realisation that the tests being used to exclude infection in patients presenting with the index symptoms were not competent for the task that they were being put. That meant that we had to study the samples using novel approaches to the analysis of infection and inflammation. Whilst the methods were new, they had all been validated and reported in the peer-reviewed literature (10) (19) (11).

Whilst I included controls in the main study, I did not wish to be complacent and felt that it would be sensible to use the methods in a sample of women who had a high probability of suffering from undiagnosed chronic UTI. A good choice would be persons with diabetes, where LUTS are commonly attributed to the metabolic disease and UTI is usually excluded on the evidence of the conventional urinalysis methods (161) (162).

In the diabetic clinic the policy was to test the urine by dipsticks. Samples were sent to the laboratory for culture only on the occurrence of a positive nitrite or 2+ of leucocytes in a patient with symptoms of acute dysuria. The limitations of such a policy are clear from previous discussion in thesis. It was notable that there was no policy for the screening for other LUTS. Complaints of frequency and urgency were attributed to diabetes. There was great faith in the veracity of the dipsticks and MSU culture (10).

A recent survey of 1000 diabetes patients (163) which used culture criteria for diagnosis revealed that prevalence of UTI was 25.3% (7.2% in males and 41.1% in females) and this did not vary between Type 1 and Type 2 diabetes patients.

It is apposite to record that we know that given any infection, the control of diabetes is difficult. It is concerning that the methods used to identify UTI are weak and unreliable.

4.1.2 Aim

The aim of this pilot survey was to assess the prevalence of LUTS in patients attending their routine diabetes clinic appointment whilst the service managed the urinalysis according to their standard practice. At this stage, I was interested in the prevalence of LUTS in diabetics who were not thought to have urinary tract symptoms. I wished to ascertain whether my sense that this would be a good group to study was well placed. This was a pilot of diabetic patients who had not been recorded as suffering from LUTS by the diabetic clinic staff.

4.1.3 Ethical Approval

This cross-sectional, prospective observational study titled Pathophysiological signals of urine in lower urinary tract symptoms had ethical committee approval since December 2010 from National Research Ethics Service Committee London – East. The Research Ethics Committee reference number is 11/LO/0109. All patient data was anonymised and held in accordance with the Data Protection Act.

4.1.4 Methods

Male and Female patients with Type 1 & 2 diabetes, aged ≥ 18 years, attending their diabetic clinic appointment at Whittington hospital were invited to fill a simple proforma (Figure 4.1). Demography details, HbA1C results were collected from the clinic database. These patients were asked to provide a midstream specimen of urine and the dipstick test was done by a trained clinic nurse. A routine culture was requested by the endocrinology nurse if urine was positive for nitrites on dipstick testing or if there was a large number of leucocytes ($> 2+$).

Whilst this study was extant, I supervised a medical student whilst he accomplished a BSc project. Whilst working on the project the student distributed these questionnaires and collected them from at the endocrine clinic and invited the patients to provide a urine sample for my study described in the next subsection (Chapter 4.2) of my thesis. However, I accomplished all the clinical and experimental work. No publications have resulted from the BSc project.

Figure 4.1 Data Collection proforma

Data collection proforma for Diabetic clinic

Patient Name: _____

Hospital No: _____ **Or** **Affix Patient Label**

Date of Birth: _____ **(Only if available)**

Telephone No: _____

Circle one of the two: Male Female

For patients to complete please:

Can you please answer these questions with a tick for yes or no?

Symptoms	Yes	No
Do you pass urine more than 8 times during the day?		
Do you get up more than once a night to pass urine?		
Do you have to rush to the toilet to pass urine?		
Do you leak urine (have any incontinence)?		
Do you have any pain in the bladder (in the lower belly) area?		
Do you feel the urine stream is reduced compared to before?		
Do you have more than 2 urine infections a year?		
We have a research study in the hospital. You will be asked for a urine sample and to answer a set of questions related to the bladder. Please tick this box if you are happy to take part.		

Clinic Nurse to fill in urine dipstick result:

Protein	Glucose	Blood	Leucocytes	Nitrites

4.1.5 Results

A total of 186 patients (96 female and 90 male) took part in the survey. Mean age of females was 59 years and males were 56 years. 40% of the total survey population expressed ≥ 2 bothersome LUTS symptoms. 68% of male patients and 72% of female patients had ≥ 1 of the 7 bothersome LUTS symptoms listed (Table 4.1).

Table 4.1 Study population and prevalence of LUTS

Total 186 patients	Men n=90	Women n=96
Mean Age	59 years	57
≥ 1 LUTS	68%	72%

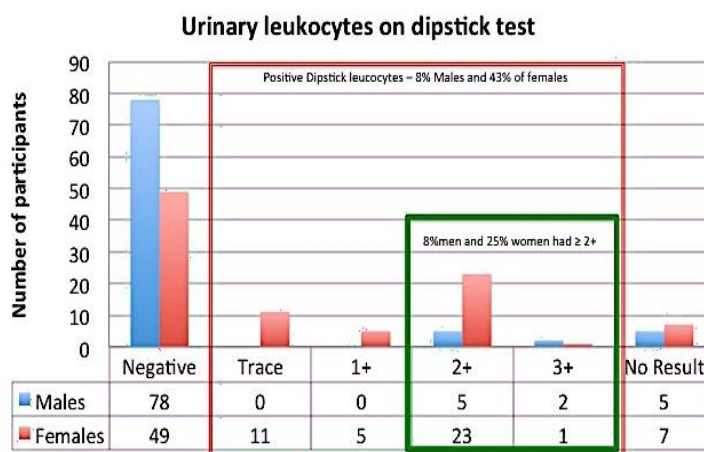
4.1.5.1 Results of Urinary Dipstick, data of the survey population

75% of the male samples and 86% of female samples had a trace or more of Protein on routine dipstick. Leucocytes were positive with a trace or more in 9/90 (10%) men, 57/96 (59%) women. Nitrites were positive in 2/90 (2%) men and 8/96 (8%) women. Leucocytes of ≥ 2 was noted in 7/90 (7.7%) men and 24/96 (25%) women (Table 4.2, Figure 4.2).

Table 4.2 Urine Dipstick results

Urinary Dipstick	Men n=90	Women n=96
Leucocytes Trace or more	9	57
Leucocytes ≥ 2	7	24
Nitrite Positive	2	8
Protein – trace or more	75%	86%

Figure 4.2 Urine Dipstick results



Nitrite dipstick was positive in 2% of males and 8 % of females

Trace or more of leucocytes (patients within the red box) if noted in a symptomatic patient with LUTS, requires further investigation and often overlooked. Leucocytes of ≥ 2 (represented by the green box) was noted in 7/90 (7.7%) men and 24/96 (25%).

4.1.5.2 Results of MSU culture data of the survey Population.

Routine MSU cultures were requested only for 18 men and 32 women (26%) as the dipstick criteria did not warrant the need for a formal culture. Mixed growth was noted in 2 men and 11 female patients; positive culture in 4 male and 9 female patients (7% of total study population) and the remaining were reported as no significant growth.

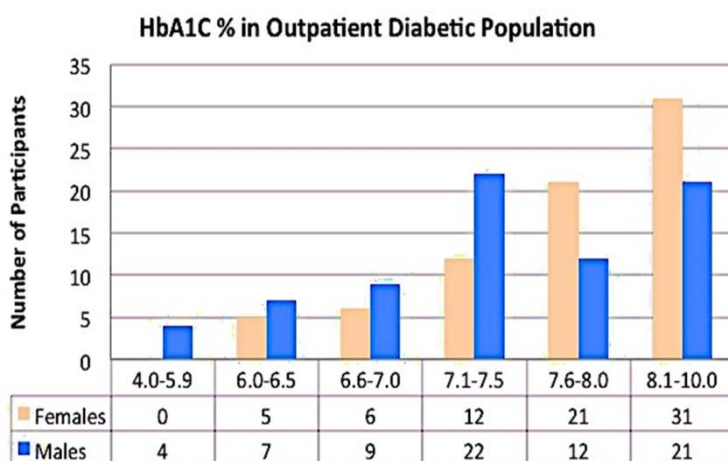
4.1.5.3 Results of HbA1C data of the survey population

HbA1C results are shown in Table 4.3 and Figure 4.3. Only 5% of females and 7% of males had good control of their diabetes. 54% of female and 36% of male diabetes patients had HbA1C level greater than 7.5%.

Table 4.3 HbA1C results

HbA1C	Male	Female
<6.5%	5%	7%
>7.5%	36%	54%

Figure 4.3 HbA1C in survey population



4.1.6 Discussion

LUTS are more common in the diabetic population with a prevalence quoted to be between 37-70% and are frequently attributed to the metabolic disorder (162, 164). Dysuria and urinary frequency are the most common questions asked and reported on. Both storage and voiding symptoms can coexist.

Despite major evaluations in the literature, no recommendation supervises the assessment and management of LUTS in this specific population. This is the first time we are exploring the correlation between LUTS symptoms and UTI using the consilience methods described in Chapter 3. Although there are studies reporting increased incidence of UTI in diabetic patients, there is no studies looking at the paucity of gold standard testing in this high-risk population (165) (166) (167).

40% of the population in this survey suffered with ≥ 2 bothersome LUTS symptoms and about 60% had at least 1 bothersome LUTS symptom.

Patients with leucocyte dipstick reactions of ≥ 2 were noted in 7/90 (7.7%) men and 24/96 (25%) women, and would warrant a thorough history, clinical assessment directed at a UTI diagnosis.

Routine MSU cultures were requested only for 26% of study population as the dipstick criteria did not warrant the need for a formal culture on the protocol used. Positive urine cultures resulted in a diagnosis of UTI in 7% of total study population and treatment was initiated by the team 48 to 72 hours later.

54% of female and 36% of male diabetes patients had HbA1C level greater than 7.5%, representing the diabetics with poor control and at a higher risk of developing hyperglycaemia and end organ damage.

These data imply that the heavy reliance on urine dipsticks and culture may promote a tendency to leave significant urine infection untreated. In turn that may have an adverse influence on diabetes control.

4.1.7 Limitations

Because of the patient records were anonymised, *post hoc* I was not able to map individual patient HbA1C values back to the records I held. This was an oversight that I should have considered at the outset and it has been made all the more clear when I come to report the pilot data. We should return to this in subsequent studies.

4.2 A Blinded cross-sectional study comparing the pathophysiological signals in the urine of diabetic patients with normal controls

4.2.1 Introduction

Diabetic patients have been found to be at a greater risk of UTI (163, 165) with studies suggesting risk ratios from 2 to 10 (164, 165) compared with control subjects.

A high proportion of positive growth MSU tests have been found to be from diabetic patients (28) although in order to investigate a causative link between diabetes and UTI, case-control studies are of more use. Comparing diabetic patients to non-diabetic controls better allows the effect of diabetes to be studied.

Diabetic patients have also been found to be at risk of hospital acquired infection, specifically pneumonia, skin infections and UTI (168). That the risk is not just limited to UTI suggests an immunological effect of the disease. Glycosuria is an explanatory conjecture with higher levels of sugar attributed to promotion of pathogenic bacterial growth. Glycaemic control has been shown to affect rate of UTI within a population of diabetic patients (169, 170). This work detected significant bacteriuria in 54.4% of patients with poor diabetic control compared with only 2.9% of those with good control (171). It is not necessarily the case that glycosuria has a causative role until this has been verified, correlation is not the same as causation.

E. Coli is regularly found to be the most common uropathogen in cultures of hospital MSU samples (28, 171-173, 174.) However, in diabetics, *Staphylococcus aureus* was found to be the most cultured bacteria (171).

Diabetes is known to cause neuropathy, retinopathy and nephropathy; all three are regularly screened in diabetic patients in outpatient clinics. diabetic neuropathy has been found to affect the detrusor muscle of the bladder specifically. Dysfunction of the detrusor muscle has been found to cause voiding symptoms in affected patients (175). Some attribute the voiding problems as aiding infection but this has not been proven.

Melzer M et al. studied patient outcomes following hospital acquired UTI and found that when compared to the whole hospital inpatient population, diabetic patients were at increased risk of death during the 28 days from diagnosis and that overall diabetes had a negative impact on prognosis of UTI (168, 176). Not only does the risk of UTI increase with diabetes, the outcomes were more severe in these patients. With recurrent UTI one of the strongest predictors for future risk, UTI in diabetic patients is clearly an important research area.

4.2.2 Hypothesis

Pathophysiological signals in the urine differ between diabetic patients and controls.

4.2.3 Ethical Approval

This cross-sectional, prospective observational study titled Pathophysiological signals of urine in lower urinary tract symptoms had ethical committee approval since December 2010 from National Research Ethics Service Committee London – East. The Research Ethics Committee reference number is 11/LO/0109. All patient data was anonymised and held in accordance with the Data Protection Act (Appendix 3).

4.2.4 Study Population and recruitment

Patients attending their regular outpatient diabetes clinic appointment at Whittington Hospital London were asked to complete a LUTS symptom survey which formed a part of the diabetic team's service evaluation audit. Patients who volunteered on this survey to participate in the urine study were approached. Patients with type one and two diabetes were included into this study.

Patients in this clinic had been referred by their GP for specialist input to control the disease progression and often require treatment alteration to ensure that their blood glucose is within the target range required to reduce end organ damage.

The patients in this clinic see a multidisciplinary team comprising an endocrinology consultant, nurse specialist, dietician, ophthalmologist, podiatrist and phlebotomist. As their entire consultation between different members of the team consisted of several hours of waiting, they had ample time to read the two-part patient information sheet and ask questions prior to recruitment. Patients who had indicated an interest and had already left the department were contacted by telephone and were given appointments specifically for recruitment purposes.

Volunteering non-pregnant, adult diabetic women who were able to provide informed consent, and complete a questionnaire were approached for the study. They were recruited once the inclusion and exclusion criteria were checked. We excluded patients who were on antibiotic therapy at the time of the study or had been on antibiotics within the last month for any reason. They were asked to provide a fresh clean catch urine sample for the study and complete the study questionnaires.

Non-pregnant volunteering controls were recruited from healthcare and research staff within the Whittington LUTS Group, Hornsey Central Community Staff and associated clinical and research departments. Participants had to be female, at least 18 years old, able to provide informed consent and a urine sample on the day of recruitment. Controls with existing diagnosis of Diabetes Mellitus, LUTS, or on antibiotic therapy were excluded.

4.2.5 Informed Consent

All patients were provided with a patient information sheet and the study was explained to them. Questions relating to the study were encouraged and answered immediately in all cases. Written consent was obtained, and a copy of the consent form was offered to all patients.

4.2.6 Symptoms and History

I interviewed the patient and obtained their medical history, recorded their demographic data and symptoms on custom built departmental software 'Artemis' which generated a symptom score for each of the LUTS symptoms: Urinary frequency, nocturia, pain, voiding, stress urinary Incontinence symptoms, urgency and urge incontinence symptoms.

The patient then completed a set of validated questionnaires which assisted in scoring the symptoms and bother scores to allow quantitative analysis. Validated questionnaires included the ICIQ-FLUTS and ICIQ-LUTSqol (International Consultation on Incontinence Modular Questionnaire © Bristol Urological Institute 2014)(135). FLUTS recorded symptoms and the bother scores in female patients on a 10-point Likert scale with zero suggesting no bother and 10 suggesting most bother. ICIQ-LUTSqol focused on the impact on quality of life from LUTS symptoms and the scale of bother to the patient. WUS (78) and WPS (79) questionnaires were completed by patients so they could quantify the presence or absence of symptoms on a dichotomous scale which most patients found easy to understand and complete (Appendix 9-12).

4.2.7 Laboratory Methods and Appendix for Lab details

This cross-sectional observational cohort study was conducted from November 2013 to July 2014 at Whittington Hospital. The laboratory protocols and processes have been described and were identical to the methods in Chapter 3. I accomplished all these.

4.2.8 Statistical Analysis

Descriptive and inferential statistical analysis was generated using SPSS[®] version 22.0 (IBM[®], New York, USA) with all data summarised by calculating means and 95% Confidence Intervals (CI). Inferential analysis was calculated using Chi Squared tests, T- tests and multinomial logistic regression as appropriate in each case. Chi Squared testing is appropriate when data follows a Chi Squared distribution. T- tests for normally distributed data and logistical regression when the variable is binomial e.g. patient or control. The independent predictor covariates were: pyuria (\log_{10} WBC μl^{-1}), fresh urinary uroepithelial cell count (\log_{10} UEC μl^{-1}), average 24 hour urinary frequency and average 24 hour incontinence. Symptoms were scored using the custom departmental software, Artemis and the symptom scores used as independent predictor covariates as well were stress incontinence, urinary urgency, voiding dysfunction and urinary tract related pain.

4.2.9 Results

4.2.9.1 Demographics

Thirty patients were recruited to the study with three excluded before analysis (two for antibiotic therapy and one for being unable to produce a sample). Twenty two volunteer controls were recruited with no exclusions. The mean ages of the

patients and controls were 53 years (95% CI 45-61 years) and 43 years (95% CI 36-48 years) respectively. There was a significant difference between the ages of the two groups after an independent samples t-test, Table 4.4 shows the distributions of the ages of the two groups. The two groups were also similar in BMI (Table 4.4). Patients had a mean BMI of 27 (95% CI 24-29) and controls 26 (95% CI 23-29), $p = .008$.

Table 4.4 Mean Age

Age	Mean (years)	SD	CI(Lower)	CI(Upper)
Controls	41	13.53	35.32	37.32
Patients	52.78	19.90	44.90	60.65
BMI	Mean	sd	CI(Lower)	CI (Upper)
Controls	25.57	6.14	22.83	28.76
Patients	26.72	5.47	24.43	28.85

4.2.9.2 Assessment of Symptoms

There was a significant difference in symptom scores between patients and controls. Table 4.5 shows the mean score for patient and control groups for each assessed symptom score. WUS, WPS, ICIQ-FLUTS SS, ICIQ-FLUTS BS, ICIQ-LUTSqol SS, ICIQ-LUTSqol BS, Artemis scores and 24hour incontinence were significantly higher in the patient group than the control group. There was no significant difference seen with respect to frequency or nocturia.

Table 4.5 Artemis and questionnaire scores for Lower Urinary Tract Symptoms in patients with LUTS and healthy controls

Symptom Measure (score range)	Patients with LUTS	Healthy controls	Significance (p)
Urgency (0 - 12)	4 (3-5)	1 (0-2)	0.001
Stress (0 - 7)	0 (0-1)	0 (0)*	= 0.04
Voiding (0 - 8)	2 (1-3)	1 (0-1)	= 0.02.
Pain (0 - 13)	4 (3-6)	1 (0)**	< 0.001
ICIQ-FLUTS Symptom Score (0 - 69)	25 (18-33)	4 (2-6)	< 0.001
ICIQ-FLUTS Bother Score (0 - 150)	74 (55-93)	11 (3-18)	< 0.001
ICIQ-LUTSqol Symptom Score (16 - 76)	52 (45-59)	21 (19-24)	< 0.001
ICIQ-LUTSqol Bother Score (0 - 200)	122 (102-147)	6 (1-11)	< 0.001
WUS	8 (5-11)^	1 (0-3)	< 0.001

(0 - 20)			
WPS (0 - 8)	5 (4-6)	0 (0)	< 0.001
Total Frequency (1 - >20)	10 (8-12)	8 (7-10)	= 0.079
Total Incontinence (0 - >5D & >5N)	2 (1-3)	0 (0)	= 0.002

Data are mean and 95% CI

* n=3; ** n=4; ^ n=17

4.2.9.3 Urine Dipstick results

There was no significant differences in dipstick results between the two study groups (0= Controls & 1 =Patients) for any of the recorded outcomes (Table 4.6 -Table 4.17). Results were scored in each case from the raw data by the distinct levels as prescribed by the manufacturer. None of the control subjects showed positive results for glucose, ketones or nitrites but there were positive results for white blood cells (WBC), protein and red blood cells (RBC). Nitrites were either positive or negative.

Table 4.6 Dipstick Nitrite data

	Patient Group		Total
	0 Control	1 Patient	
Dipstick-Nitrite Missing	5	0	5
Neg	17	26	43
Positive	0	1	1
Total	22	27	49

Table 4.7 Dipstick Nitrite- Chi-Square Tests

	Value	df	Asymp. Sig. (2- sided)
Pearson Chi-Square	7.451a	2	.024
Likelihood Ratio	9.704	2	.008
N of Valid Cases	49		

Leucocyte esterase was classified a 0- none, 1 = trace, 2= +, 3=++,4=+++.

Table 4.8 Dipstick White Blood Cells data

Dipstic WBC	Patient Group		Total
	0 Controls	1 Patients	

Missing Data		5	0	5
	0	13	22	35
	1	2	1	3
	2	0	1	1
	3	2	1	3
	4	0	2	2
Total		22	27	49

Table 4.9 Dipstick White Blood Cells - Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	10.581a	5	.060
Likelihood Ratio	13.599	5	.018
N of Valid Cases	49		

Table 4.10 Dipstick Protein data

Dipstick Protein: 0= None, 1= trace, 2=+, 3= ++

Dipstick Protein	Patient Group		Total
	0 Controls	1 Patients	
Missing Data	5	0	5
0	16	18	34
1	1	8	9
3	0	1	1
Total	22	27	49

Table 4.11 Dipstick Protein - Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.168a	3	.011
Likelihood Ratio	14.122	3	.003
N of Valid Cases	49		

Table 4.12 Dipstick Red Blood Cell data

Dipstick RBC: 0= None, 1= trace, 2=+, 3= ++

Dipstick-RBC	Patient Group		Total
	0 Control s	1 Patients	
MissingData	5	0	5
0	14	20	34
1	0	5	5
2	2	0	2
3	1	2	3
Total	22	27	49

Table 4.13 Dipstick Red Blood Cells - Chi-Square Tests

	Value	df	Asymp. Sig. (2- sided)
Pearson Chi-Square	13.017a	4	.011
Likelihood Ratio	17.529	4	.002
N of Valid Cases	49		

Table 4.14 Dipstick Ketone data

Dipstick Ketones: 0= None, 1= trace, 2=+, 3= ++

Dipstick Ketones	Patient Group		Total
	0 Controls	1 Patients	
Missing Data	5	0	5
0	17	21	38
1	0	4	4
2	0	1	1
3	0	1	1
Total	22	27	49

Table 4.15 Dipstick Ketone – Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.026a	4	.026
Likelihood Ratio	15.160	4	.004
N of Valid Cases	49		

Table 4.16 Dipstick Glucose data

Dipstick Glucose: 0= None, 1= trace, 2=+, 3= ++, 4=++++, 5=+++++

Dipstick Glucose	Patient Group		Total
	0 Control	1 Patient	
Missing Data	5	0	5
0	17	17	34
1	0	1	1
2	0	1	1
3	0	1	1
5	0	7	7
Total	22	27	49

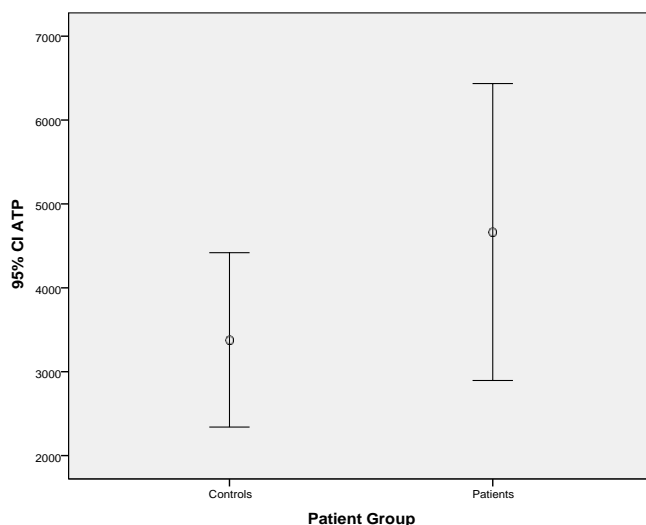
Table 4.17 Dipstick Glucose – Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	14.642a	5	.012
Likelihood Ratio	20.283	5	.001
N of Valid Cases	49		

4.2.9.4 ATP Data

Independent samples t-test was carried out to study the ATP signal in the urine of the study population. There was no statistically significant difference between patients and control subjects. The mean patient value was 4666 RLU (sd 4287 RLU) and for control patients was 3380 RLU (sd 2021 RLU) (Figure 4.4).

Figure 4.4 ATP result



4.2.9.5 Fresh Urine Microscopy results

Mean microscopic pyuria (Mean WBC) in the patient group was 18 (95% CI 1-36) and in the control subjects 2.5 (95% CI 1.4-9.1) (Table 4.18). This difference is not significant at the 95% CI however is noted to be increased in patients. Mean uroepithelial cell count (UEC) was similarly raised in the patient group although again not significant at the 95% CI. The increase in mean EPC was less striking than the pyuria with patients at 8.6 (95% CI 1.0-16) and controls at 5.3 (95% CI 1.4-9.1) (Table 4.18).

Positive results from red blood cell counts were insufficient to warrant statistical analysis.

Table 4.18 Fresh Urine Microscopy – Mean WBC count

Microscopy	Group	Mean	sd	CI(Lower)	CI(Upper)
Mean WBC	Controls	2.529	3.6	0.67	4.384
	Patients	17.926	46	0.19	36
Log WBC	Controls	.29	0.38	.10	.49
	Patients	.5289	0.68	.26	.80
Mean UEC	Controls	5.294	7.55	1.41	9.18
	Patients	8.556	19.15	.98	16.13
Log UEC	Controls	.50	.43	.27	.73
	Patients	.58	.52	.37	.78

4.2.9.6 Routine MSU Culture results

Out of the 27 patients, 20 (74%) showed a negative Routine MSU culture with 4 (14%) showing mixed growth and 3 (11%) with a positive culture (CFU ml⁻¹ >10⁵). The same three outcomes in the 22 control group subjects were 13 negative tests (59%), 8 mixed growth (36%) and 1 positive culture result (5%). Patients showed a higher proportion of positive culture results (11% in patients compared with 5% in controls). Control subjects had a higher proportion of mixed growth results (36% compared to 14%) (Table 4.19).

Routine hospital MSU protocol considers a mixed growth to be contamination, unless a single species shows growth greater than 10⁵ CFU ml⁻¹ of a known uropathogen cultures are reported as “No Significant Growth”.

Table 4.19 Routine MSU results

Study Group	No Growth	Mixed	Positive
Controls (22)	13 (59%)	8 (36%)	1 (5%)
Patients (27)	20 (74%)	4 (14%)	3 (11%)

4.2.9.7 Sediment Culture results

The results of the sediment cultures from each sample were presented in absolute terms; the reporting not limited as with the MSU. With a positive result being any culture to produce colony-forming units, 25 out of 27 patient (92.6%) and 21 out of 22 control samples (95.5%) showed growth. There was no significant difference in the positive sediment cultures between the two study groups.

There were 129 microorganisms isolated in total from the two groups. The organisms, characterised at best to genus level, were reported for each positive sample following our Sediment Culture Protocol described in Chapter 3.6.6. 71 microorganisms found from the 27 patients and 58 found from the 22 control culture plates. The commonest organisms isolated in order of frequency were *Streptococcus* and *Enterococcus* -27 counts each, *Staphylococcus* -25 counts, *Corynebacterium*-15 counts and *E. Coli* -14 counts.

There were two organisms unidentifiable by the techniques used in this study and one incidence of yeast, the only non-bacterial cultured organism. Three of the culture plates grew only one organism, two from patient group and one from a control sample.

There was an increased number of *Enterococcus*, *Lactobacillus*, *Streptococcus*, *Yeast* and *Staphylococcus* in the patient group compared to the control population, which had greater incidence of *Proteus* and *Klebsiella*, *Enterobacter*, *Serratia (KES)* but similar numbers of *Coryneform* and *E. Coli*. The small sample size precluded statistical correlations of the organisms. To compare the results with the MSU data, both sediment culture study groups showed mostly mixed growth; these would not be considered positive findings in routine testing.

For colony counts, the mean CFU ml⁻¹ in patients was 77,496 (95% CI 29,806-125,185) and in control samples was 25,690 (95% CI 1183-50,197). Both means are below the standard diagnostic threshold of 10⁵ CFU ml⁻¹ (Table 4.20).

Table 4.20 Sediment Culture results in CFU/ml

Sediment Culture cfu/ml	Mean	sd	CI(Lower)	CI(Upper)
Controls	25690	55273	1183	50197
Patients	77495	120553	29806	125185

Table 4.21 Sediment culture microorganism Patient Group Crosstabulation

Microbe * Patient Group Crosstabulation

			Patient Group		Total
			Control	Patient	
Microbe	Corynebacteria	Count	7	8	15
		% within Microbe	46.7%	53.3%	100.0%
		% within Patient Group	12.1%	11.3%	11.6%
	E. Coli	Count	6	8	14
		% within Microbe	42.9%	57.1%	100.0%
		% within Patient Group	10.3%	11.3%	10.9%
	Enterococcus	Count	15	12	27
		% within Microbe	55.6%	44.4%	100.0%
		% within Patient Group	25.9%	16.9%	20.9%
	KES	Count	1	0	1
		% within Microbe	100.0%	0.0%	100.0%
		% within Patient Group	1.7%	0.0%	0.8%
	Lactobacillus	Count	4	8	12
		% within Microbe	33.3%	66.7%	100.0%
		% within Patient Group	6.9%	11.3%	9.3%
	No Growth	Count	1	0	1
		% within Microbe	100.0%	0.0%	100.0%
		% within Patient Group	1.7%	0.0%	0.8%
	Proteus	Count	1	0	1
		% within Microbe	100.0%	0.0%	100.0%
		% within Patient Group	1.7%	0.0%	0.8%
	Staphylococcus	Count	11	14	25
		% within Microbe	44.0%	56.0%	100.0%
		% within Patient Group	19.0%	19.7%	19.4%
	Streptococcus	Count	9	18	27
		% within Microbe	33.3%	66.7%	100.0%
		% within Patient Group	15.5%	25.4%	20.9%
	Unidentifiable	Count	2	0	2
		% within Microbe	100.0%	0.0%	100.0%
		% within Patient Group	3.4%	0.0%	1.6%
	Yeast	Count	1	3	4
		% within Microbe	25.0%	75.0%	100.0%
		% within Patient Group	1.7%	4.2%	3.1%

4.2.9.8 Routine and Sediment Culture

Routine MSU and Sediment cultures used the same media (CPS3 plates) for culture. Routine MSU was negative in 45 out of 49 (92%) of all samples studied and 12 Samples showed mixed growth classified as contamination. Conversely, sediment cultures were positive in 46 out of the 49 (94%) samples. Of the 3 plates with negative findings, only 1 had no growth after 24 hours incubation. The other two had defects found after the 24hour incubation period and were unsuitable for interpretation.

Comparing the positive MSU samples with their respective sediment cultures, three of the positive samples showed identical colony identification in the sediment culture with comparable levels of growth and one sample grew only two colonies (2 CFU ml⁻¹) of the isolate identified in the positive MSU (which only reports growth >10⁵ CFU ml⁻¹).

4.2.9.9 Fluorescent cytology - Clue cell counts

In the clue cell counts, prepared microscope slides showing any sign of epithelial bacterial involvement were deemed positive. In the patient group 5 slides were found to be positive (18.5%) and 10 control slides (45%). The means of total counts for each group show less difference with the patients mean count 7.8% of all cells and control samples 15% of all counted cells. There was no significant difference between the two groups in clue cell count (Table 4.22).

Table 4.22 Mean proportion of Clue Cells

Patient Group	N	Mean	SD	SEM
Controls	22	15%	26	5.6495%
Patients	27	7.8%	25	4.8654%

4.2.10 Discussion

This was a pilot study to seek evidence of UTI that may have been missed during routine care. Despite the small sample this deficiency was well described. The study of UTI in diabetes has been compromised by the serious deficiencies in the tools being used and the whole subject must be revisited through the application of better scientific methods unencumbered by assumptions and surmise.

I tried to match the two groups in this study for age but still achieved an age difference of 10-years. This may be relevant. More importantly, BMI was matched well between the groups with very comparable means.

While there were between group differences in proteinuria which is unsurprising because the patients were poorly controlled diabetics and at risk of diabetic nephropathy. Proteinuria is no marker of infection; leucocyte esterase and nitrite did not differ between groups which is consistent with the paucity of dipsticks to diagnose infection (10).

In contrast to the dipstick, microscopy of a fresh, unspun, unstained specimen of urine showed considerable between group differences in pyuria, reflecting the poor sensitivity of dipstick leucocyte esterase tests. There was no difference in the epithelial cell counts which are important indicators of innate immune activation. The absence of elevated epithelial cell counts in the presence of pyuria could be attributed to immune suppression which we should be alert to in diabetes (41). These data are no proof but mark a consideration worth further research.

As previously reported (156), the ATP measures showed wide variance, confounding any hope for a discriminating result.

The insensitivity of routine MSU was demonstrated and only 4 of the 49 participants (8%), returned a positive result, mixed growth (reported as contamination by the laboratory in 12 of the 49 (24%) when compared to the 46 positive results (94%) from the sediment cultures. The MSU data were insufficient to evaluate the comparative bacterial ecology between study groups, but the sediment culture data are more useful has depicted qualitative and qualitative similarities between the groups. This is commensurate with other studies that have shown that the normal bladder is far from sterile and differs little from patients with chronic UTI (25) and in those with LUTS (177).

The higher mean clue cell count in patients but lower frequency of positive findings suggest that these patients have a lower baseline bacterial colonisation of the urinary tract than control subjects, but a few individuals have greater uroepithelial bacterial colonisation than non-diabetic subjects.

The standout difference was seen in the symptom analysis. That the diabetic patients consistently scored higher over six of the symptom measures. It would be wholly plausible to attribute these differences to the direct effect of diabetes on lower urinary tract homeostasis, but the microscopic pyuria injects some doubt into the assumption and lights a path for future scrutiny. Whilst only two measures discriminated the two groups, symptoms and pyuria, the similarity in cultures results is no less germane. The normal bladder is again showed not to be sterile and the conventional understanding of UTI in diabetes is conditioned on a sterile normal bladder, if that is not the case then prior assumptions must be re-examined.

4.2.11 Limitations

There are limits to the validity of the data, categorised by four main areas: uncontrolled bias, uncontrolled variables, variability in protocol and limitations in protocol. By identifying and acknowledging these limitations, future studies can be better designed and will produce more valid and powerful data.

The study was underpowered so negative results should be viewed with caution and the positive findings could still be chance and require confirmation. I should have preferred to have achieved an age-match and used random sampling. I think that in future studies, I should try and develop a more eclectic approach to the finding of normal controls. Diabetics are older and LUTS are more common with age so true asymptomatic normal may be at a premium, a fact that in itself introduces some bias.

There is always the possibility, when addressing a complicated disease such as diabetes that some unconsidered confounders may be biasing the measures. All patients attending the endocrine clinic were given a questionnaire that invited participation but not all volunteered so there may have been some self-selection bias. I am concerned that this may have meant that persons with undiagnosed LUTS may have been more forthcoming.

I made a mistake in over-anonymising the recruitment questionnaire so that I could not compare the volunteers with those who did not come forward.

Chapter 5 Study of the pathophysiological signals in the urine of patients presenting with acute flares while on long-term antibiotic treatment

5.1 Introduction

Patients referred to this service had suffered LUTS for many years and had all failed management in primary, secondary and tertiary care centres with guideline driven treatments in the UK. They had suffered recalcitrant LUTS symptoms for a mean of 5 years prior to presentation to this clinic and were unresponsive to treatment under urologists and Urogynaecologists. They had all undergone Cystoscopy, Cystodistension, Urodynamic studies and Pressure Flow studies and been treated with Antimuscarinic therapy and some had undergone further interventions like Botox and Bladder instillations using Cystistat (thought to restore the GAG layer) and had obtained no relief.

These patients responded to treatment with full dose long term narrow spectrum antibiotics treatment and were monitored every 8-12 weeks with measurement of symptoms and WCC & EPC on fresh urine microscopy.

A remarkable characteristic, exhibited by chronic UTI patients whilst on antibiotic treatment, is the occurrence of acute flares. These are random acute exacerbations of their symptoms that mimic an attack of acute cystitis.

Experiential learning has taught us that these are not due to acquired resistance to the treatment, which was our original assumption. We have much data from longitudinal treatment studies (25) (26, 178). These show that the cell-associated infection of the bladder wall subsides gradually. This is associated with slow clearance of the urinary white blood cells, but the symptoms' clearance lags significantly behind the urine. Cessation studies ((179, 180), have taught us not to attempt stopping antibiotics until the urine is clear and all symptoms have gone. Despite that caution, some patients relapse rapidly while on treatment.

To date we have little understanding of this phenomenon and it is important that we take steps to start to resolve this deficiency. To do this I decided to scrutinise presenting with an acute flare using the same methods adopted to examine newly presenting patients.

5.2 Aim

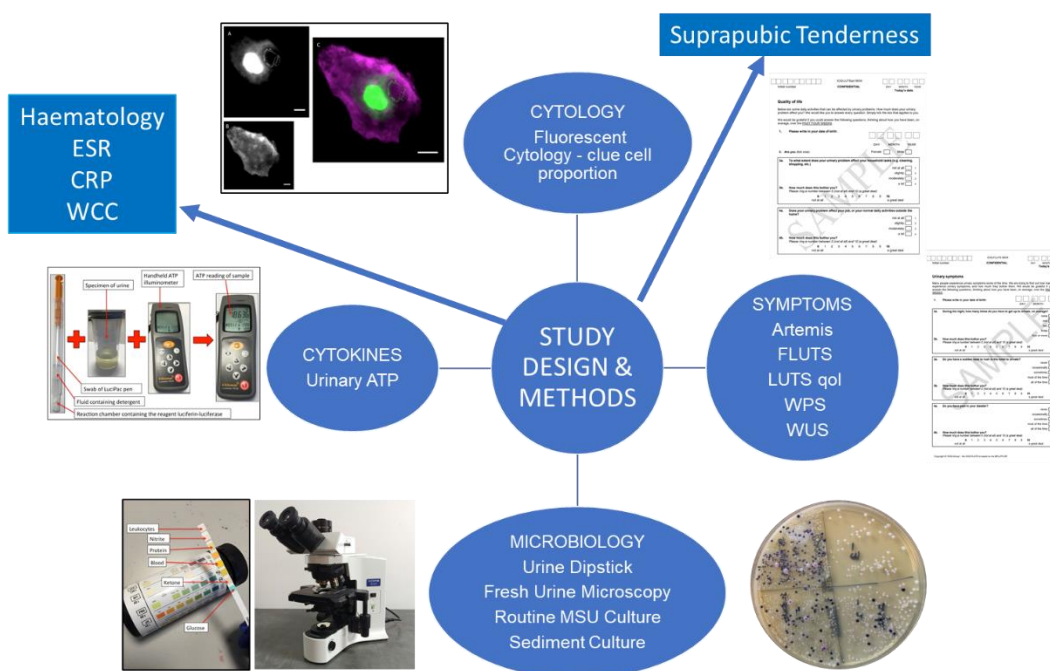
To study the pathophysiological signals in the urine of patients who present with symptom exacerbation while on antibiotic treatment.

5.3 Methods & Study Design

This study was designed to scrutinise patients who were on treatment for CUTI but who suffered acute flares which are recognised part of the response experience. Figure 5.1 describes that analyses which were deployed when a patients presented with an acute flare. This figure is similar to the one in Chapter 3, Figure 3.1, but the red arrows identify some additional components that were

used for these patients namely; examination for suprapubic tenderness and blood white cell count, ESR and CRP. Details of the Methods used have been described in Chapter 3 and under methods section (Chapter 3.5). This study has ethical approval from East London & City REC: Ref 11/LO/0109 (Appendix 14.3)

Figure 5.1 Study design and methods



5.3.1 Recruitment

Recruitment took place from January 2015 to May 2015. All patients were recruited from Community Lower Urinary Tract Service based at the Hornsey Central Neighbourhood Health Centre.

All patients included in this study had been referred to Professor Malone-Lee's unit after failed treatment at Primary, Secondary and Tertiary level centres in the UK.

Patients were approached at their routine outpatient visit or from the acute service, which sees patients for bothersome symptoms while on treatment prior to their next appointment. The inclusion and exclusion criteria is summarised in Table 5.1

Table 5.1 Summary of the inclusion and exclusion criteria for the patient group.

Inclusion Criteria	Exclusion Criteria
Adult Female \geq 18 years	All Males & Females <18 years
Current long-term full dose antibiotic treatment under Professor Malone-Lee for Chronic UTI	Ceased antibiotic therapy for any reason
Non-pregnant women	Pregnant women
Presenting with symptoms of an acute flare (pain and pyuria) while on treatment	No symptoms of both pain and pyuria with presenting with an acute flare while on treatment
Able to read and write English	Unable to read or write English

Controls for the study were volunteers that fulfilled the inclusion criteria (Table 5.2). They consisted of staff from Hornsey Central Neighbourhood Health Centre, Crouch End Health Centre and UCLH's Maternity Services. Family and friends of staff members and researchers were also recruited. Controls were screened before recruitment for LUTS symptoms, a history of recurrent UTIs and other bladder pathologies were recorded. They were excluded if on antibiotics for any reason within four weeks of recruitment. An attempt to age match the study groups was made but it was not possible to recruit older controls as they failed the inclusion criteria on more than one account.

Table 5.2 Summary of the inclusion and exclusion criteria for the control group

Inclusion Criteria	Exclusion Criteria
Adult Female \geq 18 years	Men & Females < 18 years
No LUTS symptoms	Presence of LUTS symptoms
Non-pregnant women	Pregnant woman
Not on treatment with antibiotics	On Antibiotic treatment for any reason
Pyuria \leq 10/ μ l	Pyuria > 10/ μ l
Able to read and write English	Unable to read or write English

5.3.2 Informed Consent

All participants were counselled regarding the advantages and disadvantages of the study and their questions were answered. Additionally, all participants were given a Participant Information Sheet (Appendix 14.5) detailing the study procedures and risks. After giving them adequate time to review the document, written documentation of their informed consent (Appendix 14.4) was obtained if they gave verbal consent. Once formal written consent was attained, a clean-catch MSU sample was obtained as per study and unit protocol (Appendix 14.6).

5.3.3 Data Collection

Demographic data were collected from each participant, which included their age, date of birth, ethnicity, Body Mass Index (BMI) and parity score. Parity score is defined as the number of times that she has given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the child was born

alive or was stillborn (181). Their medical history and any current treatment were also recorded on the unit's secure electronic data base (Artemis) and the study master folder. The data collated was filed as per Good Clinical Practice (GCP) guidelines in the office at Wolfson House and the consent forms were filed into the site's master file.

5.3.4 Suprapubic tenderness

I palpated the abdomen and suprapubic region to elicit tenderness and recorded the presence or absence of this sign.

5.3.5 Study Participants

A total of 21 patients and 30 controls were recruited for the study between January and May 2015. Nine controls were excluded after recruitment but before statistical analysis, for having significant microscopic pyuria ($> 10/\mu\text{l}$) and/or LUTS, leaving a control group of 21.

5.3.6 Systemic Markers of Infection

Patients of this clinic are monitored regularly with a Full Blood Count (FBC), Liver Function Test (LFT) and Renal Function tests including e-Glomerular Filtration Rate (e-GFR) as they are being treated with long-term full dose antibiotics. Consent was obtained from all patients for the additional measures of CRP and ESR on the same phlebotomy sample for the purpose of this study. A trained healthcare professional performed the phlebotomy.

The sample was sent to Whittington Hospital Haematology and Biochemistry Department for analysis and the results were uploaded onto the Whittington Anglia ICE database. All blood results were followed up within 24-48 hours. Patients and their general practitioners were informed of the results in a letter as per unit protocol.

5.4 Statistical Analysis

SPSS® Version 22.0 (IBM®, New York, USA) was used to produce descriptive and inferential statistical analysis. Medians and Interquartile Ranges (IQRs) were used to review all non-parametric data. Means and 95% Confidence Intervals (CIs) were used to review all parametric data. Inferential analysis was calculated using both parametric and non-parametric tests for the data. The independent predictor covariates and they were: pyuria (\log_{10} WBC μl^{-1}), fresh urinary UEC count (\log_{10} UEC μl^{-1}), average 24-hour urinary frequency and average 24-hour incontinence. Symptoms were scored using the custom departmental software, Artemis. The symptom scores used as independent predictor covariates as well and they were: stress incontinence, urinary urgency, voiding dysfunction and urinary tract related pain.

5.5 Results

5.5.1 Demographics

A total of 21 patients and 30 controls were recruited for the study between January 2015 and May 2015. Nine controls were excluded after recruitment but before statistical analysis, for having significant microscopic pyuria ($> 10/\mu\text{l}$) and/or LUTS, leaving a control group of 21.

As a result of persistent infections, two young patients had suffered urinary retention overdistension injury of the bladder and had a suprapubic catheter in place with six weekly catheter changes under general anaesthetic as the procedure was too painful when awake.

Three patients who had recalcitrant symptoms had undergone urinary diversion into an ileal conduit under urologists at University College London Hospital (UCLH), of whom two had their bladder and urethra left in situ and one had a partial cystectomy (removal of the accessible portion of the bladder) as she continued to experience suprapubic pain (which remained unresolved). Six patients had undergone Botox within the last six months for a small capacity bladder and residual OAB symptoms after controlling UTI and were under Botox clinic nurse specialist.

The mean age of the patients was 62 years and this was significantly greater than the controls with mean age of 41 years ($p < 0.001$) as shown in Figure 5.2 The mean BMI was 29.6 (95% CI 27.2-32.2) for patients ($n=21$) and 23.9 (95% CI 22.1-25.9) for controls ($n=21$). Mean BMI was significantly higher 29.6 in patients vs. 23.9 in patients ($p = 0.001$) (Figure 5.3). The patients' mean number of live births was not significantly greater than the controls, parity was 2 in patients vs. 1 in controls ($p=0.194$). Significantly more patients were Caucasian than controls, 81% vs. 33% ($p < 0.0001$) (Figure 5.4).

Figure 5.2 Mean ages of the patient and control groups with 95% CI

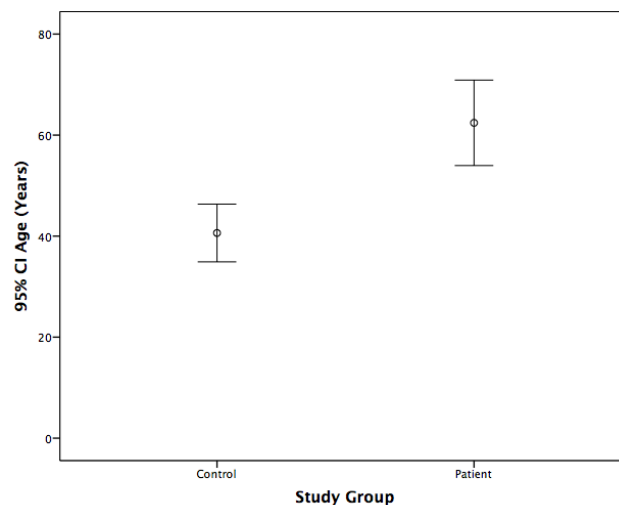


Figure 5.3 Mean BMI values of the patient and control groups with 95% CI

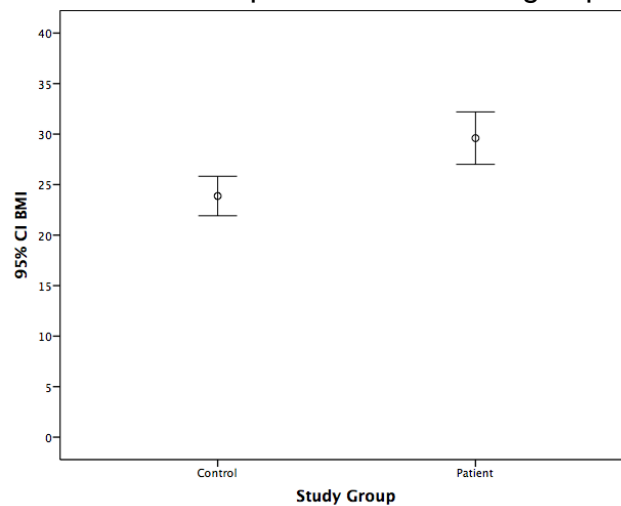
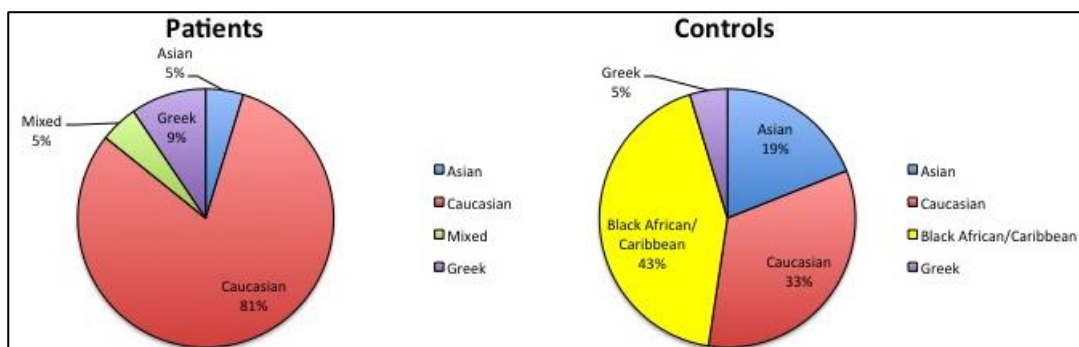


Figure 5.4 Self-reported ethnic origins of the patients with LUTS (n=21) and the healthy controls (n=21)



5.5.2 Symptoms between groups

Symptoms were assessed using the validated in-house Artemis questionnaire for assessing LUTS and four validated questionnaires (the ICIQ-FLUTS, ICIQ-LUTSqol, WUS and WPS). Total frequency was calculated by adding together the number of times the participant passed urine during the day and at night. Total incontinence was calculated by adding together the number of times the patient reported leakage during the day and at night. As one might expect, significantly more patients with LUTS had urinary symptoms than healthy controls (Table 5.3), apart from total frequency in which there was no significant difference.

Table 5.3 Artemis and questionnaire scores for Lower Urinary Tract Symptoms in patients with LUTS and healthy controls.

Symptom Measure (score range)	Mean and (95% CI)	Mean and (95% CI)	Significance (p)
	Patients with LUTS (n=21)	Healthy controls (n=21)	
Urgency (0 - 12)	4 (3-5)	1 (0-2)	0.001
Stress (0 - 7)	1 (0-1)	0 (0)*	= 0.04
Voiding (0 - 8)	2 (1-3)	1 (0-1)	= 0.02
Pain (0 - 13)	4 (3-6)	1 (0)**	< 0.001
ICIQ-FLUTS Symptom Score (0 - 69)	25 (18-33)	4 (2-6)	< 0.001
ICIQ-FLUTS Bother Score (0 - 150)	74 (55-93)	11 (3-18)	< 0.001
ICIQ-LUTSqol Symptom Score (16 - 76)	52 (45-59)	21 (19-24)	< 0.001
ICIQ-LUTSqol Bother Score (0 - 200)	122 (102-147)	6 (1-11)	< 0.001
WUS (0 - 20)	8 (5-11)^	1 (0-3)	< 0.001
WPS (0 - 8)	5 (4-6)	0 (0)	< 0.001
Total Frequency (1 - >20)	10 (8-12)	8 (7-10)	= 0.079
Total Incontinence (0 - >5D & >5N)	2 (1-3)	0 (0)	= 0.002

Data are mean and 95% CI

* n=3; ** n=4; ^ n=17

Table 5.3 also depicts the parametric and non-parametric tests carried out to examine the Artemis variables. Parametric data for ICIQ-FLUTS, ICIQ-LUTSqol, WUS, WPS, total frequency and total incontinence are individually reported

5.5.3 Suprapubic tenderness

Seven (33%) of the 21 patients experienced suprapubic tenderness when their lower abdomen was palpated. The controls did not show suprapubic tenderness.

Suprapubic tenderness elicited in seven patients of which four had obvious site abnormalities as follows:

- Two had recently had a suprapubic catheter changed and had developed wound site infection
- One patient continued to experience bladder pain despite urinary diversion into an ileal conduit. And therefore, had undergone partial cystectomy and urethra was still in place
- One patient had undergone a urinary diversion into an ileal conduit alone and she had on-going bladder symptoms and tenderness

5.5.4 Microbiology

5.5.4.1 Urinary Dipstick Leukocyte esterase test

Patients were required to have pyuria as one of the inclusion criteria so not surprisingly Leukocyte Esterase test was positive in 17 (81%) patients and three (14.3%) controls (Table 5.4).

Table 5.4 Urinary Leukocyte Esterase Test in patients with LUTS (n=21) and healthy controls (n=21)

Dipstick Leucocytes	Negative	Trace	1+	2+	3+
Controls	16	2	1	0	0
Patients	4	1	6	4	6

5.5.4.2 Urinary Dipstick Nitrite Test

The Nitrite Test was positive in four (19%) patients and none of the controls (Table 5.5).

Table 5.5 Urinary Nitrite Test Result in patients with LUTS (n=21) and healthy controls (n= 21).

Dipstick Nitrite	Negative	Positive
Controls	21	0
Patients	17	4

5.5.4.3 Urinary Dipstick Protein

The Protein Test was negative in 76% of controls and showed a trace result in 24%. In comparison it gave a negative result in 52% of patients and results of 1+ and 2+ in 38% and 10% respectively (Table 5.6).

Table 5.6 Urinary Protein Test Result in patients with LUTS (n=21) and healthy controls (n=21)

Dipstick Protein	Negative	Trace	1+	2+
Controls	11	0	8	2
Patients	16	5	8	0

5.5.4.4 Urinary Dipstick Blood

The blood test on the dipstick was negative in 71% of controls and 24% of patients. In both 24% of controls and 24% of patients the result indicated a non-haemolysed or haemolysed trace result. Overall, 52% of patients had 1+ more of blood compared to 5% of controls (Table 5.7).

Table 5.7 Blood Urinary Dipstick Result in patients with LUTS (n=21) and healthy controls (n=21).

Dipstick Blood	Negative	Non Haemolysed Blood	Haemolysed Trace	1+	2+	3+
Controls	15	2	3	0	1	0
Patients	5	4	1	6	1	4

5.5.4.5 Urinary Dipstick - Ketones and Glucose

The Ketone test was negative in all of the controls and in all of the patients apart from one. Similarly, the Glucose test was negative in all of the controls and in all of the patients apart from two.

5.5.4.6 Fresh Unspun Urine Microscopy

Pyuria signal of $>10/\mu\text{l}$ cells are used as a criterion to differentiate between patients and controls (Table 5.8).

Table 5.8 Results of fresh unspun urine microscopy in patients with LUTS and healthy controls.

Urine element (count)	Patients with LUTS (n=21)	Healthy controls (n=30)	Significance (p)
White Blood Cells*	210 (99-449)	4 (2-6)	< 0.001
Uroepithelial Cells*	41 (19-88)	5 (3-7)	$= 0.004$
Red Blood Cells*	27 (11-66)	3 (1-8)	$= 0.015$

Data are mean and 95% CI

5.5.4.7 Routine MSU Culture Report Deleted Figure and new table

MSU results were available for 20/ 21 patients. In 57% (12/21) there was no significant growth on MSU culture, 10% produced a mixed growth of doubtful significance and 29% (6/21) showed growth of 1 organism of at least 10^5 cfu/ml and one sample went missing (Table 5.9). Two healthy controls grew $\geq 10^5$ cfu/ml of *E. coli*.

Table 5.9 Routine MSU Culture in patients with LUTS (n=20) and healthy controls (n=21)

Routine MSU Culture	No significant Growth	Mixed Growth	Positive Culture
Controls	15	4	2
Patients	12	2	6

5.5.4.8 Cell Sediment Culture

All patients and 20 controls produced a positive sediment culture result of at least one colony. Following data log transformation for analysis, the mean (95% CI) total number of colony forming units (cfu) in the patients was significantly higher than in the controls, 34,198 (12589-92683) * vs. 780 (121-5035) *, $p < 0.001$ (Table 5.10).

Table 5.10 The mean Log CFU/ml values in the patients (n=21) and the controls (n=21)

Mean CFU/ml	Mean	95% CI
Patients (21)	34,198	12589-92683
Controls (21)	780	121-5035

5.5.4.8.1 Characterisation of the microbiome between patients and controls

The total number of colonies recorded was 61 in patients and 74 in controls (Table 5.11). There were a number of differences in the types of bacteria observed (Table 5.11). The differences in the microbial diversities of the two study groups were found to be significant using Pearson's chi-squared test; $\chi^2(11, N = 136) = 28.89, p = 0.002$.

Table 5.11 The total count of each bacterial genus identified in both study groups – Isolates were collected from unselective sediment cultures.

Organism	Study Group		Total
	Control	Patient	
<i>Citrobacter</i>	0	1	1
<i>Corynebacterium</i>	14	1	15
<i>E. coli</i>	7	6	13
<i>Enterococcus</i>	10	8	18
<i>KES (Klebsiella, Serratia and Enterococcus species)</i>	1	4	5
<i>Lactobacillus</i>	2	5	7
<i>Proteus</i>	0	2	2
<i>Pseudomonas</i>	0	6	6
<i>Staphylococcus</i>	26	16	42
<i>Streptococcus</i>	13	8	21
<i>Yeast</i>	1	4	5
Total number of colonies	74	61	136

5.5.5 Benchtop ATP

Following data log transformation for analysis, the mean (95% CI) ATP was higher in the patients than in the controls (Table 5.12). Mean ATP was 6237 (3236-12022) for patients vs. 2858 (2104-3873) for controls, $p=0.028$. The mean was converted to a log₁₀ scale to fit the graphs as shown in the figure below.

Table 5.12 The mean ATP values in the patients (n=21) and the controls (n=21)

ATP	Mean	95% CI
Controls	2858	2104 -3873
Patients	6234	3236 - 12022

5.5.6 Cytology – Clue cells

All samples were counted twice and an average obtained. For three patients and two controls, no UECs were found in their samples under epifluorescence microscopy.

The mean proportion of clue cells counted was 87% (95% CI 79.14-94.15) in controls and 72% (95% CI 57.90-96.00) in patients. This difference was not significant (Mann-Whitney U: 112.000, asymptomatic significance (2-tailed): 0.071).

5.5.6.1 Intra-observer variability

A paired samples t-test was used to compare the first and second clue cell counts.

There was not a significant difference in the scores for the first clue cell count (M=79.12, SD=23.14) and the second clue cell count (M=81.24, SD=22.63) conditions; $t(36) = -1.12, p = 0.271$.

5.5.6.2 Inter-observer variability

A second investigator counted 16 samples. A paired-samples t-test was used to compare the average of the two-clue cell counts with the second investigator's counts. There was not a significant difference in the scores for the average clue cell count (M=77.02, SD=23.44) and the second investigator's clue cell count (M=78.55, SD=25.19) conditions; $t(16) = -0.609, p = 0.551$.

5.5.7 Systemic Markers of Infection

Four (20%) of patients had raised CRP, five (25%) had raised ESR and three (14%) had a raised total WCC (Figure 5.13)**Error! Reference source not found..**

Table 5.13 Blood tests ESR, CRP and WCC in Patients presenting with acute flare

Study No. of Patients	CRP mg/L (0-5) *	ESR mm in 1 hr (1-30) *	WCC ($\times 10^9/L$) (3.5-12) *
001**	4	8	7
006	Missing	Missing	5.2
019	5	8	16.7
021**	1	15	3.9
025	6	22	90.5
028	6	36	7.6
029	2	17	7.5
035**	28	1	6
037	3	14	6.7
038	4	10	7.4
039**	4	16	6.4
041	3	29	8.3
042	146	46	11.4
043**	5	11	5.7
044	2	14	7.1
045	1	33	4
046**	9	37	6.8
047	4	15	6.6
049	2	38	10
050	18	44	16.3
051**	3	14	5.6

*Laboratory reference range.

** Seven patients who had Suprapubic tenderness

The results which lie outside of the reference ranges, are highlighted in grey.

5.6 Discussion

This prospective observational study aimed to examine the pathophysiological signals in the urine of a group of patients diagnosed with chronic UTIs and LUTS but when they were experiencing acute symptoms while on antibiotic treatment. Thus, they formed an enriched sample of more severe disease and I wished to use them to scrutinise the performance of the alternative measures of pathophysiology that have been adopted in this study. I was also interested the role of suprapubic tenderness as a pathognomonic sign which is not well studied in this group and in examining the systemic markers of inflammation; blood white cell counts, ESR and CRP which are common markers of inflammatory activity tested in routine clinical practice (Table 5.13).

This study sample chose patients with high pyuria however we know that some patients on therapy also had symptoms of flare despite no or low pyuria and I was unable to recruit the latter group during my time at the unit.. Because these patients were recruited when they were experiencing acute flares while on long-term full dose antibiotic therapy, the small sample size including only high pyuria at presentation, the results of this pilot should be interpreted with caution

Pyuria count on fresh urine microscopy is used to monitor these patients while on treatment. Significantly more cellular elements were observed in the urine on microscopy in the patients than controls.

Nitrites were positive in only 4 of 21 patients and none of the Controls and these data is similar to that quoted in literature (10). In routine gynaecology and Urogynaecology practice a symptomatic patient would receive empirical treatment if she meets one of the following two conditions (182):

- >1+ leucocytes with a positive nitrite test
- >2+ leucocytes with a negative nitrite test

Only 40% of patients had a Leucocyte esterase >2+ and would not have received treatment unless the Nitrite test was positive.

Only 11 (52%) of the 21 patients in this study would have received antibiotics had the guidelines been followed. 10 patients would not have received antibiotics despite presenting with symptoms and pyuria.

The differences in the microbial diversities of the two study groups were found to be significant using Pearson's chi-squared test: $\chi^2(11, N = 136) = 28.89, p = 0.002$. *Staphylococcus* was found to be the most dominant organism in both patients (26%) and controls (35%). Known uropathogens, *Pseudomonas*, *Proteus* and *Citrobacter* were present in patients (10%, 3% and 2% respectively) but not in controls. *Corynebacterium* was commoner in controls than patients as one would expect.

Two healthy controls grew $\geq 10^5$ cfu/ml of *E. coli*; neither was symptomatic (this is described as asymptomatic bacteriuria) (183),(184).

The outcome data showed similar differences to those found in the main study cohort which have been analysed in Chapter 3. Thus, I am in little doubt that there was a significant infection extant in the patients when they were studied. It is reassuring to see the findings of the previous analysis corroborated.

Suprapubic tenderness was elicited in seven patients of which four had obvious site abnormalities as follows:

- Two had recently had a suprapubic catheter changed and had developed wound site infection*
- One patient continued to experience bladder pain despite urinary diversion into an ileal conduit. And therefore, had undergone partial cystectomy and urethra was still in place
- One patient had undergone a urinary diversion into an ileal conduit alone and she had on-going bladder symptoms and tenderness**

* Catheters are changed every 6-8 weeks for patients to reduce the chance of wound site and urinary tract infection developing and also to stop crust formation. These are usually done in the Outpatient Department using aseptic technique, but both these patients had wound infection and UTIs and find the changes very painful so have a general anaesthetic every six weeks for this to take place. Both these patients were currently 2-3 weeks post catheter change and were noted to have redness around insertion site and a discharge and these are most likely post procedure infections.

**This type of neuropathic pain is seen in these patients even after a total cystectomy hence a cystectomy is not carried out as a part of the urinary diversion procedure for recurrent UTI as a routine.

Thus, in only three patients was suprapubic tenderness of possible diagnostic consequence. This contradicts a widely held view that cystitis should be associated with this sign. I think that these data are limited and that a further prospective study of suprapubic tenderness in different clinical situations should be pursued.

CRP, ESR and WCC were not raised in the majority of patients. This is an important observation because we find, when obtaining a patient's clinical history, that they have been reassured that they have no disease because the ESR and CRP are not elevated. The literature cast significant doubt on this assumption and this experiment has justified those doubts (185). These data are definitive but sufficient to motivate a more detailed prospective study to ascertain the true role of these markers, including system white cell counts, in the assessment of UTI.

The mean epithelial cell counts were higher in patients than controls. However, on studying the mean proportion of clue cells on epifluorescent microscopy, I have found in this cohort that patients on treatment had lower counts than controls which is different to my observation in chapters 3 & 4. A random sample of 1/3rd of the slides (n=16) was counted by an independent observer (a fellow student) due to the unusual finding and there was no difference in the counts observed by the two observers. This was done mainly out of due diligence and the unusual findings. I am unable to explain this finding other than question if this

is the effect of long-term oral antibiotics reducing the number of bacteria adherent to cells. This has not been observed before and it may be interesting to pursue this further.

For the broader purpose of this study, it proved unnecessary to resort to an enriched sample to check on the pathophysiology because the main cohort were so clear in what they manifest and in so doing demonstrated marked similarities to the acute symptomatic flares. There are grounds for conducting further studies on acute flares but to achieve a useful outcome we must first establish a sensitive method for plotting the changes in the bladder microbiome and that task has yet to be accomplished.

5.7 Limitations of this study

The control subjects were approximately 20 years younger than the patients. Significant attempts were made to age-match the study groups. However, although several older women were recruited, they were later excluded because they had significant LUTS symptoms and pyuria. This difficulty in age-matching patients is a common challenge in observational studies carried out by our centre. It is not surprising as LUTS prevalence increases with age (2, 81).

The patients and control were mismatched in relation to body habitus; the majority of the patients were overweight or obese where the majority of the healthy controls were of normal body weight. While intuitively it might be thought that being overweight may be a risk factor for urinary tract infection this does appear to be the case (186) although the study group is really small so we must interpret this with caution. The group was also significantly mismatched in relation to ethnicity; Caucasians made up 85% of the patients but only 35% of the controls. However as LUTS has a world-wide prevalence is unlikely to have significantly affected the results (187). However, given that the demographics of the area and the limited time and small numbers in this pilot, it would be best if we aim for a larger study with an ethnicity matched recruitment for future studies.

Rigorous exclusion criteria were applied for selection of the controls, which meant that nine subjects were not included in the analysis primarily because they had significant microscopic pyuria or LUTS. LUTS is estimated as prevalent “sometimes” and “often” in 76.3% and 52.5% respectively in women in the general population (81). Most of the controls who were excluded thought their symptoms were normal or part of ageing.

Variability in the applied protocol for urine processing is also as a potential source of bias in this study. Recruiting from multiple sites meant that samples were stored for differing times before cell sediment culture and WGA/DAPI slide preparation. Cell loss during storage (even under refrigeration) has been reported to be a significant limitation of the pyuria-dependent diagnostic tools used in clinical practice to detect UTIs (33). Future studies could attempt to further standardise the protocol to reduce this limitation.

Finally, bacterial characterisation was only carried out to genus level this it was not possible to determine whether, for example *Staphylococcus*, the predominant genus in both groups was the same species or whether it differed in the patients. Recent data made available through various projects pertaining to bladder biome mapping and 16s DNA sequencing studies have shown that cultures are unable

to differentiate between patients and controls and show a polymicrobial abundance and that the normal bladder biome comprises of over 400 microorganisms and a LUTS patients bladder contains over 500 microorganisms (26, 110, 188). The pathogens thus isolated may only be what is easily grown on the selective media used in our study and there are many that were unidentified through our selective cultures. Given what we know it is also difficult to predict causality as a microbes affinity to a selective growth medium does not prove pathogenicity or disease causation.

Chapter 6 Recalcitrant chronic bladder pain and recurrent cystitis but negative urinalysis – what should we do?

6.1 Introduction

There is growing awareness that UTI, undetected by routine diagnostics, might play a causal role in the generation of LUTS (19, 181). This proposal is controversial because the exclusion of UTI is a key step in every guideline concerned with the management of LUTS (189). Lower urinary tract symptoms, as a result, have become synonymous with non-infective disease.

However, there is increasing evidence that patients presenting with LUTS may harbour a UTI despite negative tests. The problem lies with the diagnostic criteria used in culture-based diagnosis. A significant body of published literature points to the inherent flaws of quantitative urinary microbiological analysis, in which thresholds between 10^3 cfu ml⁻¹ and 10^6 cfu ml⁻¹ of a pure growth of a single urinary pathogen are employed to diagnose UTI (24, 190).

Dipstick urinalysis performs equally poorly, hampered by insensitivity and spectrum bias (41, 191). These tests cannot reliably exclude UTI and do not take into account differences in bacterial strain virulence, host genetic variability, intracellular bacterial reservoirs, or even urine dilution due to high fluid intake before the test.

Pyuria, detected by microscopy of a fresh MSU specimen, is the most sensitive surrogate marker of UTI (41). It circumvents the problems associated with quantitative bacterial culture and its value in the diagnosis of UTI is recognised by international practice guidelines (192). Whilst ≥ 10 wbc μl^{-1} is employed almost universally to diagnose UTI, contemporary data have cast doubt on this threshold in patients with LUTS (41, 88). In the symptomatic patient, controlled studies have demonstrated that lower pyuria counts of 1-9 wbc μl^{-1} are associated with an increase in independent inflammatory and microbiological markers of UTI (41, 147).

Thus, lower levels of pyuria may also indicate infection and immune activation. The diagnostic picture has become even more complex with the recent discovery that UTI can legitimately involve polymicrobial infection; mixed growth cultures do not necessarily reflect contamination (190, 193). What's more, advances in enhanced culture and genomics technology have revealed that even the normal bladder is not sterile, and that a bona fide, possibly protective urinary microbiota can be described (190, 193).

Uncertain diagnosis is not the only area that complicates UTI management. The treatment of acute cystitis also has its limitations. A Cochrane review has reported symptomatic and microbiological failure rates of 37% and 28%, 4-10 weeks after treatment (194). Among healthy young women who suffer from their first UTI, the risk of recurrence within 6 months is 24%. If they have a history of one or more UTIs, the risk of recurrence rises to 70% in that same year (195). In a Canadian surveillance study, 14% of the 30,851 residents with UTI suffered more than one episode during the two-year study period and 2% had six or more episodes (196).

Taken together, these findings are of real concern, and whilst guidance on the diagnosis and management of UTI is increasingly prescriptive, one size does not necessarily fit all. We hypothesise that chronic LUTS may result from urinary infection falling below the routine culture threshold, and that therefore antibiotic treatment could confer benefit. Indeed, Stamm et al (1981) published an RCT reporting success from a 10-day regimen of doxycycline for culture-negative patients with acute frequency and dysuria who had microscopic pyuria (using ≥ 8 wbc μl^{-1} as their threshold) (197). Further studies are needed to support these findings.

In 2004, we started to use antibiotics to treat patients with chronic LUTS who demonstrated microscopic pyuria, despite negative dipstick testing and negative MSU cultures employing a 10^5 cfu ml^{-1} threshold. Using sensitive microbiological methods, we have since generated data demonstrating widespread bacterial infection in these patients (19). Other groups have also reported similar findings LUTS (24). Our clinical approach sought to manage patients towards symptom resolution and clearance of pyuria by combining antibiotic treatment with careful outpatient monitoring. This paper describes the evolution of our clinical practice as a result of these observations.

6.2 Methods

These data represent a large case series of women treated in a single tertiary centre from 2004 to 2014. All women with chronic LUTS and pyuria ≥ 1 wbc μl^{-1} who agreed to antibiotic therapy provided symptom and urinalysis data. This group included patients with existing diagnoses of OAB, BPS/IC, voiding symptoms, and rUTI. Stress urinary incontinence (SUI) was a common non-dominant symptom amongst patients but was not a therapeutic target for antibiotic treatment.

All patients presenting to the service were subject to a standardised clinical assessment. Demographic data, medical comorbidities, and concurrent medication were recorded, together with details of previous treatments. All clinical data were recorded in our electronic patient database.

Lower urinary tract symptoms were characterised using a 39-question inventory that was developed and validated at this centre (11) (Table 6.1). The urinary urgency and lower urinary tract pain subscales of this instrument have been validated as independent measures (78, 156). Microscopic pyuria was enumerated from a freshly collected clean-catch MSU specimen as described in the methods section of Chapter 3.6.4. An MSU was submitted for routine culture employing a $\geq 10^5$ cfu ml^{-1} diagnostic threshold as enumerated in Chapter 3.6.5. The biomedical scientists at Whittington Hospital carried out the Antibiotic sensitivity testing was conducted on all bacterial isolates from positive cultures as per standard NHS laboratory protocol using the disc diffusion method in accordance with EUCAST guidelines (198).

Antibiotic therapy was not contingent on positive dipstick urinalysis or routine MSU cultures. As previously described, these tests are insufficiently sensitive to exclude UTI and were not used to arbitrate treatment decisions. The results of MSU culture were used to monitor bacterial resistance rates only.

Table 6.1 Artemis questionnaire (39 question inventory)

Urinary urgency (11)	Voiding symptoms (7)
1. Urgency	20. Hesitancy
2. Urgency incontinence	21. Reduced stream
3. Latchkey urgency	22. Intermittent stream
4. Latchkey urgency incontinence	23. Straining to void
5. Waking urgency	24. Terminal dribbling
6. Waking urgency incontinence	25. Post micturition dribbling
7. Running water urgency	26. Double voiding
8. Running water urgency incontinence	Pain symptoms (13)
9. Cold urgency	27. Suprapubic pain
10. Anxiety urgency	28. Filling bladder pain
11. Premenstrual aggravation	29. Voiding bladder pain
Stress urinary incontinence (8)	30. Post-void bladder pain
12. Cough sneeze incontinence	31. Pain relieved by voiding
13. Exercise incontinence	32. Pain partially relieved by voiding
14. Laughing incontinence	33. Pain unchanged by voiding
15. Passive Incontinence	34. Loin pain
16. Bending incontinence	35. Iliac fossa pain
17. Standing incontinence	36. Pain radiating to genitals
18. Lifting incontinence	37. Pain radiating to legs
19. Pre-cough preparation	38. Dysuria
	39. Urethral pain

Written information was provided explaining the justification for treatment, expected clinical outcomes, and potential adverse effects. Patients understood that they were being treated outside of standard guidelines. The clinical lead for the unit took sole responsibility for all off-label prescribing.

The assessments conducted at presentation were repeated at every follow-up visit. Duration of follow-up was variable, as treatment response dictated the number of review visits. All data, up to the point of patient discharge, was included in the analysis. The Patient Global Impression of Improvement (PGI-I) scale was used to measure treatment response (199, 200). Adverse events were recorded

at every patient interaction. These were graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.3 (201).

The clinical management of these patients evolved during the study period. Treatment was initiated using first-line urinary antibiotics including nitrofurantoin, trimethoprim and cefalexin. Patients were reviewed for response at 14 days, or earlier if adverse effects were encountered. Treatment modifications were tailored to efficacy and tolerance (106). The urinary antiseptic agent methenamine hippurate was used as an adjunct and some patients required combination antibiotic therapy. Wherever possible, we tried to forge our observations into useful amendments of these personalised regimens.

An effective, tolerated treatment was continued until symptoms were ameliorated and the inflammatory response in the urine had resolved which was measured by resolution of pyuria on fresh urine microscopy. The treatment was then discontinued. If the symptoms recurred, treatment was reinstated. This cycle was repeated until there was no symptomatic deterioration after antibiotic withdrawal for 12 weeks. At this point, patients were asked to initiate short-course, self-start antibiotic treatment, at the first sign of any recurrence of symptoms, identical to the self-start regimens recommended for rUTI. Symptom-based antimicrobial treatment, introduced early in the infection cycle, was advocated to prevent chronic symptom recurrence (40).

6.3 Statistical analysis

All statistical analyses were conducted using IBM SPSS 22 (IBM, New York). Changes in outcome variables were analysed using a mixed model, linear regression analysis within a repeated measures design.

The time interval from first attendance at each clinic visit varied between patients and the intervals could vary within the patients. To address this, the effect of the number of days from first attendance was controlled in all models involving multiple attendances. Time from first visit was entered as a covariate, accounting for patient-specific random effects. The data were indexed by patient number and visit number using a main effects model and random intercept.

Ordinal regression was employed to analyse PGI-I responses recorded on the last visit only. Comparison of non-parametric data used the Kruskal-Wallis or Mann-Whitney U tests.

6.4 Ethical approval

Validated symptom and biomarker data were collected in accordance with a protocol approved by the East Central London Regional Ethics Committee (REC1) (Ref: 11/H0721/7) (Appendix 3).

6.5 Results

6.5.1 Patients

A total of 1996 women presented to the clinical service between 2004 and 2014. There were 433 women who attended just once for urodynamic studies or urinalysis, and these patients were neither treated in the centre nor followed up. A further 444 women were treated for OAB and did not demonstrate pyuria, or presented with SUI as their only symptom. These women were not treated with antibiotics. After these exclusions, 624 women (mean age=53.4 years; SD=18) who demonstrated pyuria ≥ 1 wbc μl^{-1} at presentation were included in the analysis.

Patients described longstanding LUTS prior to their referral to this service (mean duration=6.5 years; SD=6.3). Many of the 624 women had established diagnoses of OAB or BPS/IC from elsewhere. Urinary urgency symptoms were described by 73% of women, whilst voiding symptoms and lower urinary tract pain affected 71% and 65%, respectively. Forty-three per cent of women described SUI. Patient demographics and symptoms are summarised in Table 6.2.

Table 6.2 Patient demographics and summarised symptom data collected at the first attendance.

Demographics	Median	Mean	Std. Deviation	Std. Error of Mean	95% CI
Age (years)	54.0	53.4	18	0.7	52 - 54.8
Symptom duration (years)	4.0	6.4	6.3	0.3	5.9-7.0
24-hour urinary frequency	10	10.8	5.2	0.2	10.4-11.2
24-hour incontinence episodes	0.4	1.2	2.0	0.1	1.1-1.4
Total symptom score (0-39)	11.0	11.1	6.3	0.3	10.6-11.6
Urgency subscale score (0-11)	4.0	3.8	3.2	0.1	3.5-4.0
Pain subscale score (0-13)	3.0	3.2	3.2	0.1	3.0-3.5
Voiding subscale score (0-7)	3.0	2.9	2.4	0.1	2.7-3.1
SUI subscale score (0-8)	0.0	1.2	1.9	0.1	1.0-1.3

6.5.2 Dipsticks and urine culture

We performed 1988 dipstick analyses: 558 (28%) demonstrated leucocyte esterase \geq 'trace' and 138 (7%) were nitrite positive. However, 1433 (72%) of these samples showed pyuria on direct microscopy. Of the 2209 MSU cultures that were performed during observation, only 362 (16%) were positive using the threshold of $\geq 10^5$ cfu ml⁻¹ although microscopic pyuria was recorded in 1741 (79%) of these samples.

6.5.3 Antibiotic use

Our prescribing practice evolved over the course of the observation period as we scrutinised our treatment-response data (Appendix 13). This led to the treatment regimens being simplified and refined as the data were collected.

In 2014 when data collection ceased, 80% of patients were being treated with 12 antibacterial regimens. Six of these consisted of methenamine hippurate combined with one antibiotic, most commonly a first-generation antimicrobial such as cefalexin, nitrofurantoin or trimethoprim. Full-dose treatment was administered.

We identified a cluster of patients with marked urethral pain and low-level pyuria whose symptoms preferentially responded to a macrolide or tetracycline, perhaps suggestive of a fastidious microorganism.

6.5.4 Treatment duration and efficacy

We tested the need for ongoing treatment empirically by stopping antimicrobial therapy. Treatment cessation was permitted once any reduction in LUTS had reached a steady state and pyuria had cleared. If symptoms recurred, the occurrence was documented, and treatment was reinstated. Thus, we stopped treatment 858 times and restarted 633 (74%) times on recurrence.

Amongst patients with pain symptoms, relapses were associated with significantly higher pain scores (mean=4.2; 95% CI = 3.6-4.9) compared with their symptoms at the beginning of treatment (mean=2.7; 95% CI=2.2-3.2) ($p=.001$).

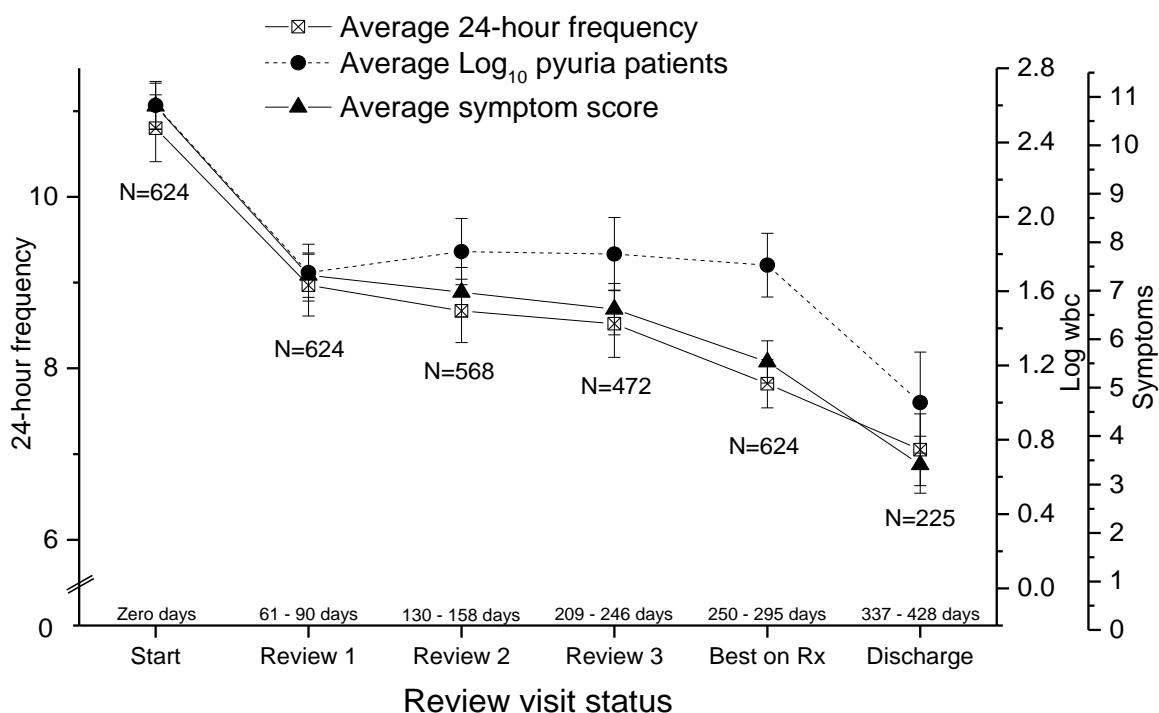
Two hundred and twenty-five women completed treatment and were discharged. The median number of patient visits was five (mean=6.6; SD=5) with 40% of women discharged after four visits and 80% discharged within ten. Mean treatment length was 383 days with a significant variation in duration (SD= 347; 95% CI=337- 428).

Some patients required long-term therapy, as attempts to withdraw treatment were associated with relapse. Others were treated successfully but requested long-term monitoring due to anxieties about disease recurrence. The data were recorded in the electronic record.

Figure 6.1 shows a plot of 24-hour urinary frequency, total LUTS, and pyuria, at time points including: (A) baseline, (B) three subsequent review visits, (C) their optimum state on treatment, and (D) at discharge. There was a significant reduction in 24-hour urinary frequency ($F=75$; $p=.0001$), total LUTS ($F=98$;

p=.0001), and \log_{10} pyuria (F = 15.4; p=.0001). Incontinence episodes did not change significantly (F=1.8; p=.18). Optimum treatment-associated effects amongst patients who were not discharged are represented by data at time point (C).

Figure 6.1 Change in urinary frequency, \log_{10} pyuria and total LUTS, plotted against visit status (mean and 95% CI). The “best on treatment” is the patient’s best symptom score whilst taking medication.



Separate analyses demonstrating the change in individual symptom subscales associated with treatment are presented in Figure 6.2. Antibiotic treatment was associated with a significant reduction in urinary urgency (F 90; p=.0001), lower urinary tract pain (F=108; p=.0001), and voiding symptoms (F=10; p=.002). Symptoms of SUI were unchanged during treatment (F=1.4; p=.24) (Table 6.3).

Figure 6.2 Change in individual LUTS subscales plotted against visit status.

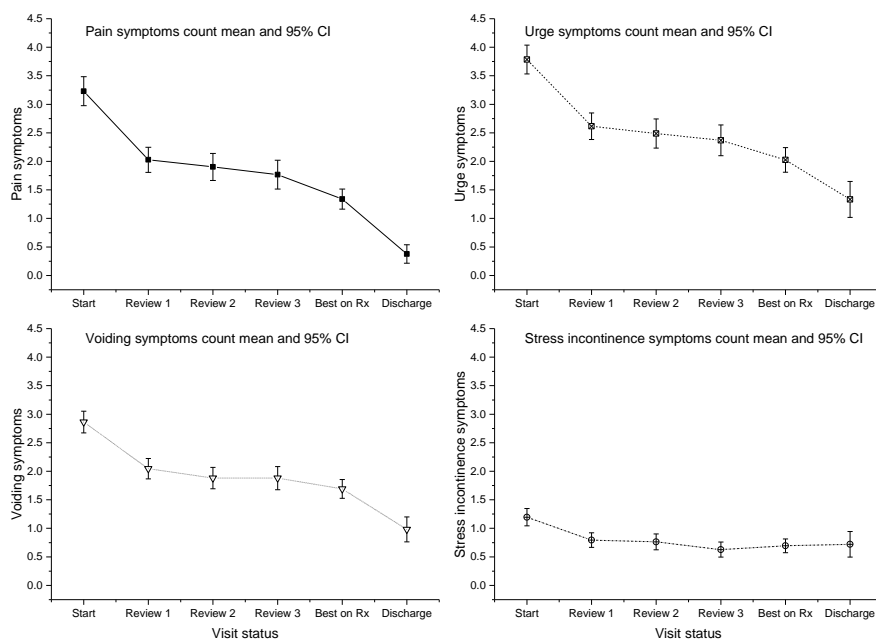


Table 6.3 Changes in LUTS associated with treatment.

Mixed models, linear regression analysis within a repeated measures design.			
Dependent variable – Outcome measure	F Statistic	Fisher's p	Figure
Log ₁₀ wbc μl ⁻¹	15.4	.0001	4
24-hour urinary frequency	75.0	.0001	4
24-hour incontinence episodes	1.8	.18	Not plotted
Total LUTS symptom score	98.0	.0001	4
Urgency subscale score	90.0	.0001	5
Pain subscale score	108.6	.0001	5
Voiding subscale score	10.1	.002	5
SUI subscale score	1.4	.24	5

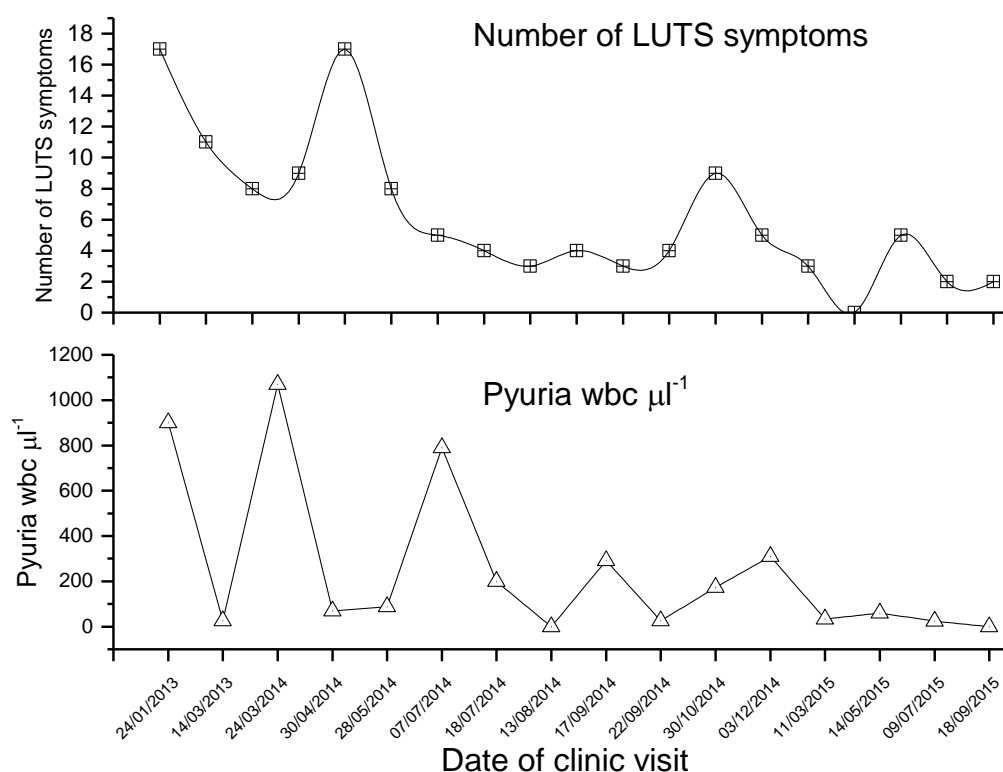
Independent variable = Time from first visit in days; Repeated measures = Visit number.

The PGI-I responses demonstrated a significant improvement over the treatment period ($X^2= 2272$; $df=5$; $p=.001$). Eighty-four per cent of women rated their condition as 'much better' (20%) or 'very much better' (64%).

These data demonstrate that long-term antibiotic treatment is associated with resolution of chronic LUTS and pyuria, which is an independent surrogate marker of infection. These findings were accompanied by PGI-I responses indicating that the vast majority of patients perceived these symptomatic improvements to be clinically meaningful.

Plots of the pyuria and symptoms in individual patients for each visit showed a typical response pattern in 73% of cases, termed a “damped oscillation” (Figure 6.3). It plots against time the urinary pyuria (white blood cells or pus cells) and the symptoms. The patient aged, 42, had suffered undiagnosed chronic urinary infection for two years. She was treated by us with antibiotics over three years (2013 to 2015). The acute flares of decreasing amplitude are well shown. Despite the lower peaks, the symptoms tended to be more severe during the later flares. The intensity of symptoms often prove misleading, being more severe when the inflammation is less. Thus they did not necessarily imply treatment failure. This patient required 16 visits to the clinic over three years. Only 27% of our patients would take this long; 73% require much less (Figure 6.3). This features a series of oscillations of decreasing amplitude. Ten per cent of patients demonstrated the converse, and this was associated with symptomatic deterioration. Ten per cent showed a rapid, uninterrupted fall to baseline (“critically damped oscillation”), and 7% were unclassifiable.

Figure 6.3 Damped oscillations of decreasing amplitude of one patient



The symptoms and pyuria taken from one illustrative patient showing the associated damped oscillation that occurred in 73% of women during treatment.

Baseline variables including age, duration of symptoms, urinary frequency and incontinence, and pyuria did not predict treatment outcome. Higher pain scores at presentation were predictive of greater maximal symptom palliation associated with antibiotic therapy ($b=0.57$, $p=.029$). Higher scores for urinary urgency ($b=1.1$, $p=.001$) and voiding symptoms ($b=1.0$, $p = .005$) predicted less favourable responses, although the magnitude of these effects were very small.

6.5.5 Adverse events

Two hundred and sixty-six patients reported 475 AEs during 273,762 treatment days. All but one of these AEs were classified as mild or moderate (CTCAE grades 1-2). A change in antibiotic was instituted in all cases. Adverse events are listed by system in Table 6.4 with the CTCAE grading definitions.

To our knowledge, the only serious adverse event (SAE) (CTCAE grade 3) was an eosinophilic pneumonitis associated with the use of nitrofurantoin. A patient with severe LUTS, only ameliorated by nitrofurantoin, was exposed intermittently to the drug over eight years with numerous attempts at cessation and use of alternatives. She developed a sudden eosinophilic pneumonitis with some fibrosis.

Six cases of *C. difficile* toxin positive diarrhoea and one case of diarrhoea expressing *C. difficile* antigen were seen during treatment. All were treated as outpatients. Seven patients with a history of *C. difficile* diarrhoea were managed without recurrence.

No other AEs were recorded.

Table 6.4 Adverse events

Adverse events	Frequency (%)
Total	475 (100)
General reactions	255 (53.7)
Malaise or non-specific systemic upset	195 (41.1)
Cutaneous reactions	47 (9.9)
Pruritus	13 (2.7)
Gastrointestinal disorders	84 (17.6)
Diarrhoea*	42 (8.8)
Nausea	33 (6.9)
Constipation	5 (1.1)
Vomiting	4 (0.8)
Respiratory disorders	50 (10.7)
Dyspnoea	38 (8.2)
Cough	11 (2.3)
Eosinophilic pneumonitis and fibrosis**	1 (0.2)
Musculoskeletal disorders	39 (8.2)

Arthralgia	39 (8.2)
Central nervous system disorders	19 (4.0)
Headache	17 (3.6)
Paraesthesia	2 (0.4)
Laboratory abnormalities	28 (5.9)
Transaminitis	28 (5.9)

*6 cases of C. Diff toxin positive diarrhoea and 1 case of C. Diff positive antigen; **SAE.

CTCAE grading system: (1) Mild - asymptomatic or mild symptoms, intervention not required; (2) Moderate - minimal intervention indicated; (3) Severe or medically significant but not immediately life-threatening - of hospitalisation indicated; (4) Life-threatening, urgent intervention

6.5.6 Antibiotic resistance

We analysed data from all 362 positive MSU cultures. The median number of antibiotics to which the isolate was resistant remained at 1 over all visits (interquartile range 0-2 for visits one and two, and 0-3 for the third and subsequent visits). These differences were not significant (Kruskal-Wallis $X^2 = 2.5$; $df=3$; $p=.47$). These data demonstrate that long-term antibiotic use, as practiced using our methods, does not appear to generate antibiotic resistance amongst uropathogens detected by standard urine culture.

6.6 Discussion

Our clinical experience over a decade with a large cohort of patients demonstrates the promise that antibiotic therapy may hold for treating symptoms previously ascribed to a non-infectious aetiology. In the process, it strengthens the hypothesis that UTI might be implicated in the generation of chronic LUTS. In 2008, it was estimated that nearly 45% of the world's population were affected by LUTS, and this is expected to increase as our population ages. Urinary urgency incontinence affects 15-22% of Americans (202) with a staggering estimated cost of US\$76b annually. If even a fraction of these patients have a bacterial aetiology that is treatable, their care might be transformed.

The principal limitation of this work is that it represents an evolution of clinical practice rather than an intervention study. This has generated a treatment protocol that now requires testing in a randomised trial. Definitive conclusions regarding efficacy need to be corroborated in future work, although the treatment appears to be safe. Assuming any treatment effect is real, these data raise the question of whether the reduction in LUTS associated with antimicrobial therapy is clinically significant.

Our PGI-I data point strongly to the overall reduction in LUTS conferring a meaningful improvement for patients. Furthermore, the relief of urinary urgency exceeded the minimal clinically important difference (MCID) described for the

urgency subscale of our LUTS questionnaire (76). Whilst no MCID has been defined for the lower urinary tract pain subscale of the instrument, the mean number of pain symptoms fell from 3.2 to 0.4, which is likely to be clinically significant. Whether the reduction in voiding symptoms amongst patients was of clinical importance is difficult to determine. The reduction in 24-hour urinary frequency associated with treatment was significantly greater than that conferred by antimuscarinic medication in patients with OAB (190). Twenty-four hour urinary incontinence episodes did not change significantly but baseline incontinence frequency was too low to expect a detectable change associated with treatment.

The outcome data were not generated within a study and no formal control group is available for comparison. Nonetheless, SUI symptoms amongst patients that were exposed to treatment targeting other LUTS did not change. Given our understanding of the pathophysiology of SUI, we would not expect antibiotic treatment to influence these symptoms. These data provide a positive control group. In addition, our centre has collected urinary biomarker data from control subjects in prospective research studies with 12 month follow-up (147, 178). Thirty-six women (mean age=45.5 years; SD=11.4) were included as control subjects in these studies, providing monthly MSU samples for analysis. Median pyuria expression during follow-up was zero. Statistical comparison of these control data with the patient data presented here demonstrates higher levels of pyuria expression amongst patients than controls across all time points ($F=39$; $p=0.0001$).

Demographic variables and disease chronicity did not predict outcome in association with antibiotic therapy and many of the baseline symptom scores did not demonstrate any predictive properties. Higher pain scores at baseline did predict a greater reduction in overall symptoms associated with treatment. If this is a genuine finding, it may hint that pain, a central feature of the inflammatory response, is a marker of infective, treatable pathology in these patients.

The difference between patients with pyuria and negative culture, compared to those with pyuria and positive culture have been analysed in Chapter 3 where it has been shown that the higher the pyuria the greater the probability of a positive culture and a greater symptom experience (203).

We tried to minimise treatment duration, but symptom relapse associated with a return of pyuria often necessitated further treatment cycles. As our treatment strategy evolved, we focused on first generation, narrow-spectrum antibiotics in maximum dose, combined with methenamine hippurate, guided by changes in symptoms and urine microscopy. Methenamine has a general bactericidal effect in the urinary tract, suppressing bacterial growth without selecting for resistant microbes. These choices are likely to generate less bacterial resistance than using conventional antibiotics alone. Our resistance data would seem to support this approach.

Symptom control usually required the maximum tolerated dose of an antibiotic and in some patients, more than one drug. This reality is not surprising given what we now know about host/pathogen interactions in rUTI. Polymicrobial infections are not unusual (190). Given that genomic and other more modern approaches began to reveal bacterial diversity in this patient population (181), antibiotics

targeting fastidious organisms were also introduced. In addition, several common uropathogens, including *E.faecalis* (104), *E.coli*, *S.saprophyticus*, *K. pneumoniae* and *S.enterica*, are known to invade urothelial cells and form intracellular bacterial communities (104). Such reservoirs may be resistant to antibiotics present in the lumen, as many such drugs are not cell-permeant. This means that any sequestered bacteria are free to emerge later to reinitiate infection. The deeper layers of the bladder mucosa may harbour bacterial reservoirs and cell turnover is slow. Ceftriaxone, ciprofloxacin, and azithromycin can reduce the percentage of intracellular bacteria in vitro (204). Uropathogens can also form biofilms that elaborate a polymeric capsule, conferring intrinsic antibiotic resistance (205-207). Most bacteria within these biofilms divide little, thereby failing to express a therapeutic target for most antimicrobial drugs (208). These insights might account for the protracted treatment periods required to achieve disease regression.

Recurrent relapse, experienced by some patients in association with antimicrobial withdrawal, might be explained by similar mechanisms. The failure of some patients to tolerate antimicrobial withdrawal represents a significant clinical challenge. This will need to be addressed in future work.

It is worth noting that intracellular reservoirs and biofilms clinging to shed urothelial cells are unlikely to be recovered during routine MSU culture. This test samples very small volumes of urine supernatant (typically 1-10 ul), whereas infected cells settle quickly to the bottom of sample tubes. Our laboratory and others (19, 193) have found that enhanced collection methods involving collecting sediment via centrifugation provide the necessary sensitivity and this has been corroborated in Chapter 3 of my thesis as well.

The damped oscillations in pyuria demonstrated by the majority of patients under treatment suggest that antibiotic inhibition in the lower urinary tract is partial. Damped oscillations are seen in systems where the retarding force, in this case antimicrobial therapy, is smaller than the force it opposes (209). The damped oscillator and long treatment periods needed support the use of maximum antibiotic doses. These oscillations also argue against our results being explained by simple regression to the mean.

In summary, the evolved treatment strategy is as follows: UTI diagnosis (191) rests on symptoms, signs, and microscopic pyuria. Without the latter we do not initiate antibiotic treatment. We combine methenamine hippurate with a first-generation, narrow-spectrum urinary antibiotic to find a tolerated regime that mediates a symptomatic response and a reduction in pyuria. If urethral pain and dysuria are prominent, macrolides and tetracyclines are favoured. We continue treatment until the symptom control is optimal and the pyuria has cleared before trialling treatment withdrawal. More than one cycle is frequently required to achieve lasting symptom resolution.

We provide all patients who have completed their treatment with a short course of first-generation urinary antibiotic that they can initiate at the very first hint of symptom resurgence. Patients are advised to take three to seven days of antimicrobial treatment, dependent on how quickly the new symptoms settle, and this approach is advocated to prevent chronic symptoms reasserting themselves

after an acute infection. In the medium term, this approach seems to be effective, although we have yet to collect data on the long-term success of this strategy.

Given these data, an RCT is the next logical step. We believe that the correct design should be a comparative trial of the management protocol evolved here, against treatment stipulated by current guidelines. We hope that these data will help in the design of future studies.

Chapter 7 Guidelines failing patients with painful lower urinary tract symptoms, pyuria and negative urinalysis: cross-over data supporting long-term antibiotic treatment

7.1 Introduction

Recalcitrant chronic bladder pain and symptoms of recurrent cystitis in patients with negative urinalysis present a worrying management problem. The treatment of painful lower urinary tract symptoms (LUTS) is a significant challenge, and there is little quality data to guide clinicians. The evidence for oral or intravesical therapies for painful bladder symptoms is poor (210). Cystodistension and urethral dilation are also deployed without evidence to support their utility (211-213). Patients with recurrent, acute symptoms of cystitis and negative urinalysis are often exposed to multiple, short courses of antibiotics in primary care without evidence of benefit (214).

While there are published guidelines for managing acute and recurrent urinary tract infection (UTI) (215), there are none for patients who may be suffering a chronic form of the disease. Recently, we published data describing our experience treating women with chronic painful LUTS for a mean duration of six years prior to presentation to our clinic. These patients had been exposed to conventional therapies without a resolution of symptoms. Data were gathered from 624 patients over 10 years. Given microscopic pyuria and symptoms, we presumed an infectious cause even in the face of negative dipstick urinalysis and urine cultures, as these tests lack sensitivity and fail to detect infective organisms in bona fide disease (19, 216). This previous report described the evolution of a simple management regimen using first-generation, narrow spectrum, urinary antibiotics combined with methenamine hippurate, guided by symptom palliation and the resolution of pyuria (148). The study demonstrated that treatment was associated with symptomatic improvement and the resolution of biomarkers of infection. There were few treatment-emergent adverse events (AEs), and no significant increase in antibiotic-resistant isolates. These data are being employed to develop a randomised trial (RCT).

This novel approach requires clinic-based fresh urine microscopy to quantify pyuria, full doses of urinary antibiotic, protracted treatment periods and careful safety monitoring (148). Although some patients (20%) could be discharged after only six months, we found that it took an average of 383 days (95% confidence interval (CI)=337-428) to achieve symptom resolution (mean reduction in validated symptom score=70%) without the need for further antibiotic treatment. A reduction in symptoms and pyuria associated with antibiotic treatment, followed by deterioration in both measures on early treatment cessation, provide additional cogent observational evidence of efficacy.

Despite positive preliminary data, this therapeutic approach contravenes guidelines for treating acute urinary infection and recognised treatment regimens for recurrent UTI (182). It also defies antibiotic stewardship policies (41, 217). The evolution of this treatment method has been supported by a long-running translational research programme and basic scientific activity at University College London. As such, the clinical approach has been founded on peer-reviewed preclinical and clinical research, in addition to the systematic collection of efficacy and safety data through surveillance. Nevertheless, the nature of the

treatment generated understandable concern amongst medical managers within the host NHS trust and primary care commissioners.

In September 2015 a patient being managed with nitrofurantoin, having failed numerous attempts to withdraw this agent over 30 months, developed a rare, acute eosinophilic pneumonitis, which is reported to occur only during the first six months of treatment (218). Nitrofurantoin was stopped, and the occurrence was reported to our trust, in line with local safety monitoring procedures. This one serious adverse event (SAE) occurred amongst a cohort of 624 patients during 273,762 treatment days. There were 475 total AEs during this period, managed with medication withdrawal or modification as outpatients.

In response to this SAE, the trust medical management imposed prescribing restrictions limiting treatment to short-course antibiotic therapy recommended by guidelines for acute UTI. This imposition effectively suspended the service, and treatment was stopped in 221 patients on long-term regimens. Although the service reopened five weeks later, this sudden suspension tested the hypothesis, supported by previous data, that premature cessation of long-term antibiotic therapy results in disease regression. This paper reports the outcomes that ensued from this event.

7.2 Methods

Our diagnosis of UTI rests on symptoms, signs, and microscopic pyuria. Without the latter we do not initiate antibiotic treatment. We combine methenamine hippurate, a bactericidal urinary antiseptic, with a full-dose, first-generation, narrow-spectrum urinary antibiotic. Antibiotic selection is based on symptomatic response and a reduction in pyuria, along with drug tolerance. Cefalexin is favoured as first-line therapy, with trimethoprim or nitrofurantoin, second and third choice agents. We continue treatment until symptom control is optimal and pyuria has cleared before testing treatment withdrawal; we restart the treatment if relapse occurs. Usually, more than one cycle is required to achieve lasting symptom resolution off treatment (148).

The clinical service was suspended for five weeks from 21st October 2015. When these restrictions were lifted, we contacted patients who had stopped treatment. We identified those who reported symptom deterioration and wherever possible assessed them at the centre as a priority. We measured their symptoms, using a validated measure (11), and urine samples were examined immediately by microscopy using a haemocytometer to quantify leucocytes and shed epithelial cells. Previous work using an antibody against the specific urothelial marker protein uroplakin-3 has shown that the majority of epithelial cells present in the urine specimens of these patients originate from the bladder, not as contaminants from the vulva or vagina (104). In the majority of cases, disease recurrence, indicated by worsening symptoms and pyuria motivated reintroduction of treatment. The clinic suspension permitted the collection of data before treatment cessation, whilst off treatment following cessation, and after treatment was restarted.

The following variables were collected: 24-hour frequency, 24-hour incontinence episodes, lower urinary tract pain, urinary urgency, voiding symptoms and stress urinary incontinence (11). If attending the centre, urinalysis included urinary

leucocyte and epithelial cell counts quantified from fresh urinary microscopy, and routine urine culture. These data were reported at three time points: (1) Whilst on treatment prior to the closure; (2) Whilst off treatment after the closure; and (3) after recommencing treatment. Data reported after restarting treatment were captured from the last consultation within 12 months of the cessation. Some of the symptomatic data were gathered by telephone consultation only, using the measures outlined above. Telephone reviews did not permit urinary biomarker data to be collected at all consultations.

7.3 Ethics

The East Central London Regional Ethics Committee (REC1) (Ref: 11/H0721/7) provided ethical approval for data collection (Appendix 3).

7.4 Statistics

We used the IBM SPSS Version 25 (IBM, New York) for analyses. The data were tested for normality using Q-Q plots. A close linear relationship between the measured variables and the theoretical Z-scores existed and so the data were suitable for parametric analysis. We analysed the differences over the three assessment points using a repeated measures ANOVA. Mauchly's Test was used, and common variance was not violated.

7.5 Results

The unplanned cessation of treatment occurred in 221 patients (female=210; male=11) of the 1035 active patients at the service, with a mean age of 56 years (range=19-92; SD=17.81). Sixty-six per cent of the women were postmenopausal. They had experienced treatment-resistant, painful lower urinary tract symptoms (LUTS) for a mean of six years (sd=7) prior to presentation at this centre. They had attended the centre for an average of seven clinic visits (sd=6) over a mean of 1.7 years (sd=2) and were treated with antibiotics with regular trials of cessation. One hundred and ninety-nine patients (90%; F=188; M=9) reported deterioration in their symptoms after stopping treatment. Thus, 21 did not report deterioration (10%; F=19; M=2). We collected data on 192 (97%) of those who deteriorated. The other seven were unavailable to provide the minimum dataset of symptom measures at three assessments.

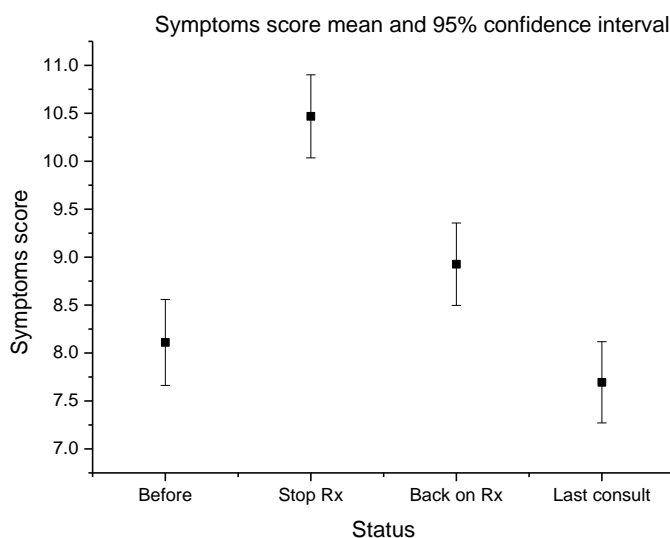
Patients had been assessed an average of 58 days (sd=49) before clinic closure. The service was closed for five weeks and the patients were first reviewed an average of 68 days (sd=38) after the closure. Their last review, within a year of the suspension, was a mean of 284 days after clinic closure (sd =76).

The results of the statistical analysis are reported in Table 7.1, There was an increase in symptoms after cessation, followed by recovery in the wake of treatment reinstatement (F=33; df=2; p<.001). Figure 7.1 illustrates the symptoms response, including data from the last patient review (mean=353 days; sd= 70; F=21; df=3; p<.001). Symptom scores increased after antibiotics were stopped but after the antibiotic regime was recommenced, symptom scores fell to levels reported prior to the suspension. Figure 7.1 includes a fourth data point, from the most recent assessment, typically conducted by telephone and therefore not accompanied by urinalysis data.

Table 7.1 The results of repeated measures ANOVA comparing the variables

Variable	df	F	Sig.
Total symptoms score	2	33.228	0.000
Urgency symptoms	2	11.301	0.000
Stress urinary incontinence symptoms	2	4.282	0.014
Voiding symptoms	2	11.080	0.000
Pain symptoms	2	39.779	0.000
24-hour frequency	2	1.008	0.366
24-hour incontinence	2	3.795	0.023
Log ₁₀ wbc	2	3.713	0.026
Epithelial cell count	2	5.855	0.003

Figure 7.1 Mean total symptom score before cessation, when off treatment and following reintroduction.



Because of geographical dispersion and use of telephone review, urinalysis data were collected less often than symptom measures. Overall, urinalysis data were available for 132 patients (66%) before cessation; 130 patients after cessation (65%); and 122 (61%) after treatment reintroduction. Figure 7.2 plots the pyuria count before cessation, when off treatment, and following reintroduction. There was an increase in urinary leucocyte expression after treatment cessation, and a commensurate decrease after reinstating treatment (F=3.7; df=2; p=.026).

Figure 7.2 Mean pyuria (WBC) count on fresh urine microscopy before cessation, when off treatment and following reintroduction.

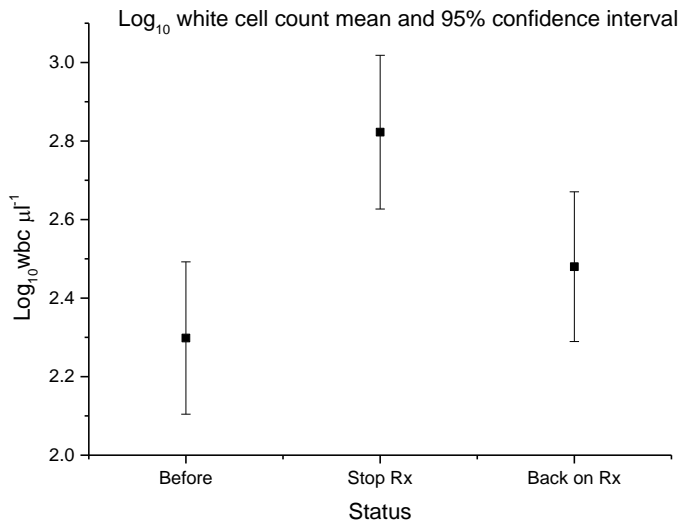
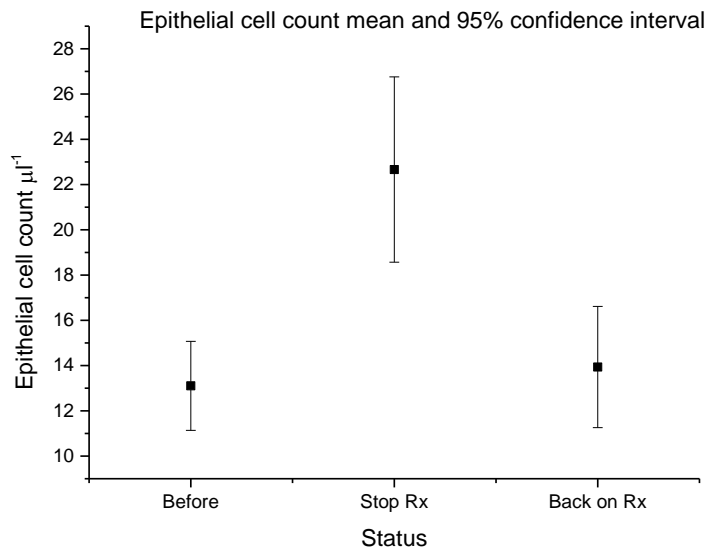


Figure 7.3 provides a similar analysis of the urothelial cell counts at different time points. Urothelial cell expression mirrored changes in symptom scores and urinary leucocyte numbers ($F=6.0$; $df=2$; $p=.003$). Twenty-four-hour frequency did not change over the series ($F=1$; $df=2$; $p=.4$) although 24-hour incontinence worsened off treatment ($F=4.0$; $df=2$; $p=.003$).

Figure 7.3 Mean epithelial cell count on fresh urine microscopy before cessation, when off treatment and following reintroduction.



Menopausal status did not predict symptom scores, pyuria, or epithelial cell counts.

Urine cultures were obtained at the first three time points but only 20 (15%) were positive before treatment cessation, 24 (18%) after stopping and 25 (20%) after restarting treatment. This result is not surprising as we do not use MSU cultures to initiate treatment, and the insensitivity of urine culture is now well recognised (216). There were 11 patients who needed admission to hospital during the clinic closure and these were classified as SAEs in line with ICH GCP criteria (219). Admission indications are summarised in Table 7.2. With one exception, these patients had not experienced any hospital admission for UTI-associated SAEs whilst receiving treatment from this service. The exception was one patient who previously required admission following interruption of the management regimen as part of our treatment protocol.

Table 7.2 Hospitalized patients with SAEs in association with treatment cessation

No. of patients (n=11)	Description of SAEs and management
1	Paraplegia due to activation of autoimmune vasculitis secondary to urosepsis. Treated with cycled of IV Methylprednisolone and Cyclophosphamide; UTI remains difficult to manage.
1	Admitted with urosepsis and CT evidence of right renal abscess. Required six weeks of IV antibiotic treatment
5	Admitted with acute UTI & treated with a single dose of IV antibiotic and five days of oral antibiotic, after which UTI recurred
2	Reactivation of Systemic Lupus Erythematosus (SLE)
2	Worsening of Multiple Sclerosis (MS) symptoms and CISC for retention

Taken together, these results support the hypothesis that treating painful LUTS with long-term antimicrobial courses is effective. These data support a mounting body of evidence (148, 216) that painful LUTS may have an infectious aetiology when dipstick and microbiological culture results do not indicate disease.

7.6 Discussion

An unplanned cessation of treatment in an unselected group of patients with chronic painful LUTS managed with protracted antimicrobial courses resulted in a measurable deterioration in symptoms. The reintroduction of the original treatment regimen was associated with improvement. Only 24-hour urinary frequency was unaffected. Arguing against a mere placebo effect or a by-product of the stress induced by the clinic suspension, the inflammatory biomarker pyuria mirrored the rise and fall of symptoms in these patients. Similarly, shed urothelial cell counts, which are known to reflect a host innate response to inflammation, moved in concert with the other variables, thus supporting a growing awareness of their ability to reflect the disease process (64, 104, 220).

A subset of the patients was hospitalised during the suspension period, and the underlying reasons for admission highlight worsening urinary infection, including pyelonephritis and urosepsis, in the face of antibiotic withdrawal. These events point to potentially significant consequences when patients receiving antibiotic therapy for presumed chronic infection have their treatment suddenly withdrawn without access to treatment reinstatement. It should be noted that all but one of

these patients had remained free of hospital admissions whilst under the care of the service and receiving treatment.

To date, a limited number of RCTs support longer-term antibiotic treatment for painful LUTS caused by chronic infection (21-23). The current data are not from an RCT, but they resulted from a random withdrawal of treatment, followed by reinstating the same treatment. These circumstances provide credible observational data from a cross-over process. As well as corroborating the earlier RCTs, they are in agreement with our own previous observational data demonstrating improvements associated with treatment (148). Our earlier paper also reported high relapse rates related to planned trials without treatment. These data suggest that presumed chronic infection may require much longer antibiotic courses than are recommended for other urological infections. The next step is a RCT using these data as proof of concept.

The principal limitation of this study is the lack of corroborative microbiological data to complement the existing variables that we report. Our research programme, and others, have demonstrated the insensitivity of routine urine culture (19, 177, 181, 193, 221), and treatment in the clinical service has never been contingent on routine culture evidence of UTI. In fact, our recent work shows that the MSU culture cannot distinguish this sort of patient from healthy controls, whereas a genomic analysis demonstrates clear differences (222). Nonetheless, we recognise that the absence of MSU culture data will be contentious for many readers. Pyuria, however, is a well-established and independent biomarker of UTI, and is not subject to the same bias as patient-reported symptoms.

Another limitation is that this study is not a clinical trial, though an RCT is being planned. However, the unique circumstances allowed us to generate data similar to that produced in cross-over studies, providing a rare opportunity to test our hypothesis.

Finally, only two thirds of patients generated urinalysis data, with the potential to generate selection bias. During normal clinic service, patients on telephone review had lower symptom scores than those attending in person for urinalysis ($t = -3.2$, $p = .002$, 95% CI of diff = -4.8 to -1.1), indicating more stable disease permitting remote surveillance. This was also the case after the prescribing restrictions were lifted and normal service resumed ($t = -4.9$, $p < .001$, 95% CI of diff = -5.9 to -2.5). By contrast, during the suspension there was no difference between symptom scores comparing those who attended for face-to-face review and urinalysis, with those who were reviewed by telephone ($t = -0.84$, $p = .4$, 95% CI of diff = -2.4 to +1.0). Thus, it is less likely that the data were confounded by a selection bias.

The UK National Institute for Health and Care Excellence (NICE) has generated guidelines for the management of acute UTI, which limits antibiotic prescriptions to 14 days. The majority of patients in this study had failed to respond to standard antibiotic treatment and other recommended interventions for painful LUTS, prior to referral to this service, and a significant proportion of those referred for treatment came from other tertiary units. NICE has acknowledged a guideline deficit for patients affected by complicated UTI by publishing a “placeholder” statement, employed to identify a knowledge gap (182). It is noteworthy that the treatment of acute UTI using recommended antibiotic prescribing guidelines is

associated with a 25%-35% microbiological and symptomatic failure rate (194). These data, and the results of our work, demonstrate the need for further research into the management of UTI, and for existing guidelines on UTI to be urgently reviewed. It is our hope that clinicians will recognise the challenges that these patients pose in their own practices and that this will galvanise efforts to advance our knowledge in this neglected area of medicine.

While widespread concern about antimicrobial resistance (AMR) makes stewardship policies imperative, a one size-fits-all approach may be equally unhelpful. Long-term antimicrobials should not be withheld when they are necessary (as is the case, for example, in tuberculosis patients). This is particularly true when patients are treated in a consultant-led clinical setting, subject to additional governance processes, careful monitoring, and individualised therapy.

There will never be guidelines for all circumstances, as there will always be exceptional patients. We acknowledge the real difficulties that treating patients outside normal guidelines present, especially within an evolving field, and without high-quality evidence to guide treatment. Nonetheless, clinical experience and practical wisdom, especially when founded on solid scientific insights, should always be regarded as a key property of good medicine (223, 224).

Chapter 8 Antimicrobial resistance patterns of MSU cultures of chronic UTI patients on long term antimicrobial treatment compared to patients presenting with acute UTI to the emergency department.

8.1 Introduction

An estimated 14 million people in the UK (men, women and children) are living with bladder problems and the rate of emergency admissions due to a urinary tract infection (UTI) has almost doubled to 60/100,000 in the last five years (177). In 2012/13 unplanned admissions for urinary tract infections (UTIs) cost £432 million per year, averaging 2.1 million per UK clinical commissioning group (225). Urinary tract infections are the most common healthcare acquired infection (HCAI), comprising 19% of all HCAs; 43-56% of UTIs are associated with urethral catheterisation; approximately 17% of secondary nosocomial bloodstream infections are caused by catheter use, with an associated mortality of 10% (226).

The UK National Institute for Health and Care Excellence (NICE) has generated guidelines for the management of acute UTI, which limits antibiotic prescriptions to 14 days maximum. NICE has acknowledged a guideline deficit for patients affected by complicated UTI in publishing a “placeholder” statement that points to a knowledge gap (182).

Recently, national, international, and antimicrobial stewardship committees have adopted consensus guidance on the presumption that the shorter the duration of treatment, the less the risk of antimicrobial resistance (AMR), thus recommending courses as short as 3 days to treat symptomatic acute UTI(227). They omit recommendations on surveillance to identify those who fail to respond to these rigid protocols. The RCTs on treatment of acute UTI, using 3 to 14-day treatment courses, consistently demonstrate 25%-35% microbiological and symptomatic failure rates (194).

AMR is regarded as one of the major public health concerns of the 21st century (228, 229). Numerous studies have provided estimates of the burden of resistance, but the methods adopted and data reported are inconsistent. Thus, estimates of resistance, related mortality and other outcomes for Europe (230), the USA (231) and the world (232), must be tentative. The World Health Organisation (WHO) survey provides the most comprehensive data on global levels of antibiotic resistance (233). Variations in microbial isolates tested, methods of resistance testing, and sparse data on clinical application impairs determination of the population attributable fraction (PAF) for mortality due to antibiotic resistance (227, 234).

Treatment failure is often assumed to be the result of antibiotic resistance although the culture-derived evidence is weak (25). Other plausible explanations include guideline driven inadequate or prophylactic dosing and short treatment durations. In the context of intracellular colonisation and biofilm infections (104, 208), the pattern of response to treatment of CUTI is known to oscillate so the acute flares experienced, which are part of the expected pathophysiological response, are erroneously assumed to be due to antibiotic resistance (148).

The development of AMR is not straight forward and a number of publications refer to the mechanism of hetero resistance which describes a phenotype in which a bacterial isolate contains subpopulations with reduced antibiotic susceptibility compared with the main population (235). The resistance phenotype is often unstable, and in the absence of antibiotic pressure it rapidly reverts to susceptibility. A common mechanistic explanation for the instability is the occurrence of genetically unstable tandem amplifications of genes that cause resistance. Due to their instability, low frequency and transient character, it is challenging to detect and study these subpopulations, which often leads to difficulties in unambiguously classifying bacteria as susceptible or resistant (235).

Transposons can transfer from a plasmid to other plasmids or from a DNA chromosome to plasmid and vice versa that cause the transmission of antibiotic resistance genes in bacteria (236). The treatment of bacterial infectious diseases is difficult because of existing antibiotic resistance that part of this antibiotic resistance is caused by transposons (236). Resistance genes may also be acquired by microbes through horizontal gene transfer (237). This involves exchange of genetic material between microbes in contrast to vertical transfer through reproduction.

Patients with chronic recalcitrant bladder pain and recurrent cystitis are difficult to diagnose and treat. We have published data that implicate microbial parasitisation of the urothelial cells as a plausible cause (11, 19, 25, 104, 147, 177, 238). We have reported a 10-year follow-up study on the treatment of chronic urinary tract infection (UTI) in patients who exhibited symptoms and pyuria, despite negative routine MSU cultures. Treatment with first-generation, narrow-spectrum urinary antibiotics, took an average of 383 days to achieve resolution of symptoms and pyuria (148). Given the growing concerns over AMR (239, 240) (241), we measured the resistance found in positive urine cultures from our chronic UTI patients whilst on antibiotic treatment.

Doctors are divided on whether patients with symptoms of UTI, who are culture-negative but manifest pyuria, should be treated with antibiotics. Some refrain from prescribing treatment to avoid further AMR. Others argue that denying antibiotic treatment to women with relevant symptoms and pyuria is unjustifiable. In the battles against AMR, more germane targets lie in agricultural overuse in China, the USA, Brazil and the Asian subcontinent (242-244).

8.2 Study Setting

Our tertiary centre receives referrals of recalcitrant chronic UTI patients, who have already failed guideline regulated treatment offered by primary care physicians, urologists and Urogynaecologists from all over the UK and abroad. These patients have already been exposed to repeated, full dose, short antibiotic treatment courses, daily, rotating antibiotic prophylaxis. They have undergone multiple non-invasive and invasive imaging and surgical procedures prior to referral to our centre. It takes an average of 6.4 years from onset of symptoms to being seen in our centre. These patients have continued to suffer with debilitating symptoms despite the variety of treatments and interventions offered at primary, secondary and tertiary care centres in the UK.

In our Centre, diagnosis of UTI rests on symptoms, signs, and microscopic pyuria. Without the latter, we do not initiate antibiotic treatment. We use a combination of a full-dose, first-generation, narrow-spectrum urinary antibiotic with methenamine hippurate, a bactericidal urinary antiseptic. Antibiotic selection is based on symptomatic response and a reduction in pyuria, along with the individual's drug tolerance. Cefalexin is our first-line therapy, with trimethoprim or nitrofurantoin, second and third choice agents (Appendix 14.13). We continue treatment until symptom control is optimal and pyuria has cleared before testing treatment withdrawal. We restart the treatment if relapse occurs. Usually, more than a 3- month cycle is required to achieve lasting symptom resolution off treatment (148, 179). 80- 85% of the cohort responds to the treatment regime within an average of 383 days and about 15-20% require longer treatment courses (148, 179).

The clinical service evolved out of a traditional urodynamics based incontinence clinic. Following discovery of the deficiencies of urodynamics in explaining chronic recalcitrant painful lower urinary tract symptoms (245-247) our research concentrated on finding a pathophysiological explanation for the persistent chronic nature of symptoms with episodes of acute flares. The emerging publications from 2009 to date (19, 61, 104, 245, 248, 249) influenced our referral mix and we started to see a rise in patient referrals with chronic recalcitrant bladder pain and recurrent cystitis with negative MSU cultures. In 2014, after recognising serious errors inherent in MSU culture (25), we had to base our treatment on symptom response, signs and urine microscopy for pyuria, and not on culture and sensitivity data. Hence, we used a protocol that adopted the use of first -generation, narrow-spectrum, antibiotics which were tailored on tolerance and response. We achieved good results(179).

At every review, a clean-catch MSU sample was sent to the hospital laboratory. If a culture isolated a pure growth of $\geq 10^5$ cfu/ml of a known urinary pathogen, sensitivity analysis was routinely conducted for the following five antibiotics: amoxicillin, co-amoxiclav, ciprofloxacin, nitrofurantoin, and trimethoprim. Here we report on the analyses of the MSU culture and sensitivity data during the treatment of these complex cohort of patients whilst on treatment with protracted antibiotic regimes (179).

8.3 Aim

To examine the resistance rates of microbes in patients treated with long-term oral antibiotics for chronic UTI and compare them to the resistance rates identified in patients attending the emergency department of the Whittington Hospital, London, with symptoms of acute UTI.

8.4 Ethics

The East Central London Regional Ethics Committee (REC1) (Ref: 11/H0721/7) provided ethical approval for data collection (Appendix 3).

8.5 Methods

We conducted an analysis of our clinical data collected from January 2004 to September 2018 to examine the resistance patterns exhibited by microbes grown on positive MSU cultures in chronic recalcitrant painful LUTS patients on long-term antibiotic therapy and compared it to the resistance recorded from microbes grown on positive MSU cultures from untreated Emergency department (ED) patients presenting with symptoms of acute UTI between Jan 2016 to Dec 2018.

We recorded the results of midstream urine cultures, over 14 years (2004 to 2018), from patients attending our lower urinary tract symptoms (LUTS) clinic. At each visit the patients were instructed in the correct method for collecting a clean catch midstream urine specimen (25). The MSU specimens were submitted to the Whittington Hospital Microbiology Laboratory, London, UK for routine culture as detailed in Chapter 3.6.5.

A significant culture involved the isolation of a pure growth of $\geq 10^5$ cfu/ml of the following urinary pathogens: *Acinetobacter*, *Citrobacter*, *Klebsiella/Enterobacter/Serratia*, *Corynebacterium*, *Diphtheroid*, *Enterobacteriaceae*, *Enterococcus*, *Escherichia coli*, *Morganella*, *Proteus*, *Pseudomonas*, *Staphylococcus*, or *Streptococcus*.

The antibiotics that were tested were amoxicillin, co-amoxiclav, ciprofloxacin, nitrofurantoin, and trimethoprim using the MIC break points defined in EUCAST SOP 10.1 (198). For each reported sensitivity data, we counted the number of the five antibiotics to which resistance was detected, with a potential score range between zero antibiotics (no resistance) and all five antibiotics (resistance to all five antibiotics).

Statistical analysis of the resistance counts were compared across years using the non-parametric Kruskal-Wallis test.

8.6 Results

Between 2004 and 2018 we submitted 30,647 MSU specimens for urine culture of which 2838 (9%) grew $\geq 10^5$ cfu/ml of a single species of known urinary pathogen. These data were obtained from 3,096 patients (mean age=61 years, SD=0.3). There were 2710 women (57.3% over 50 years) and 384 men. For comparison purposes we collected data from patients attending the ED department with symptoms of acute UTI during three years 2016 to 2018 providing 3,600 positive cultures, of which, 2465 were women (50.5% postmenopausal) and 1135 were men with a mean age of 54 years (SD=24).

The data on resistance counts are presented in Figure.8.1. Figure.8.1 plots the mean and 95% confidence intervals of the resistance counts for each year of the study and the same for the ED data collected for three years. The resistance counts are higher than the A&E comparisons. The lowest counts in our patients are recorded during the years of providing a urodynamics service. There is a marked rise in these counts during 2010 to 2013, reflecting the transition to a service for recalcitrant painful LUTS and recurrent UTI. In 2014, alerted by new data (25) we ceased to use culture sensitivity data in guiding antibiotic prescriptions(179). If the patient remained symptom free with low pyuria counts,

we did not alter antibiotic treatment based on culture results. This rising trend in the AMR rate was stabilised from 2014 (Kruskal-Wallis chi-squared = 164, df = 13, p-value = 0.001).

Figure.8.1 Mean and 95% CI of resistance counts for each year of the study.

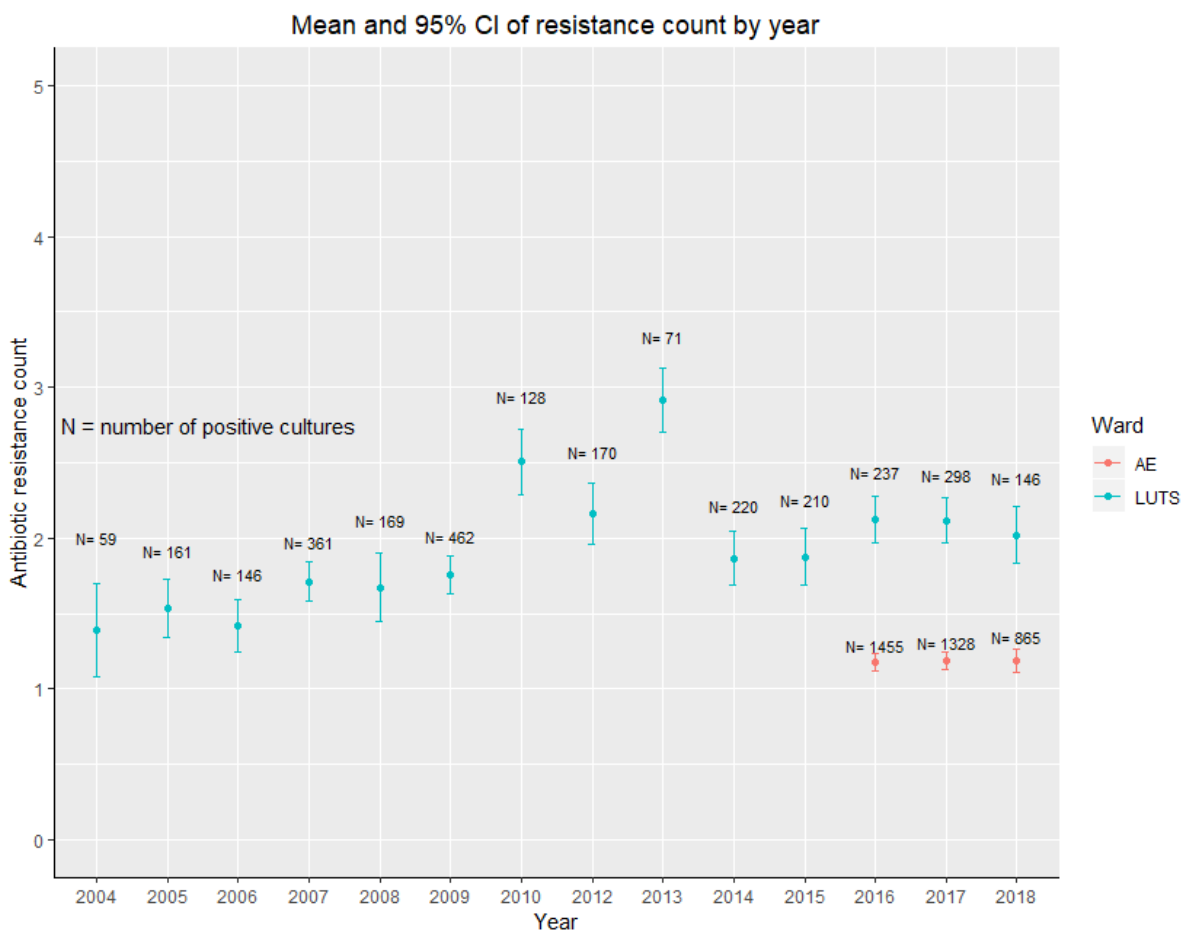
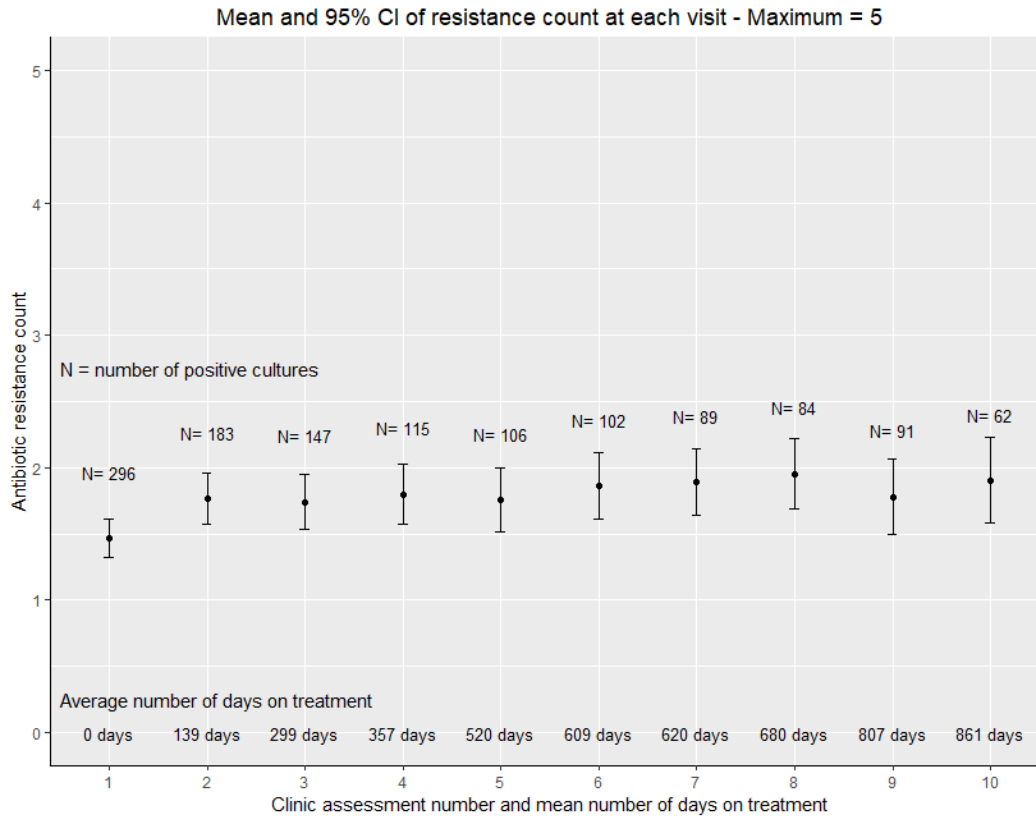


Figure 8.2 illustrates the mean number of resistant antibiotics of the five that were tested for a subset of 296 patients presenting to the service for the first time from 2014, onwards, when the urine culture data did not guide antibiotic prescribing. The data are from positive MSU cultures collected over 10 consecutive visits to the service.

Figure 8.2 Mean number of resistant antibiotics for a subset of patients



In the subset size (n=296, Figure 8.2), the resistance count rises only once at the second visit following the introduction of an antibiotic at the first visit, it then maintains a plateau throughout (Kruskal-Wallis chi-squared = 21.224, df = 9, p-value = 0.01).

The qualitative species differences, between LUTS patients and the acute AE patients, identified in the positive cultures are presented in Table 8.1.

Table 8.1 The qualitative differences in isolate genus between the LUTS patients and the Acute UTI AE patients

Genus	% in Chronic LUTS Patients	% in Acute UTI AE Patients
<i>Escherichia coli</i>	41.03	63.13
<i>Pseudomonas</i>	18.63	4.14
<i>Enterococcus</i>	13.04	6.2
KES	12.47	13.51
<i>Proteus</i>	4.58	5.32
<i>Candida</i>	3.63	2.41
<i>Streptococcus</i>	3.05	0.71
<i>Staphylococcus</i>	1.6	2.93
<i>Enterobacter</i>	0.93	0.49
<i>Citrobacter</i>	0.58	0.16
<i>Acinetobacter</i>	0.11	0.22
<i>Serratia</i>	0.09	0.14
<i>Morganella</i>	0.06	0.36
<i>Diphtheroids</i>	0.06	0
<i>Trichomonas</i>	0.04	0
<i>Stenotrophomonas</i>	0.04	0.03
<i>Corynebacterium</i>	0.02	0.05
<i>Ewingella</i>	0.02	0
<i>Achromobacter</i>	0	0.03
<i>Providencia</i>	0	0.08
<i>Alcaligenes</i>	0	0.08

8.7 Discussion

The data presented in this study demonstrate patterns in AMR that might not have been expected given the current assumptions about resistance and long-term antibiotic treatment. This study demonstrates that AMR in this patient group on long term full dose antibiotics did not increase over time as previously predicted (250-252).

Only 9% of MSU specimens for urine culture grew $\geq 10^5$ cfu/ml of a single species of known urinary pathogen. The very low isolation rate is well recognised in contemporary literature, as chronic UTI patients frequently manifest with lower-level infections, below the 10^5 cfu/ml threshold (25, 216).

The resistance counts are higher than the A&E comparisons. The lowest counts in our patients are recorded during the years of providing a urodynamics service (Figure 8.1). There is a marked rise in these counts during 2010 to 2013, reflecting the transition to a service for recalcitrant painful LUTS and recurrent UTI. In 2014, alerted by new data (25) we ceased to use culture sensitivity data in guiding antibiotic prescriptions(179). If the patient remained symptom free with low pyuria counts, we did not alter antibiotic treatment based on culture results.

A fall in AMR ensued from 2014 (Kruskal-Wallis chi-squared = 164, df = 13, p-value = 0.001).

The subset size (n = 296, Figure 8.2) was reduced for three reasons: (1) the service was closed to new admissions from October 2015; (2) only 15% of our chronic patients test above the 10^5 cfu/ml threshold needed to trigger sensitivity testing; and (3) only a minority of patients need such extensive follow-up (eg 861 days) so the numbers reduce with time. Despite these impediments data were still sufficient for informative analysis. The key finding is that the resistance count rises only once at the second visit following the introduction of an antibiotic at the first visit, it then maintains a plateau throughout (Kruskal-Wallis chi-squared = 21.224, df = 9, p-value = 0.01).

Patients reporting to ED acutely and yet to commence antibiotic treatment showed resistance to one antibiotic, consistent over the three year period demonstrating that antibiotic resistance existed prior to antibiotic use.

These findings are commensurate with Darwin's theory of biological evolution. Widespread multi-antibiotic resistance predates humans by millions of years (253-256). This is not surprising since antibiotics are produced by bacteria and these organisms have been extant for 3.5 billion years. The antibiotics are produced to attack competitors, but in the process the microbes have to co-evolve resistance factors to protect themselves from self-destruction. Antibiotics and resistance co-evolve, and it has been happening over billions of years.

A section of the Lechuguilla Caves, New Mexico, has been sealed off from the rest of the world for 4 million years. Bacteria from this site were found to be resistant to many antibiotics; some exhibiting multi-resistance to 14 currently prescribed agents (254). Similar data have been obtained from deep bore holes into the Canadian permafrost (253) and deep ocean sampling (256). Yanomami hunter-gatherers, isolated for 11,000 years, carry in their bowels microbes with numerous antimicrobial resistance genes, including resistance to synthetic antibiotics (257). The tribes are so isolated that they have had no antibiotic exposure. These discoveries reinforce the idea that such resistance is ancient and widely dispersed in the environment.

Seen in this light, AMR is a Darwinian inevitability predating humans. The current AMR crisis arises from the selection of resistance strains, through antibiotic use and frequent mis-use, out of the natural colonising populations. Microbes whether commensal or pathogenic can switch resistance genes on and off (207). It is an important survival mechanism. If bacteria are manufacturing resistance proteins, their nutritional demands rise commensurately, and they must pay the price (258). If they were to continue to express this resistance, when the offending antibiotics are not in the environment, the advantage cost would prove detrimental and the microbes would be disadvantaged, so it is in their interest to switch off the resistance genes. The influence of these effects is still being elucidated. An environment with constant exposure to antibiotics, whether broad or narrow spectrum, will result in the selection of microbes with the relevant resistance genes, despite the additional nutritional needs necessary to express the genetic phenotype.

It is a misconception that resistance data, obtained from urine cultures, reflects the situation accurately. If a patient is consuming an antibiotic, the diagnostic cultures, should they prove positive, will be biased so as to select microbes, from the bladder microbiome, that have resistance to the antibiotic. It is not correct to assume that the bacterium that is cultured is the cause of the UTI (25). The normal bladder is not sterile but hosts over 400 different species, increasing to over 500 in untreated patients with chronic recalcitrant bladder pain and recurrent cystitis (25). Thus, there is an increase in species dispersion, but we do not know what microbes are causing the UTI. An isolate might well be an easy to grow normal commensal. Regrettably, a culture may encourage clinicians to change the prescription, and thereby enter a self-generating chain of increasingly broad-spectrum antimicrobials, when no change was needed. This practice is bound to drive AMR.

An important clinical outcome to consider is whether a given antimicrobial therapy can successfully treat an infection. Standard antibiotic susceptibility testing guidelines recommend that sensitivity should be measured when a planktonic microbe is grown in rich medium, in mono-culture. Therefore, test results only indicate whether an organism is sensitive to an antimicrobial compound under those precise conditions. These laboratory tests do not consider the conditions microbes experience within an infection site, including constant assault by the host immune system. In addition, antimicrobial efficacy is also influenced by immunosuppression (102) and drug-drug interactions (103), which may contribute to differences between laboratory results and clinical outcomes. Various studies have evaluated whether in vitro minimum inhibitory concentration (MIC) – still considered the gold standard for drug susceptibility testing – correlated with the success or failure of antimicrobial treatment. Surprisingly, these studies found little or no association between clinical antimicrobial susceptibility testing results (specifically, a pathogen's in vitro MIC value for a specific drug) and clinical outcomes measured following antibiotic treatment, even in the context of single species infections (93-98). In other words, a patient did no better when they were infected by susceptible organisms (low MIC) compared to resistant organisms (high MIC).

It was the data reported by Sathanthainanthamoorthy et al in 2013 (25) that showed that the bladder microbiome in patients and controls show polymicrobial abundance and that treatment on MSU data, may result in multiple treatment changes as selection pressure on one organism with antibiotic treatment based on C&S, could lead to the others surfacing at next culture In CUTI patients. This led us to cease to prescribe on midstream urine culture data from the beginning of 2014. Instead, we used our analysis of patient symptoms and microscopic pyuria to diagnose chronic UTI. We used first generation narrow spectrum urinary antibiotics, primarily cephalexin, trimethoprim or nitrofurantoin combined with Methenamine and changed the prescription in response to tolerance and plots of the symptoms scores and urine microscopy results (148, 179). We maintained the patient on the same prescription once it was established. These patients oscillate back to health, exhibiting acute flares along the way(179). Expecting these flares, we remained loyal to the original antimicrobial, responding to occurrences with a temporary increase in dose. Patients' symptoms improved and a proof of concept study has generated encouraging evidence(179). The data of Figure 8.1 demonstrates the influence on AMR of treating on culture and then the effect of abandoning this practice. Most MSU cultures in CUTI patients were

being reported negative despite severe symptoms. When Sediment culture based antibiotic treatment changes were made the rates of ARM rose. Following SS thesis and data publication we ceased sediment culture guided clinical treatment and treated based on symptoms and pyuria and used narrow spectrum antibiotics. This resulted in the ARM returning to the original level and continued improvement when selection pressure was maintained with Narrow spectrum antibiotics as explained in treatment chapter 6. The comparative data obtained from A&E patients, contrasts the situation when antibiotics are not in the system.

The data depicted in Figure 8.1 are expected given our understanding of Darwinian selection. We started an antibiotic in untreated patients, and sure enough the resistance count rose by the next attendance. This was the only significant change in the series. After that, absence of change is commensurate with a consistent selection pressure because the prescription was unaltered. We assumed that if the patient was responding the isolate from a culture was irrelevant.

We have the lowest use of cephalosporins and quinolones in Europe. English surveillance programme for antimicrobial utilisation and resistance (ESPAUR) (259) reported that despite low levels of use of cephalosporins, the proportion of bloodstream infections resistant to these antibiotics has not changed significantly in the last five years. UK Health Security Agency authorities (259) admit that AMR is not so straight forward.

Treatment of TB serves as a good reminder of the role of antibiotic misuse in the development of AMR (260). The reasons why multidrug resistance continues to emerge, and spread are mismanagement of TB treatment and person-to-person transmission. Most people with TB are cured by a strictly followed, 6-month drug regimen that is provided to patients with support and supervision (261). Inappropriate or incorrect use of antimicrobial drugs or use of ineffective formulations of drugs (such as use of single drugs, poor quality medicines or bad storage conditions), and premature treatment interruption has caused drug resistance (261) (260).

The patients with CUTI are suffering with severe debilitating symptoms for many years and have often faced hospital admissions and have been denied treatment due to negative dipstick for nitrites, negative urine cultures or normal inflammatory markers and most importantly hospital antimicrobial policies geared to reduce antibiotic usage across the entire spectrum of disease. These patients respond well to long term, full dose, narrow spectrum antibiotics as described in Chapter 6 of this thesis (179).

In conclusion, this approach to managing a difficult problem seems not to promote the upsurge of AMR that many anticipate. It is unfortunate that these CUTI patients are often denied treatment with antibiotics despite unreliable AMR data, the lack of understanding of the mechanisms of resistance and unprecedented fear of treatment being the sole cause of all AMR. We hold that the greater threat comes from changing the antibiotic prescription in response to misleading culture resistance data. We would also question the use of cycles of different antibiotics, a practice derived from reliance on culture data (262).

8.8 LIMITATIONS

The major limitation of this work is that we have limited data on the actual number of isolates in the patients' urine and we are unable to determine what the remainder of the microbes are doing in response to the constant selection pressure of a narrow spectrum antibiotic over a period of time.

Burden of AMR and awareness campaigns are resulting in shorter courses of antibiotics, genuine patients with disease not being adequately treated and this chapter does not address the effect of different treatment strategies and their effect on AMR.

The dataset was very small and not meaningful to breakdown and study further individual antibiotic resistance patterns.

I acknowledge that AMR is a very complex subject and this observational study report is only one of the many projects that the unit intends to undertake which is discussed in the Future work section of this thesis.

Chapter 9 Summary

I have studied a unique group of patients in my thesis who are refractory to the current guidelines driven management of their painful LUTS and recurrent cystitis symptoms.

9.1 Quality of life impact of a CUTI

Before characterising these patients and the pathophysiological signals they presented with I studied the burden of disease by examining the quality of life impact of the condition on a patients everyday life and health by using online surveys, validated LUTSqol questionnaires. The frequent attendances to seek help from medical professionals compounded with problems with screening and diagnostic tests has likely led to the symptoms being attributed to a psychological cause. Assessment of the impact of the condition on the quality of life on LUTSqol analysis showed a significant impact across many domains of their everyday life compared to controls.

I felt it important to supplement my findings with an analysis using qualitative measures by studying patient reported biographies of their journey with this condition until treatment at this centre. In doing this I also used textual analysis of biographies to explore the interaction of the patients with the healthcare professions. This was germane because I was finding compelling evidence of urinary tract infection when the received wisdom was that there could be no infection. The patients' experience of the disease could best be explained by infection, so I was studying a group who have consistently asked about the possibility of a causal role for infection with this being repetitively rebuffed.

My hypothesis was that patients who found themselves in this situation must have experienced additional QOL burden. The qualitative data explored through patient stories and biographies provided a moving account of degree of physical and emotional distress and adversity caused by CUTI. The effect on patients work, personal and family relationships and the word clouds brought out the recurrent themes and the frequency of impact. Studying psychological impact in a traditional way using validated questionnaires, provide very little meaningful data as the numerical data does not explore individual experiences and struggles (136, 263). This is much needed for the clinician to begin to understand the impact and certainly helped transform the experience of a patient as stated by the patient during subsequent visits to the centre.

A patient survey further revealed that the symptoms being dismissed due to paucity in awareness about the condition and negative diagnostics in addition to the fear that the doctor does not know what to do or cannot help often leads to a loss of confidence in the clinician and makes consultations much more difficult.

These patient biographies and survey brought in a more personal and humane perspective on the patients journey with this condition and I have gone on to study this population further in subsequent chapters in my thesis. This data to us was extremely important in setting the scene to explore this condition further.

9.2 Pathophysiological signals in the urine of patients referred with IC & PBS symptoms.

I started off by studying the pathophysiological signal in the urine of 146 new female patients presenting with chronic recalcitrant painful lower urinary tract symptoms (LUTS) and recurrent cystitis to the centre prior to commencement of antibiotic therapy and compared them to controls. These are an enriched sample of patients who have been assessed extensively in primary, secondary and tertiary care and failed to respond to all known guideline regimens. They have all been diagnosed with interstitial cystitis (IC) or painful bladder syndrome (PBS). I tested the hypothesis that the root cause of the symptoms was a chronic UTI. To do this I used consilience to analyse the symptoms, quality of life, the urinary pathology using fresh, unspun unstained urine microscopy to count white cells and epithelial cells, epifluorescent microscopy to examine urothelial cell morphology and urinary ATP. I also explored the microbiology using an enhanced spun sediment culture. I compared my sample of “IC” patients with a set of normal controls.

In comparison to the controls my “IC/PBS” sample demonstrated differences in pyuria, urothelial cell shedding, ATP, and polymicrobial abundance on enhanced urinary culture. These data have demonstrated well the fact that the standard methods for excluding UTI in patients with IC/BPS are failing and that when patients with IC/BPS are compared to normal controls we can see that they exhibit microbiological abundance and an innate immune activation that the standard tests should have detected if they were doing their job. Thus, we can say that UTI has not been excluded in patients with IC/BPS who are managed according to published protocols.

These findings provided correlative evidence in support of an infective aetiology. These data do not clinch the proposition that IC/BPS is due to infection, but they do fill the first rung of Judea Pearl’s ladder of causation (159).

9.3 Pilot data on pathophysiological signals in diabetics and patient’s presenting with a flare while on treatment

LUTS are more common in the diabetic population with a prevalence quoted to be between 37-70% and are frequently attributed to the metabolic disorder (162, 164). Dysuria and urinary frequency are the most common questions asked and reported on. Both storage and voiding symptoms can coexist. Despite major evaluations in the literature, no recommendation supervises the assessment and management of LUTS in this specific population.

The pathophysiological signals in the pilot study of a sample of diabetics were also commensurate with those in the main data set described above, whilst the diabetics demonstrated a comparatively reduced appreciation of pain. The standout difference was seen in the symptom analysis that the diabetic patients consistently scored higher over six of the symptom measures. It would be wholly plausible to attribute these differences to the direct effect of diabetes on lower urinary tract homeostasis, but the microscopic pyuria injects some doubt into the assumption and lights a path for future scrutiny.

I sampled a further subset of the main “IC” sample whilst they were experiencing an acute flare of their symptoms and they demonstrated a coherent response in the consilience variables that were undetected by standard urinary dipstick and culture analysis. The same standard methods failed to find evidence of infection in the patients at presentation. It was notable that suprapubic tenderness and systemic markers (WCC, CRP and ESR) did not reflect the manifestations of pathology, during acute flares, that immediate urine microscopy and enhanced culture showed.

The consilience data were correlative and not definitive evidence of causation by infection. I therefore moved on to use Judea Pearl’s principles for assessing causation moving through the three rungs of his ladder: (1) Correlation (2) Intervention (3) Counterfactuals.

9.4 Treatment of Chronic UTI with antibiotics

In study interventions, I studied a sample of 624 female patients who were treated with antibiotics for their symptoms with the necessity of such intervention being tested by regular trials without treatment. The outcomes from these activities were measured and the data collated. This observational study answered well the challenges of intervention.

UTI diagnosis (191) rests on symptoms, signs, and microscopic pyuria noted in fresh unspun urine. Without the latter we do not initiate antibiotic treatment. We combine methenamine hippurate with a first-generation, narrow-spectrum urinary antibiotic to find a tolerated regime that mediates a symptomatic response and a reduction in pyuria. If urethral pain and dysuria are prominent, macrolides and tetracyclines are favoured. We continue treatment until the symptom control is optimal and the pyuria has cleared before trialling treatment withdrawal. More than one cycle is frequently required to achieve lasting symptom resolution.

We provide all patients who have completed their treatment with a short course of first-generation urinary antibiotic that they can initiate at the very first hint of symptom resurgence. Patients are advised to take three to seven days of antimicrobial treatment, dependent on how quickly the new symptoms settle, and this approach is advocated to prevent chronic symptoms reasserting themselves after an acute infection. In the medium term, this approach seems to be effective, although we have yet to collect data on the long-term success of this strategy.

Given these data, an RCT is the next logical step. We believe that the correct design should be a comparative trial of the management protocol evolved here, against treatment stipulated by current guidelines. We hope that these data will help in the design of future studies.

9.5 Counterfactuals on the effect of treatment cessation and effect on antimicrobial resistance in patients treated with long term antibiotics

During my time in the unit, an unprecedented, unplanned six-week closure of the centre provided me with an unusual opportunity to examine the counterfactual because treatment was withdrawn from 221 women without warning. I was able to measure outcome data before, during the cessation and after re-introduction of treatment.

The current data are not from an RCT, but they resulted from a random withdrawal of treatment, followed by reinstating the same treatment. These circumstances provide credible observational data from a cross-over process. As well as corroborating the earlier RCTs, they are in agreement with our own previous observational data demonstrating improvements associated with treatment (148). Our earlier chapter also reported high relapse rates related to planned trials without treatment. These data suggest that presumed chronic infection may require much longer antibiotic courses than are recommended for other urological infections. These cross-over data answer many of the questions about counterfactuals and provide a rich justification for a upcoming large RCT using these data as proof of concept. At this time the evidence for infection as the cause of IC is remarkably strong.

I had to acknowledge that the trials without treatment were promoting longer courses of urinary antibiotics that we should have wished. Patients were recovering and we were restricting to first generation, narrow spectrum urinary antibiotics. Nevertheless, worries about AMR were inevitable. I therefore mounted a study that analyses the sensitivity data that were obtained from the MSU cultures that were conducted on the patients treated with long term narrow spectrum antibiotics at this centre.

Patients reporting to ED acutely and yet to commence antibiotic treatment showed resistance to one antibiotic, consistent over the three year period demonstrating that antibiotic resistance existed prior to antibiotic use. I certainly detected an increment in AMR predicted on what we know about Darwinian selection, but I also found evidence that should prove reassuring and was commensurate with the surprising stability of the patients' responsiveness to the same medication used consistently in our study cohort.

This approach to managing a difficult problem seems not to promote the upsurge of AMR that many anticipate. It is unfortunate that these CUTI patients are often denied treatment with antibiotics despite unreliable AMR data, the lack of understanding of the mechanisms of resistance and unprecedented fear of treatment being the sole cause of all AMR. We hold that the greater threat comes from changing the antibiotic prescription in response to misleading culture resistance data. We would also question the use of cycles of different antibiotics, a practice derived from reliance on culture data (262).

9.6 Conclusions from the thesis

This research has introduced me and enhanced my skills for understanding and treating chronic UTI. I have a vastly different understanding of the patients and their plight since I first started. I have published and continue to work on original peer-reviewed papers. I have acquired a number of bench laboratory skills for analysing the pathology and microbiome of the bladder. I have learned so much about the scientific method, its application to clinical research and modern developments in the science of causation.

During my research programme, I have presented at departmental, national and international conferences and forums and my work has been received positively.

I have also gained experience with clinical governance structure in NHS, MDT set up, root cause analysis and I have participated in academic and external scrutiny of the practices of the centre. I have learned an enormous amount about clinical governance, the patient voice and representation, the working of parliament, local government, ethics, audit clinical governance and safety monitoring. I have learned much about truth, speaking truth to power and courage so we may advocate for our patients, which is rightly expected in any doctor-patient relationship.

There will never be guidelines for all circumstances, as there will always be exceptional patients. I acknowledge the real difficulties that clinicians in primary and secondary care face when treating patients with CUTI and many would not feel comfortable taking on novel treatment protocols when the science of a field is evolving. I believe that we must learn to fear less and be ready to allow scientific advance to question our practices and guidelines even if that make us feel uncomfortable and threatened. I have learned to respect those who speak outside of the mainstream and in my future career I shall look to nurture them in difficult times. I now have a richer understanding of evidence-based medicine than I ever dreamt of and I hope to champion this in my forthcoming professional life.

Chapter 10 Limitations of the thesis and Future Work

10.1 Limitations of this thesis

Exceptional patients with recalcitrant symptoms therefore the data is not applicable to the average patient in outpatients with symptoms of OAB or those who present with painless LUTS. The limitations of the individual chapters has been elaborated in detail in the thesis and I will attempt to summarise it here.

Though the biographies in chapter 2 provided a better understanding of the impact of a CUTI, it is important to acknowledge that these patients were grateful that they had met a specialist clinic, had a diagnosis and their symptoms treated after failing most known regimes hence more likely to have provided positive feedback for our clinic. Their relief on receiving a diagnosis will also have an impact on the quotes they have chosen to share and cannot be generalised.

In the main dataset in chapter 3, we were not able to match the patient and control groups for age and menopausal status. Another limitation was that Sub categorisation of the patient groups was not an option, given the sample size. However, the patients were homogenous in that they presented with mixed lower urinary tract symptoms.

The Diabetes survey lacked data on glycaemic control in these patients because of the patient records were anonymised, post hoc I was not able to map individual patient HbA1C values back to the records I held.

The pilot data on samples from diabetes and patients with acute flare on treatment both had a small sample size and we were unable to age match patients and controls. These studies was underpowered so negative results should be viewed with caution and the positive findings are pointing to an infective aetiology however require confirmation with a larger sample set. The pilot data on Diabetes is crucial to understand the burden of CUTI on these patients and will help us to power future studies.

Treatment and sudden cessation studied in subsequent chapters were both studies on exceptional patients from a single centre on an outside guideline treatment with no control data. However, this data provided the intervention and counterfactual data which is most crucial in developing a study design for a multicentre RCT.

While the last chapter studied the MSU culture & sensitivity data and reported resistance rates in our patients taking long term narrow spectrum treatment, we are only aware of a small portion of the actual microbiome and further studies should aim to use molecular methods to map the communities and resistance and perhaps also look at the effect on the gut and vaginal microbiome at the same time.

10.2 Future work

10.2.1 Chronic Disease burden in women

CUTI is not unlike other chronic painful conditions that effect women including menopause, pelvic pain syndrome, IC/PBS, endometriosis and mesh related pain. I would like to include this relevant quotations from Julia Cumberledge review “First Do No Harm” (140).

“There is an institutional and professional resistance to changing practice even in the face of mounting safety concerns. There can be a culture of dismissive and arrogant attitudes that only serve to intimidate and confuse. For women there is an added dimension – the widespread and wholly unacceptable labelling of so many symptoms as ‘normal’ and attributable to ‘women’s problems.’”

This review brings to light the years of suffering endured by women, years of being dismissed, denied acknowledgement of chronic pain and delays in potential treatment options to end their suffering. Chronic UTI sufferers have elaborated in their stories, the diagnostic nightmare they have faced due to medical teams failing to acknowledge presenting symptoms and relying on faulty diagnostics leading to endless suffering.

10.2.2 Extended work on pilot data obtained on diabetic population and patients with acute flare on antibiotic treatment

Pilot in chapters 5 and 6 have sampled a rather at-risk group of individuals who are difficult to diagnose using the current tests and if left untreated it is likely to cause a significant deterioration of general health and QOL. Future research students in our programme will focus on a detailed study on Diabetic women with LUTS and recurrent cystitis and my colleague Dr Dhanuson Dharmasena is continuing work in acute flare patients currently on treatment with antibiotics as per our unit protocol.

10.2.3 The bacterial ecology in patients with chronic urinary tract infections and antimicrobial resistance compared to healthy volunteers.

A number of cross-sectional studies have highlighted that urine, even in healthy controls, is not sterile (20, 62). Moreover, these investigations have uncovered a diverse bacterial ecology (microbiome) of commensals and potential uropathogens in the bladder. Little is known about how these bacterial profiles differ between health and disease and, more importantly, which specific bacteria cause disease. One of the most frequently asked questions by patients when they are to commence an antibiotic regime for a UTI is ‘Will I become resistant to antibiotics?.’ We wish to answer this question. It is a misconception that resistance data, obtained from urine cultures, reflects the clinical situation accurately (264, 265). Standard antibiotic susceptibility testing guidelines recommend that sensitivity should be measured when a planktonic microbe is

grown in rich medium, in mono-culture. Therefore, test results only indicate whether an organism is sensitive to an antimicrobial compound under those conditions. These laboratory tests do not consider the conditions microbes experience within an infection site, including constant assault by the host immune system.

This study will address the problems of causation of UTI and antimicrobial resistance (AMR). This information is vital in understanding AMR and developing strategies to reduce it. We are beginning to understand a difference between a 'clinical response' and a 'microbiological response' in this patient group, studied in a longitudinal prospective cohort we will be able to appreciate the evolving ecology in the bladder whilst patients are on treatment. This study will help re-define the diagnostic approach to this condition and enable us to identify the causative bacteria and examine the development of antibiotic-resistance using sophisticated molecular techniques. The evidence generated will hopefully lead to urgently-needed improved diagnostic and treatment protocols for the significant numbers of patients with this condition.

Ethical approval has been granted by Research Ethics Committee: Health and Care Research Wales for this study and Dr Shaimaa Najdi is starting a PhD on this topic.

10.2.4 Guideline for management of Chronic UTI

Given the evolving data on the paucity of diagnostic tests and success achieved with the treatment of this unique group of patients with recalcitrant LUTS, recurrent cystitis and pyuria on Microscopy, urgent work is needed to produce a NICE or Specialist society guideline for the diagnosis and management for Chronic UTI.

We as a team under the leadership of Dr Khasriya who heads the Whittington Chronic UTI clinic have put forward proposals to NICE, IUGA and BAUS and we have been asked to develop a guideline to IUGA SIG Committee for the management of Bladder pain including a section of Chronic UTI. Our group is a part of the task force to commence this work.

10.2.5 Application of image-based machine learning to urine microscopy to diagnose urinary tract infection (UTI)

Manual urine microscopy is currently the most informative test as it accurately depicts the underlying pathophysiology(62, 266). However, it is labour-intensive, requires expertise and is unsuitable for centralised laboratory services due to cellular degradation(267). Image-based machine learning (ML) and deep learning (DL) can be employed to overcome this and have already proven successful in radiology(268) and histopathology(269) where an algorithm is trained to 'see' an image in far more detail than the human eye. Better and more rapid UTI diagnosis could allow earlier intervention, leading to potential avoidance of hospital admissions and reduction in antimicrobial resistance (AMR) due to prolonged antibiotic treatment.

The aim of this study is to design, train, validate and test an end-to-end, image-based deep-learning model to diagnose UTI in a diverse cohort of patients. Dr Natasha Liou is the PhD student who has commenced work on this study. Our pilot studies have combined advanced AI techniques with high-content screening microscopy to fully automate this process. Our trained model has been developed on an enriched dataset of urine samples from patients with chronic UTI and is now able to automatically identify and enumerate cell types.

This model overcomes the inaccuracies of urine dipstick while capitalising on the utility of microscopy and incorporating patient symptomatology. The technology could be translated into a cost-effective, rapid, user-friendly, point-of-care portable device suitable for primary care, outpatient clinics, emergency centres, and importantly in peoples' homes in the community.

10.2.6 Randomised Control trial comparing long term Antibiotic treatment to standard management according to current hospital guidelines

At the start of my research career, I was involved in the two existing RCT's comparing oral Nitrofurantoin treatment vs placebo for CUTI in MS (Multiple sclerosis) and OAB patients with a crossover design where patients became their own controls. Although clever in design and achievable with 1 year follow up, we had to close both studies due to failure to recruit or failure to continue the study. Patients declined to participate when they had the option of achieving successful treatment of full dose antibiotics given the units standard protocol. Nitrofurantoin was poorly tolerated in the three MS patients recruited to the trial and the study did not allow for other antibiotics.

Learning from the previous study and reasons for failure to complete recruitment, Dr Khasriya has submitted an application to MRC for a single centre open labelled RCT (initially a selection trial) to compare the treatment of Chronic UTI in our centre to standard NHS treatment. The initial study will have 4 arms namely:

1. Short course (5-7 day) as per NHS antimicrobial guidelines)
2. Long term full dose oral antibiotics for treatment of Chronic UTI (LUTS Clinic protocol)
3. Methenamine only
4. Long term antibiotic treatment (LUTS Clinic protocol) with Methenamine
- 5 Once nightly oral antibiotic prophylaxis

The study will provide the feasibility data and help in the design of a multicentre RCT for the management of Chronic UTI.

10.2.7 Quality of life and economic burden caused by CUTI

The data obtained by the biographies is new and encouraging and has prompted us to continue to collate patient narratives for a larger dataset and we are aiming to use Python software for electronic analysis of the patient stories to study the impact of CUTI on daily life. The patient narratives when analysed with digital software are generating some important themes related to the suffering experienced by these CUTI patients and this warrants a detailed analysis of a larger dataset to validate the findings.

There is a lot of information on the cost of UTI treatment on the NHS and other healthcare systems (Ref Fox and NHS digital) however the economic burden of a CUTI on the individual patient is poorly understood, including impact of days off work, loss pay, promotion and termination. There are large patient support groups all over the world with one having >8000 subscribers (Ref Embedded UTI), and these societies will enable us to obtain good quality real life data on the economic burden faced by CUTI sufferers.

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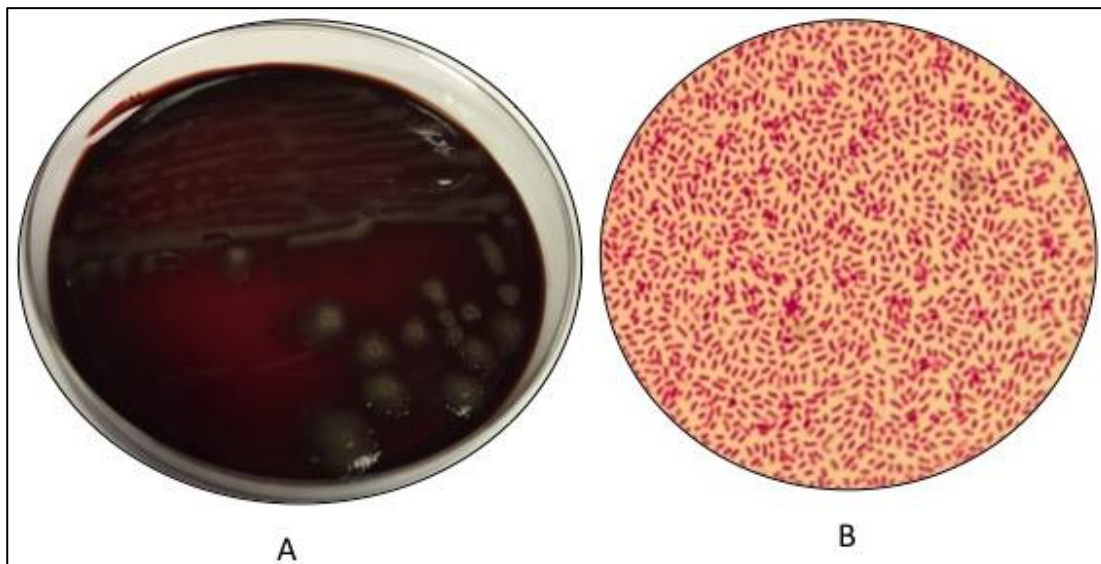
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Appendices

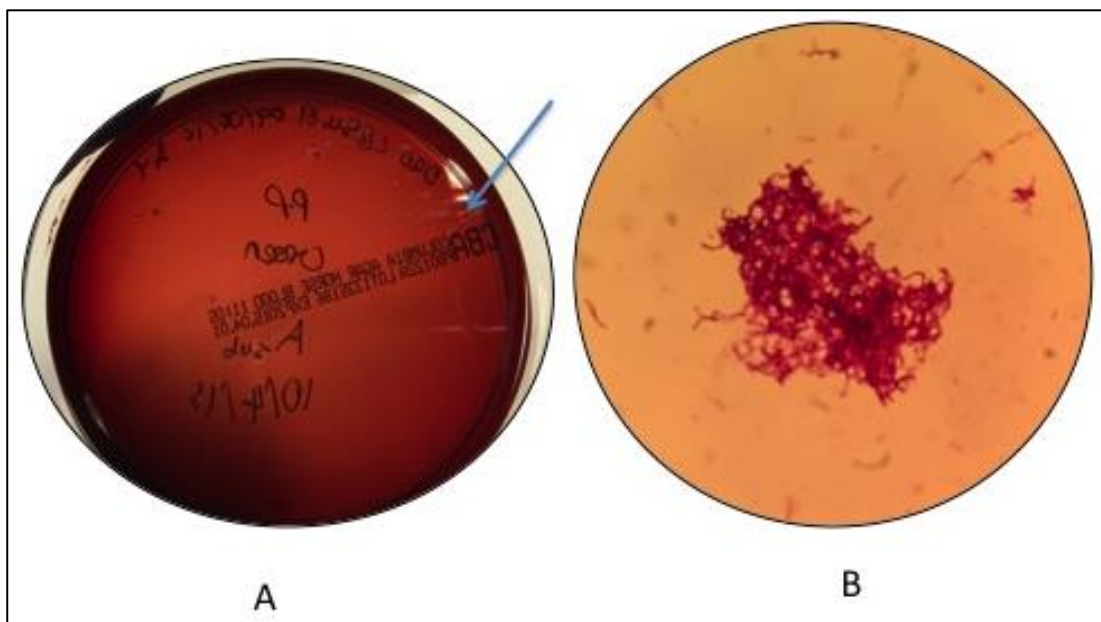
Appendix 1. Sediment culture images

Sediment Culture Growth Plates Microorganisms were identified by growth colony characteristics, microscopy and Gram stain

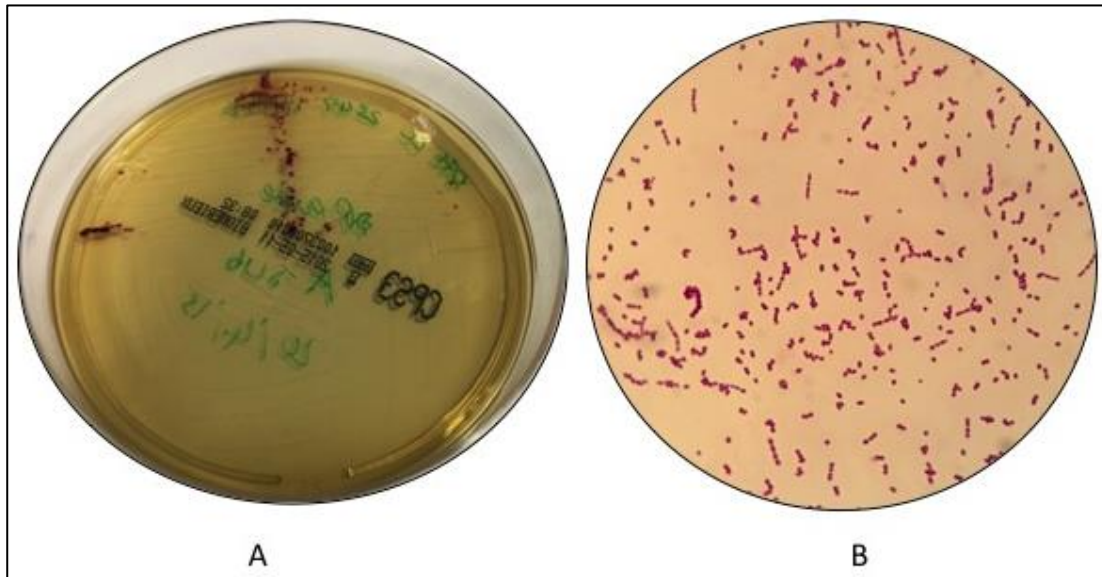
A) Pure growth colony of *Klebsiella*, *Serratia* and *Enterococcus* species on a CBA plate. B) Gram stain of *Klebsiella*, *Serratia* and *Enterococcus* species, a Gram-negative rod. *Klebsiella*, *Serratia* and *Enterococcus* species was more prevalent in patients (6%) than controls (1%).



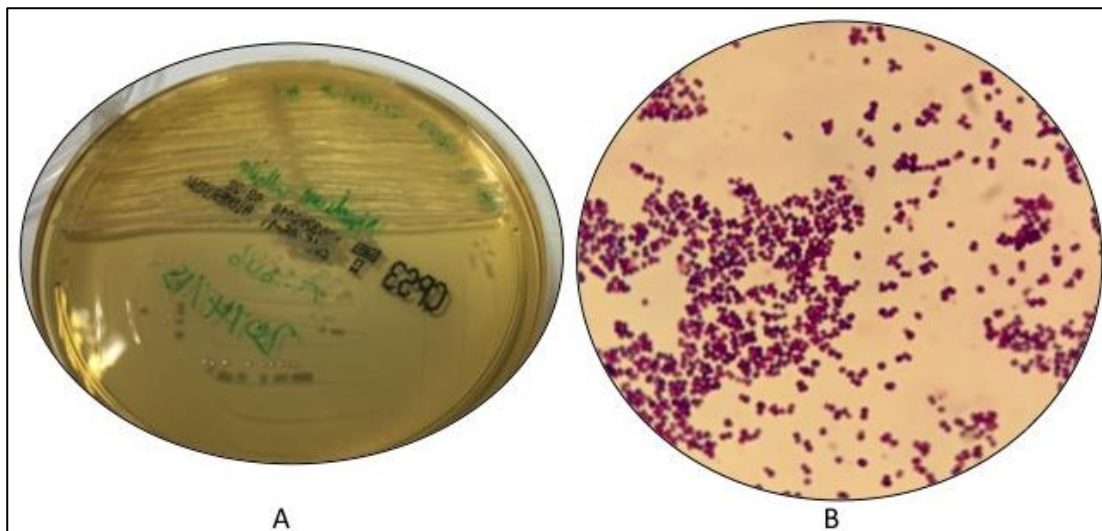
A) Pure growth colony of *Lactobacillus* on a CBA plate, arrow delineates majority of growth. B) Gram stain of *Lactobacillus* with its distinctive long thin spaghetti-like rods. *Lactobacillus* was more prevalent in patients (8%) than controls (3%).



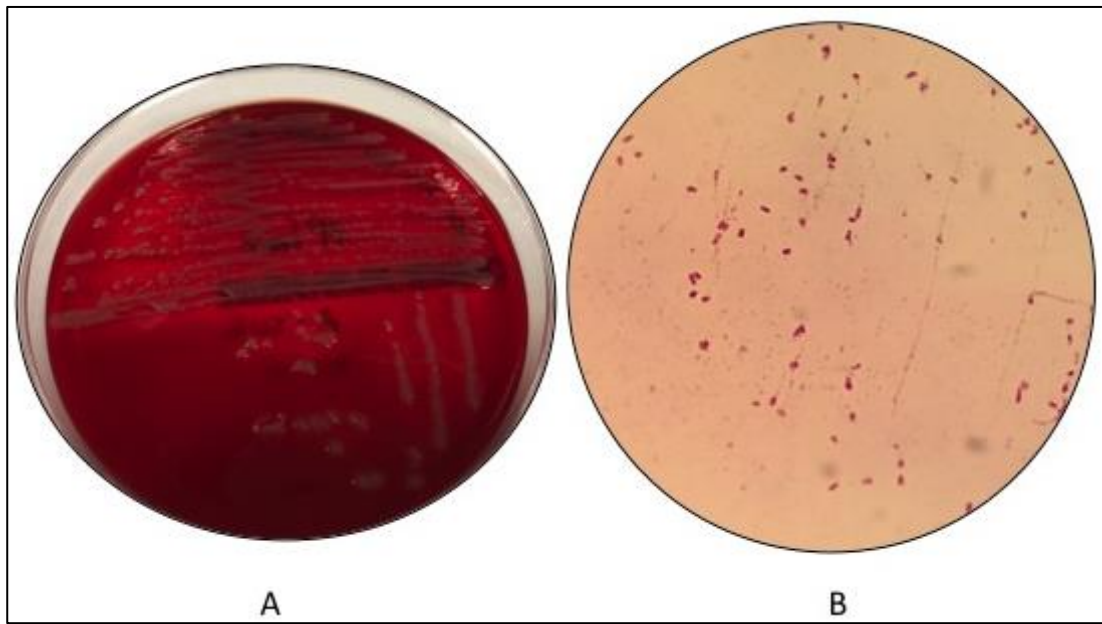
A) Pure growth of *Streptococcus* on CPS3 plate. B) Gram stain of *Streptococcus*, a Gram-positive cocci. *Streptococcus* was present in both patients (13%) and controls (17%).



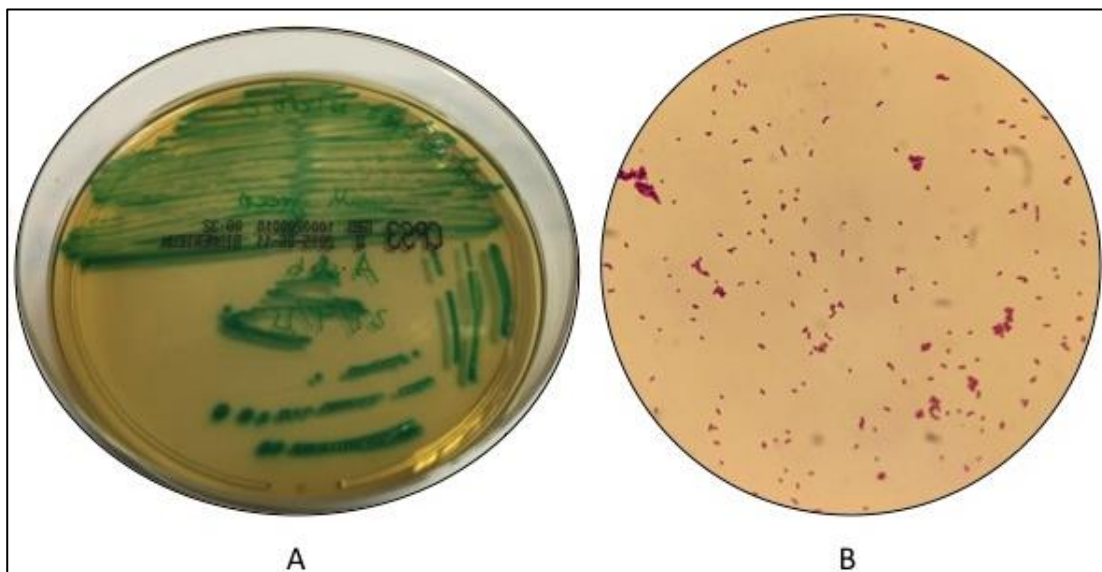
A) Pure growth colony of *Staphylococcus* on a CPS3 plate. B) Gram stain of *Staphylococcus*, a Gram-positive cocci. *Staphylococcus* was found to be the predominant species in both the patients (26%) and the controls (35%).



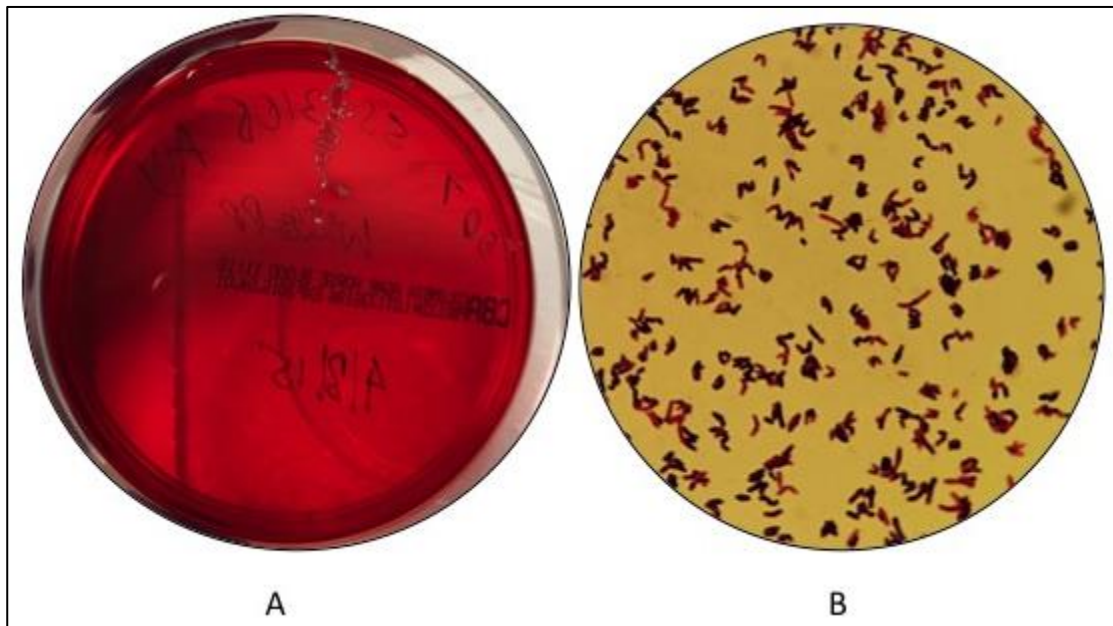
A) Pure growth colony of *E. coli* on a CBA plate. B) Gram stain of *E. coli*, a Gram-negative rod. It was found to be both prevalent in patients and controls in the same proportion (10%).



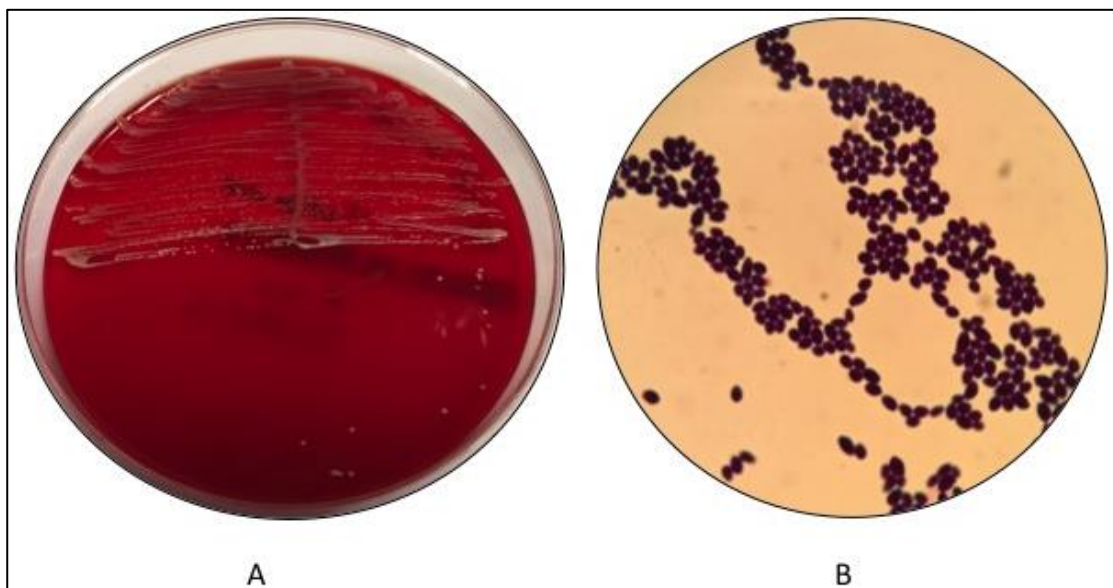
A) Pure colony growth of *Enterococcus* on a CPS3 plate. B) Gram stain of *Enterococcus*, a Gram-positive cocci. *Enterococcus* was found to be prevalent in controls and patients in the same proportion (13%).



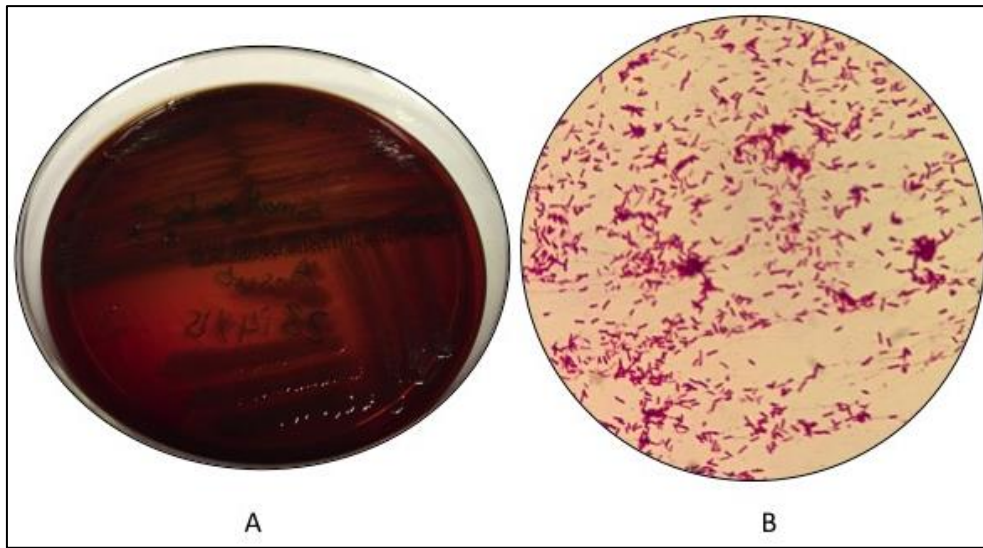
A) Pure growth colony of *Corynebacterium* on a CBA plate. B) Gram stain of *Corynebacterium*, a Gram-positive rod. *Corynebacterium* was found to be more prevalent in controls (19%) than patients (2%).



A) Pure growth colony of Yeast on a CBA plate. B) Gram stain of Yeast, a Gram-positive oval-shaped cocci. Yeast was found to be more prevalent in patients (7%) than controls (1%).



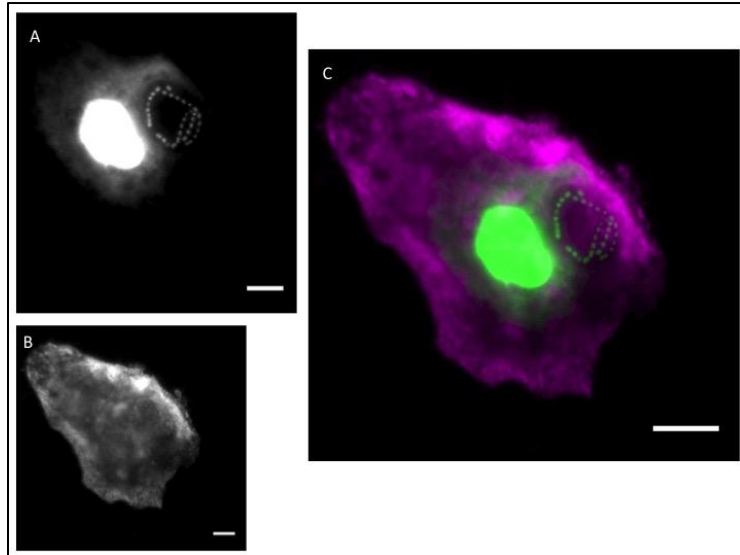
A) Pure growth colony of *Pseudomonas* on a CBA plate. B) Gram stain of *Pseudomonas*, a Gram-negative rod. *Pseudomonas*, along with *Citrobacter* and *Proteus* were only found in patients and not controls.



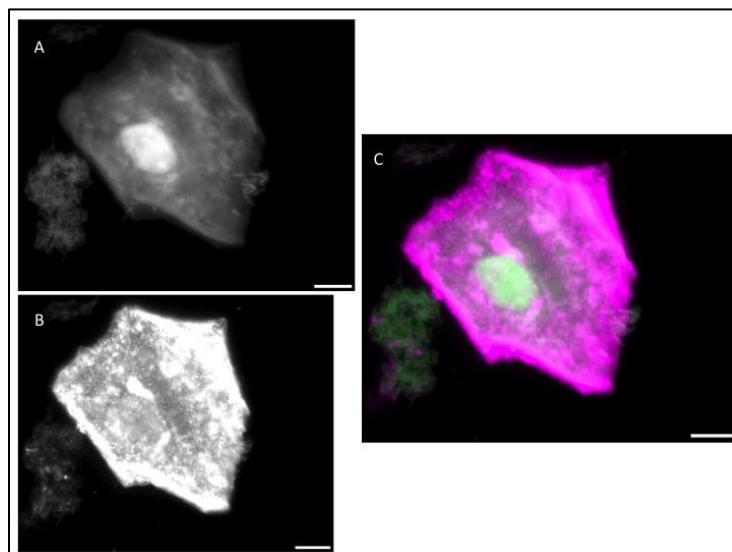
Appendix 2. DAPI Epifluorescence images

DAPI EPIFLUORESCENCE IMAGES

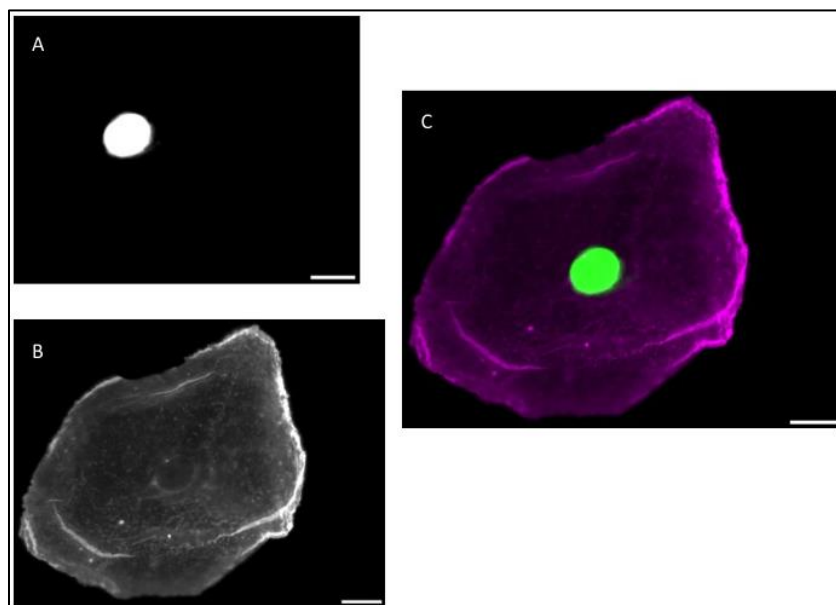
- A) A clue cell with bacterial cocci shown in the DAPI channel in monochrome.
B) A clue cell with bacterial cocci shown in the WGA channel in monochrome.
C) A clue cell with bacterial cocci shown in the merging of the DAPI (Green) and WGA (Magenta) channels. White scale bar represents 20µm.



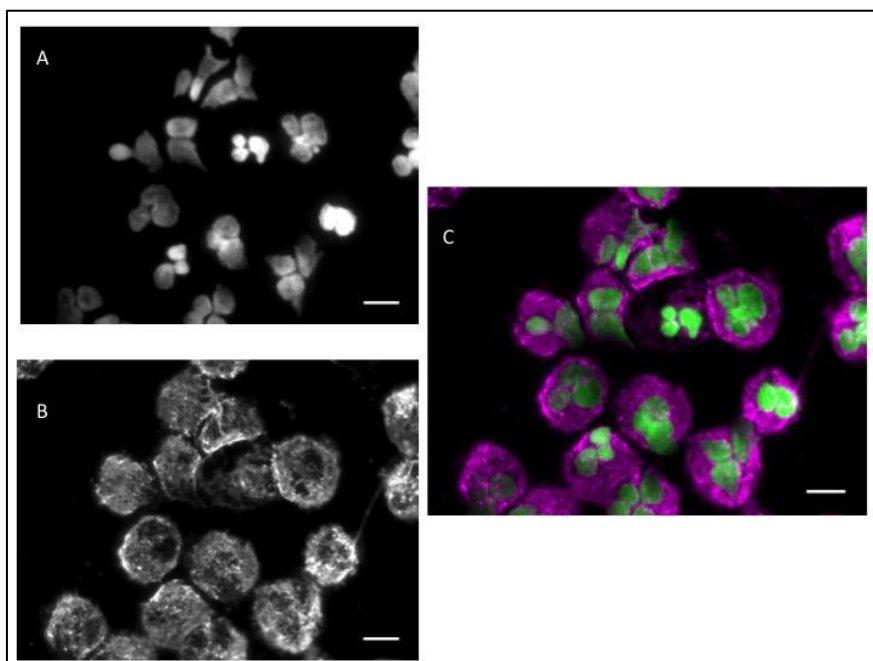
- A) A clue cell with bacterial rods shown in the DAPI channel in monochrome.
B) A clue cell with bacterial rods shown in the WGA channel in monochrome.
C) A clue cell with bacterial rods shown in the merging of the DAPI (Green) and WGA (Magenta) channels. The fact that the bacterial rods have taken up WGA into their plasma suggests they are Gram-positive rods. White scale bar represents 20µm.



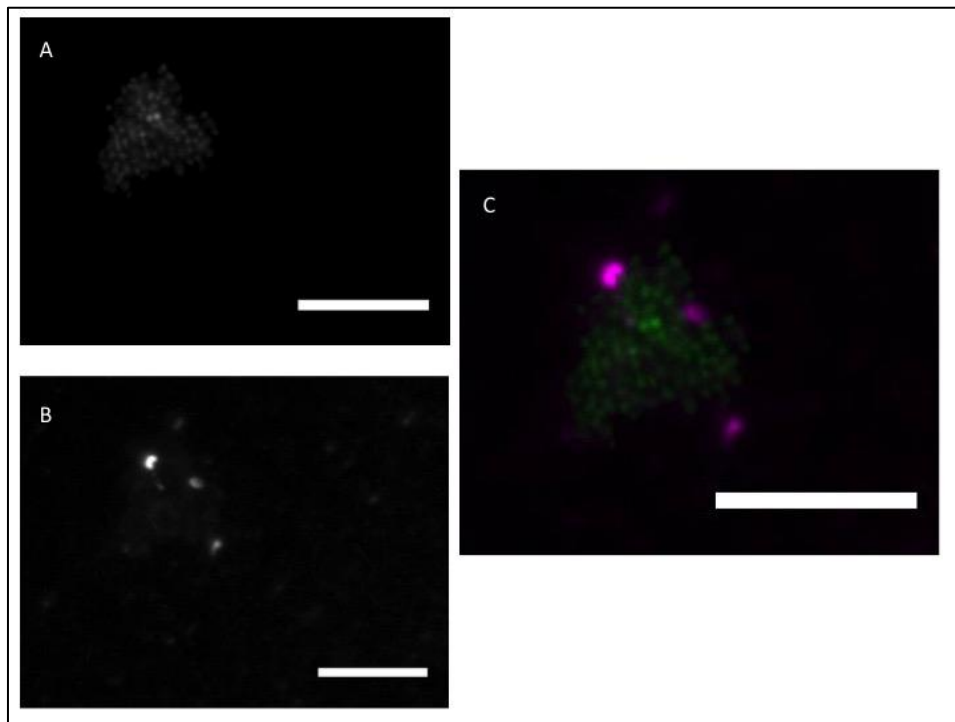
A) A blank uroepithelial shown in the DAPI channel in monochrome. B) A blank uroepithelial cell shown in the WGA channel in monochrome. C) A blank uroepithelial cell shown in the merging of the DAPI (Green) and WGA (Magenta) channels. White scale bar represents 20 μ m.



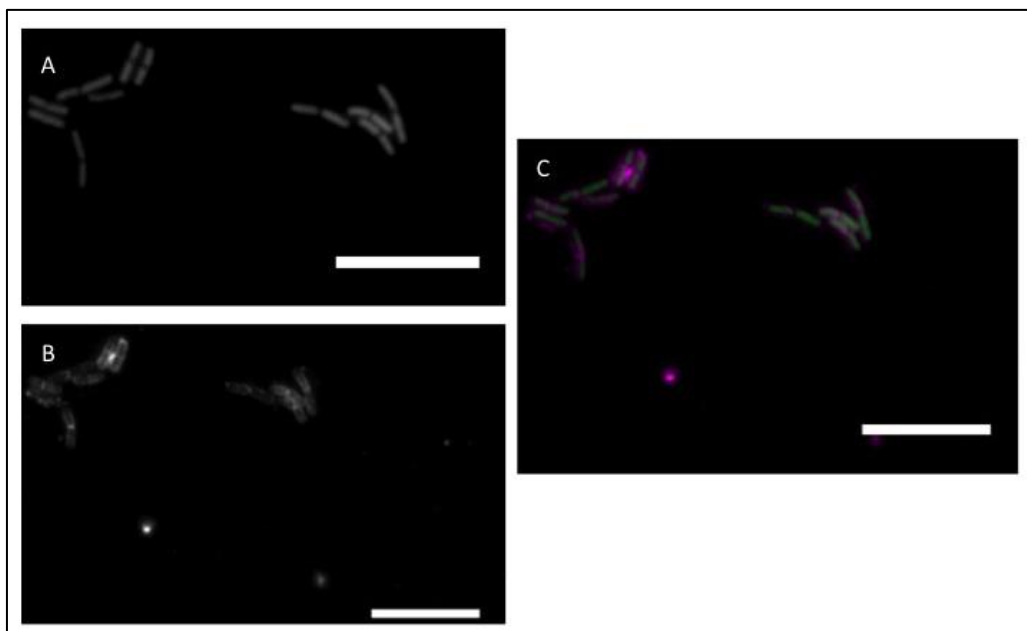
A) WBCs shown in the DAPI channel in monochrome. B) WBCs shown in the WGA channel in monochrome. C) WBCs shown in the merging of the DAPI (Green) and WGA (Magenta) channels. DAPI here has been taken up by the nuclei of the WBCs and WGA by the plasma. White scale bar represents 20 μ m.



A) Planktonic bacterial cocci shown in the DAPI channel in monochrome. B) Planktonic bacterial cocci shown in the WGA channel in monochrome. C) Planktonic bacterial cocci shown in the merging of the DAPI (Green) and WGA (Magenta) channels. DAPI has been taken up by the bacterial DNA here. White scale bar represents 20 μ m.



A) Planktonic bacterial rods shown in the DAPI monochrome channel. B) Planktonic bacterial rods shown in the WGA monochrome channel. C) Planktonic bacterial rods shown in the merging of the DAPI (Green) and WGA (Magenta) channels. The fact that the bacterial rods have taken up the WGA stain into their plasma suggests they are Gram-positive rods. White scale bar represents 20 μ m



Appendix 3. Ethical committee & R&D approval

- Ethical committee approval for Chapters 3-7



NRES Committee South East Coast - Surrey

Bristol Research Ethics Committee Centre
Whitefriars
Level 3, Block B
Lewins Mead
Bristol
BS1 2NT

Tel: 0117 342 1380

19 November 2014

Professor James Malone-Lee
Professor of Medicine
University College London
Research Department of Clinical Physiology
Division of Medicine
1st Floor
Wolfson House
2-10 Stephenson Way

Dear Professor Malone-Lee

Study title: A blinded cross-sectional survey of the pathophysiological signals detectable in the urine of patients with overactive bladder, bacterial cystitis painful bladder syndrome, ketamine cystitis and cystoplasty.

REC reference: 11/LO/0109

Amendment number: Substantial Amendment 3

Amendment date: 20 October 2014

IRAS project ID: 67316

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

The Sub-Committee approved the following changes:

1. Additional assays to urine testing; and
2. Additional blood test for selected group of patients who have acute dysuria.

Approved documents

The documents reviewed and approved at the meeting were:

- Ethical committee approval for Chapter 8



National Research Ethics Service

East Central London REC 1

South House, Block A
Royal Free Hospital
Pond Street
London
NW3 2QG

Telephone: 020 7794 0552

Dr James Malone-Lee
Department of Medicine
Centre for Clinical Science, Technology & Geriatrics
Clerkenwell Building
Highgate Hill, London
N19 5LW

23 February 2011

Dear Dr Malone-Lee

Full title of study: A blinded observational cohort study of the immunological changes associated with chronic cystitis and overactive bladder symptoms in women

REC reference number: 11/H0721/7

Thank you for your letter of 21 February 2011. I can confirm the REC has received the documents listed below as evidence of compliance with the approval conditions detailed in our letter dated 12 January 2011. Please note these documents are for information only and have not been reviewed by the committee.

Documents received

The documents received were as follows:

Document	Version	Date
Covering Letter		21 February 2011
Participant Information Sheet: Patient	2.0	21 February 2011
Participant Information Sheet: Control	2.0	21 February 2011
Participant Consent Form: Control	1.0	21 February 2011

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

11/H0721/7	Please quote this number on all correspondence
------------	---

Yours sincerely



Kathy Clark
Committee Co-ordinator

pp

Copy to: **Mr David Wilson, R&D office**

This Research Ethics Committee is an advisory committee to London Strategic Health Authority
The National Research Ethics Service (NRES) represents the NRES Directorate within
the National Patient Safety Agency and Research Ethics Committees in England

- R&D Approval – Chapter 9



1st Floor, Bloomsbury Building
St Pancras Hospital
4 St Pancras Way
NW1 0PE

Tel: 020 3317 3045
Fax: 020 7685 5830/5788
www.noclor.nhs.uk
05 November 2015

Professor James Malone-Lee
Centre for Clinical Science and Technology
UCL, Royal Free Campus,
Rowland Hill Street, London
NW3 2PF

Dear Professor Malone-Lee,

I am pleased to confirm that the following study has now received R&D approval, and you may now start your research in the trust identified below:

Study Title: An analysis of outcomes and adverse events associated with the treatment of recalcitrant lower urinary tract infection		
R&D reference: 168107		
REC reference: N/A		
Name of the trust	Name of current P/IC	Date of permission issue(d)
Whittington Hospital NHS Trust	Professor James Malone-Lee	05 November 2015
If any information on this document is altered after the date of issue, this document will be deemed INVALID		

Specific Conditions of Permission (if applicable)
If any information on this document is altered after the date of issue, this document will be deemed INVALID

Yours sincerely,



Mabel Saill
Research & Development Manager

May I take this opportunity to remind you that during the course of your research you will be expected to ensure the following:

- **Patient contact:** only trained or supervised researchers who hold the appropriate Trust/NHS contract (honorary or full) with each Trust are allowed contact with that Trust's patients. If any researcher on the study does not hold a contract please contact the R&D office as soon as possible.
- **Informed consent:** original signed consent forms must be kept on file. A copy of the consent form must also be placed in the patient's notes. Research projects are subject to random audit by a member of the R&D office who will ask to see all original signed consent forms.
- **Data protection:** measures must be taken to ensure that patient data is kept confidential in accordance with the Data Protection Act 1998.
- **Health & safety:** all local health & safety regulations where the research is being conducted must be adhered to.
- **Serious Adverse events:** adverse events or suspected misconduct should be reported to the R&D office and the Research Ethics Committee.
- **Project update:** you will be sent a project update form at regular intervals. Please complete the form and return it to the R&D office.
- **Publications:** it is essential that you inform the R&D office about any publications which result from your research.
- **Ethics:** R&D approval is based on the conditions set out in the favourable opinion letter from the Research Ethics Committee. If during the lifetime of your research project, you wish to make a revision or amendment to your original submission, please contact both the Research Ethics Committee and R&D Office as soon as possible.
- **Monthly / Annually Progress report:** you are required to provide us and the Research Ethics Committee with a progress report and end of project report as part of the research governance guidance.
- **Recruitment data:** if your study is a portfolio study, you are required to upload the recruitment data on a monthly basis in the website: <http://www.crn.nihr.ac.uk/can-help/funders-academics/nihr-crmp-portfolio/recruitment-data/>
- **Amendments:** if your study requires an amendment, you will need to contact the Research Ethics Committee. Once they have responded, and confirmed what kind of amendment it will be defined as, please contact the R&D office and we will arrange R&D approval for the amendment. If your study is Portfolio Adopted, amendments must be submitted for R&D review via the NIHR CRN (CSP), please refer to the Amendments Guidance for Researchers: <http://www.crn.nihr.ac.uk/can-help/funders-academics/gaining-nhs-permissions/amendments/>
- **Audits:** each year, noclor select 10% of the studies from each service we have approved to be audited. You will be contacted by the R&D office if your study is selected for audit. A member of the governance team will request you complete an audit monitoring form before arranging a meeting to discuss your study.

Appendix 4 Consent form

Community Lower Urinary Tract Service
Hornsey Central Neighbourhood Health Centre
2nd Floor
151 Park Road
London
N8 8JD

Study Number:

Participant Identification Number for this study:

CONSENT FORM

A blinded cross-sectional survey of the pathophysiological signals detectable in the urine of patients with overactive bladder, bacterial cystitis, painful bladder syndrome, ketamine cystitis, cystoplasty, early viable single intrauterine pregnancy and tubal ectopic pregnancy. ("Urine study")

Name of Researcher:

Please initial box

1. I confirm that I have read and understand the information sheet dated 20th October 2014 (version 5.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. 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.

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the sponsor of the trial (UCL), from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I agree to my GP being informed of my participation in the study.

5. I agree to take part in the above study.

Name of Patient

Date

Signature

Name of Person taking consent

Date

Signature

Name of Chief Investigator

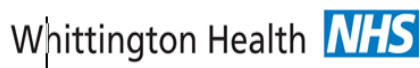
Date

Signature

(if different to the person taking consent)

When completed: 1 for participant; 1 (original) for researcher site file; 1 to be kept in medical notes.

Appendix 5 Participant information sheet



Community Lower Urinary Tract Service
Hornsey Central Neighbourhood Health Centre
2nd Floor
151 Park Road
London
N8 8JD

Participant Information Sheet

Short title: Pathophysiological signals of the urine in lower urinary tract symptoms

A blinded cross-sectional survey of the pathophysiological signals detectable in the urine of patients with overactive bladder, bacterial cystitis, painful bladder syndrome, ketamine cystitis, cystoplasty, early viable single intrauterine pregnancy and tubal ectopic pregnancy. ("Urine study")

Version 5.0 20th October 2014

We would like to invite you to take part in our research study. Before you decide we should like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. We suggest that this should take about 20 minutes. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear. Take time to decide whether or not you wish to take part.

What is the purpose of the project?

Many people suffer with lower urinary tract problems causing a variety of bladder symptoms that can include urinary incontinence (leaking from the bladder). There may be problems with bladder emptying and with recurrent urinary infection. We recognise that the symptoms are very unpleasant.

We have been working with patients who suffer from bladder troubles for many years. We have discovered that many of these people demonstrate a previously unrecognised inflammatory reaction in the urine that is evident provided that the urine is examined very fresh by a light microscope. When these specimens are sent to the ordinary laboratory more than half are reported as not showing infection, termed "culture negative". We have developed a better method for analysing the samples that uses very sensitive microbiological methods and found that we can identify bacterial infection in over 80%.

We have some evidence that our affected patients are not suffering from recurrent urine infection but from the same chronic infection, going on for months or years, which from time to time becomes acutely worse for periods. What is more we believe that the bacteria are living inside the cells of the bladder where they are protected from immune and antibiotic attack so that treatment has to use methods that are different to those used to treat ordinary urinary infection.

We seek to find out whether this is definitely the case in patients with lower urinary tract symptoms and to discover what the symptoms are really telling us. We want to know whether such infections may be adding to the problems that affect the bladder. We are also interested in studying newer and more sensitive markers of infection.

We are inviting you to take part in a Pilot Study. This is a small study and the results will help us decide if we need to carry out a much bigger study in the future.

Why have I been invited?

You have been chosen as you fulfil one of the following descriptions

- ◆ You have symptoms of disease of the lower urinary tract
- ◆ You have no symptoms but would like to volunteer as a normal control.

We hope to include 1800 persons in total

Do I have to take part?

It is up to you if you decide whether or not to take part. If you do decide to take part you will be given a copy of this information sheet to keep. You will be asked to sign a consent form, a copy of which will be given to you. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care you receive.

What will happen to me if I take part?

If you decide to take part in the project the research clinician will arrange to see you. You will be asked some questions about your bladder symptoms. Your answers will be recorded on a form stored on a computer. This will take about 30 minutes. The computer record is closely guarded by the NHS security system so there is no unauthorised access to your record.

A urine specimen will be taken by the usual midstream method. Part of the sample will be sent to the hospital laboratory to check for any bacteria in your urine, and the remainder will stay in our department for a number of tests. Immediately after the sample is provided, it will be looked at under the microscope by the research staff to detect small 'white blood cells,' which can signify an infection in the urine.

A lot of the time, tests performed in the hospital laboratory can come back as negative, and looking for white blood cells under the microscope straight away

Pathophysiological signals of urine in lower urinary tract symptoms

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is a way of detecting infection that may otherwise have been missed. We would like to record the results of these tests in your research records.

The urine will also be used to examine the inflammatory reaction that is occurring in your bladder.

We shall be saving an aliquot of your urine and freezing it in order to keep it preserved. At a later date this urine sample will be tested of the presence of a number of chemical that we hope will lead us to a better understanding of the diseases that are affecting our patients. Included in these tests, we will be looking into studying difficult to culture bacteria such as Chlamydia that can be detected via other scientific techniques. You may be asked to provide a blood sample to measure the C-Reactive Protein (CRP) and Erythrocyte Sedimentation Rate (ESR) values. Both of these tests measure the level of inflammation or infection present at that point in time. The level of CRP and ESR rise when there is inflammation and infection throughout the body.

The data that we obtain from these studies will not provide a definitive answer to our enquiries but will guide us considerable over the future direction of our research efforts.

Expenses and Payments

We shall be able to reimburse you for travel expenses incurred from attending the study visits and for additional sustenance arising from this travel. We should be grateful for receipts describing the expenses for our auditors. We are not in a position to pay you for participation in the study

Other studies

It would not be advisable for you to participate in this study if you are already a subject in another study.

Pregnancy

We are not including women who are pregnant in the main study. There is a separate arm of the study for women who are pregnant. Please ask for if you would like more information.

What will I have to do?

You will have to:

1. Give your consent
2. Attend the our department for your scheduled visit
3. Provide urine specimens at this visit.

Day Identification and visit	Actions
Pre-study	You will be sent or given a Patient Information Sheet
Between 0 and 48 hours	You will be contacted to ask if you are interested in

later	taking part in the study or asked at your routine appointment. If yes, an appointment will be made for a Visit or you will be asked to undertake the study the same day
Day 0 Visit 1	<ul style="list-style-type: none"> ◆ Screening to check eligibility ◆ Study explained and any questions answered ◆ Informed consent form signed ◆ Completes symptom questionnaires ◆ Provide a blood sample (selected patients only) ◆ Provide Midstream Urine sample obtained ◆ Analysis of urine sample <ul style="list-style-type: none"> ◆ An aliquot will be stored frozen at -80°C for subsequent transfer to the Immunology Laboratories at UCL, the laboratories of the Centre for Clinical Science and Technology and the Pfizer Laboratories in Kent dependent on the assays required.

What about my current treatments for other conditions?

You will be able to continue all of your normal treatments and these will not be affected by this study.

What are the alternatives to participating in the study?

It is entirely your decision if you wish to take part in the study and it will not affect your future care if you do not wish to do so. If you do not wish to take part you will have your assessment in the usual way and your condition will be managed as is done routinely by your consultant.

What are the possible disadvantages and risks of taking part?

You will be seen for an additional visit to this clinic. However, you will be seen outside of the clinic and will not have to wait as in an ordinary clinic. We shall do our best to book you in line with your convenience. When the blood sample is taken you may you may feel slight pain or a sting when the needle is inserted.

What are the possible benefits of taking part?

There may not be any advantage. However, you will also have access to unusually close monitoring of your bladder symptoms, which despite your involvement in this study will be managed by our usual methods usual ways.

What happens when the research study stops?

We shall be pleased to continue with your normal clinical care in the usual ways.

What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information concerning this is given in Part 2 of this information sheet. If you have any concerns or [complaints](#) you should contact your study doctor in the first instance.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2.

Contact Details

Your Doctor

Professor James Malone-Lee

Tel. Number: 020 3074 2251

Dr Sheela Swamy

Tel. Number: 020 3074 2253

This completes Part 1 of the Information Sheet. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Participant Information Sheet – Part 2

What if new information becomes available?

Sometimes during the course of a research project, new information becomes available about the disease area that is being studied. If this happens, and it is relevant to your situation, your research doctor or nurse will tell you about it, and discuss with you and change in management that might be appropriate.

What will happen if I don't want to carry on with the study?

You are free to withdraw from the study at any time before and after signing the consent form without needing to give any explanations. The study may be ended at any time with or without your consent.

Unless you wish otherwise, the data collected from you up to the point at which you leave the study will be used in the analysis.

What if there is a problem?

Every care will be taken to ensure your safety during the course of the study. If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions. Please contact Professor Malone-Lee or Dr Dhanuson in the first instance

Harm

In the event that something does go wrong and you are harmed as a result of taking part in the approved research study UCL, the Research Sponsor, has indemnity (insurance) arrangements in place for non-negligent harm. If you are harmed and this is due to someone's negligence then you may have grounds for legal action for compensation against the Trust but you may have to pay your legal costs.

Every care will be taken in the course of this clinical trial. However in the unlikely event that you are injured by taking part, compensation may be available.

If you suspect that the injury is the result of the Sponsor's (University College London) or the hospital's negligence then you may be able to claim compensation. After discussing with your study doctor, please make the claim in writing to Professor Malone-Lee who is the Chief Investigator for the clinical trial and is based at Hornsey Central Neighbourhood Health Centre, 151 Park Road, Crouch End, London N8 8JD. The Chief Investigator will then pass the claim to the Sponsor's Insurers, via the Sponsor's office. You may have to bear the costs of the legal action initially, and you should consult a lawyer about this.

Participants may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of University College London or another party. You should discuss this possibility with your study doctor in the same way as above.

Complaints

Pathophysiological signals of urine in lower urinary tract symptoms
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If you have any questions about your rights as a research subject or have a complaint about the way in which the study has been carried out, please contact: Professor Malone-Lee

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital or from the Department of Health website: <http://www.dh.gov.uk>. You may obtain the necessary guidance from the hospital Patient Advice and Liaison Service (PALS), Whittington Health, Tel 020 7288 5956 or 020 7288 5957

Will my taking part in this study be kept confidential?

If you consent to take part in this study, the records obtained while you are in this study as well as related health records ~~will remain strictly confidential at all times~~. The information will be held securely on paper and electronically at the hospital site managing this research under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the Sponsor (UCL), who is not involved in the study. You will be allocated a study number, which will be used as a code to identify you on all study forms. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised (if it is applicable to your research).

Your records will be available to people authorised to work on the study but may also need to be made available to people authorised by the Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. By signing the consent [form](#) you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

If you withdraw consent from further study treatment, unless you object, your data and samples will remain on file and will be included in the final study analysis.

In line with the regulations, at the end of the study your data will be securely archived for a minimum of 5 years. Arrangements for confidential destruction will then be made.

Will my GP be informed of my involvement?

With your permission, your GP, and other doctors who may be treating you, will be notified that you are taking part in this study.

What will happen to any samples I give?

Blood samples taken will be sent to the hospital laboratory to be analysed to calculate the CRP and ESR results. This will then be communicated to the participating researchers. These results will be kept confidential.

We shall be taking a sample of urine for the study purposes and not as a part of normal clinical care. This sample will not be labelled with information that can directly identify you, only with the study number that has been allocated to you. Only the researchers working on this project will have access to your sample.

Part of the urine sample will be sent to the hospital laboratory to check for any bacteria in your urine and another portion will be looked at under the microscope to detect small 'white blood cells,' which can signify an infection in the urine.

A portion of the urine sample will be frozen and stored in a monitored freezer in a secure laboratory in the Department of Medicine. This will then be passed to the Immunology Laboratories at UCL, the laboratories of the Centre for Clinical Science and Technology dependent on the tests required and a percentage of samples will be passed on to the Pfizer Laboratories in Kent. These samples will be anonymised. Once analysed the urine sample will be disposed of as per UCL regulations.

At these laboratories a number of experiments will be conducted on the sample in order to search for chemicals that might, in the future, be used to help us in the diagnosis of bladder disease. We are constantly trying to improve the methods that we have available to help in identifying the cause of a patient's symptoms and to monitor the response to treatment

Will any genetic tests be done?

No genetic tests will be done.

What will happen to the results of the research?

We shall use the data to make decisions on how we should plan our future discovery research. We are hoping to identify signals that should encourage us to look at some areas more closely.

Who is organising and funding the research?

This study is organised by the Department of Medicine at the Whittington Hospital in collaboration with the Division of Immunology at UCL, the Department of Urology at UCL and Pfizer Ltd. The research is funded by Professor Malone-Lee's research funds. The study is sponsored by University College London.

Who has reviewed the study?

All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by London East Research Ethics Committee

Further information and contact details

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your study doctor, who will be able to provide you with up to date information about the drug(s)/procedure(s) involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor. If you

require any further information or have any concerns while taking part in the study please contact one of the following people:

Your Doctor	
Professor James Malone-Lee	Tel. Number: 020 3074 2251
Dr Sheela Swamy	Tel. Number: 020 3074 2253

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

Appendix 6 Instructions on clean catch MSU

Midstream Urine Specimen

For correct test results, follow instructions carefully.

A jug, a plastic container and a wet gauze will be given to you

Cleansing before collecting the urine specimen

1. Squeeze and rub some alcohol gel onto your hands once you are in the toilet. There are alcohol gel bottles in each toilet.
2. Thoroughly cleanse the entire genital area using the special gauze that is in the jug.
 - Females – hold the outer edges of labia apart and cleanse from front to back with the gauze. One wipe only!
 - Males – retract foreskin if not circumcised and wipe the end of the penis with the gauze. One wipe only!

Please do not throw the gauze into the toilet bowl. Throw it into the yellow bin which is on the floor of the toilet

Collecting the urine specimen

- Females – continue to hold labia apart while urinating
 - Males – continue to retract foreskin while urinating
1. Urinate (pee) a small amount of urine into the toilet.
 2. Then without stopping, catch some urine into the bowl by passing it into the urine stream.
 3. As the stream comes to the end move the bowl away and urinate (pee) the rest into the toilet
 4. Put the lid on the jug and bring it to a member of staff.
5. Wash hands after collecting the urine specimen.

Appendix 7 Sediment culture protocol

Sediment Culture Stages

1. Culture of sediment

Spin 5ml urine down with centrifuge for 5 minutes at 2000rpm

Remove supernatant from spun specimen using pipette, leaving behind sediment

Resuspend sediment in 400 µl phosphate buffered solution (PBS)

Split one CPS3 plate into 4 parts (if doing serial dilutions see below)

Pipette 50µl of the resuspended sediment onto each plate half of both plates and spread using spreader (hockey stick)

Place both in the incubator for 24 hrs

2. Serial dilutions

Add 900 µl into 3 epindorfs marked -1,-2, -3

Pipette 100µl of the resuspended sediment (neat) into epindorf -1 and mix well

Pipette 100µl of -1 solution into epindorf -2 and mix well

Pipette 100µl of -2 solution into epindorf -3 and mix well

Split the CPS 3 plate into 4 quadrants marked Neat, -1,-2, -3 and pipette 50µl into each quarter and spread with spreader (hockey stick).

3. Incubate for 24 hours.

4. Colony count and Subculture

Identify the different colonies on each plate using the CPS3 colour chart and perform a colony count for each type on each half of the plate.

The colony forming units per ml for each type of colony can be worked out by using the following method:

Count number of colonies on each half and take an average or count in each quadrant and take an average

$(\text{count} \times 20 \times \text{dilution factor} \times 0.4) = A$

$A / \text{total volume of urine used (5 mls)} = \text{cfu/ml}$

Aerobic Subcultures if you are going to freeze the individual isolates or if the plate has produced a mixed growth making identification difficult.

Take a single colony of each isolate with 1µl loop and streak onto one CBA plate.

Retain in incubator for 24 hrs.

Repeat this process in the event of a mixed culture.

Bacterial Identification (Genus only) using Colour charts and bench top tests

1. Identification by colour (CPS3 plate)

Colours

- ***E Coli***: Burgundy, mauve, white, pink (has also been green). Size Varies
- ***Pseudomonas***: pigmented white/cream (small to medium), wet
- ***Proteus***: White/cream, often with brown tinge and unbearable sweet odour
- ***Citrobacter***: While/cream (small to medium)
- **KES**: Green/green-brown/Turquoise green, Medium to large, Muroid
- ***Enterococcus***: Tiny to small turquoise (**Staphylococcus* species sometimes can grow in this colour-do a catalase test to eliminate this possibility)
- ***Staph.aureus*** –Small creamy yellow
- ***Streptococcus Agalactiae*** - Tiny to small dark purple/violet/ Indigo/purple-blue (*Staph* species can sometimes appear in this colour –do a catalase test to eliminate this possibility)

White Colony – Gram stain is a must

Colour not on chart – Do Gram stain and Api

*Gram stain all white colonies

2. Gram Stain Method:

Transfer 4-5 drops of sterile water or PBS solution onto a microscope slide

Take a single pure colony and rub into PBS on a slide

Leave slide to dry on a slide burner (5-10 min)

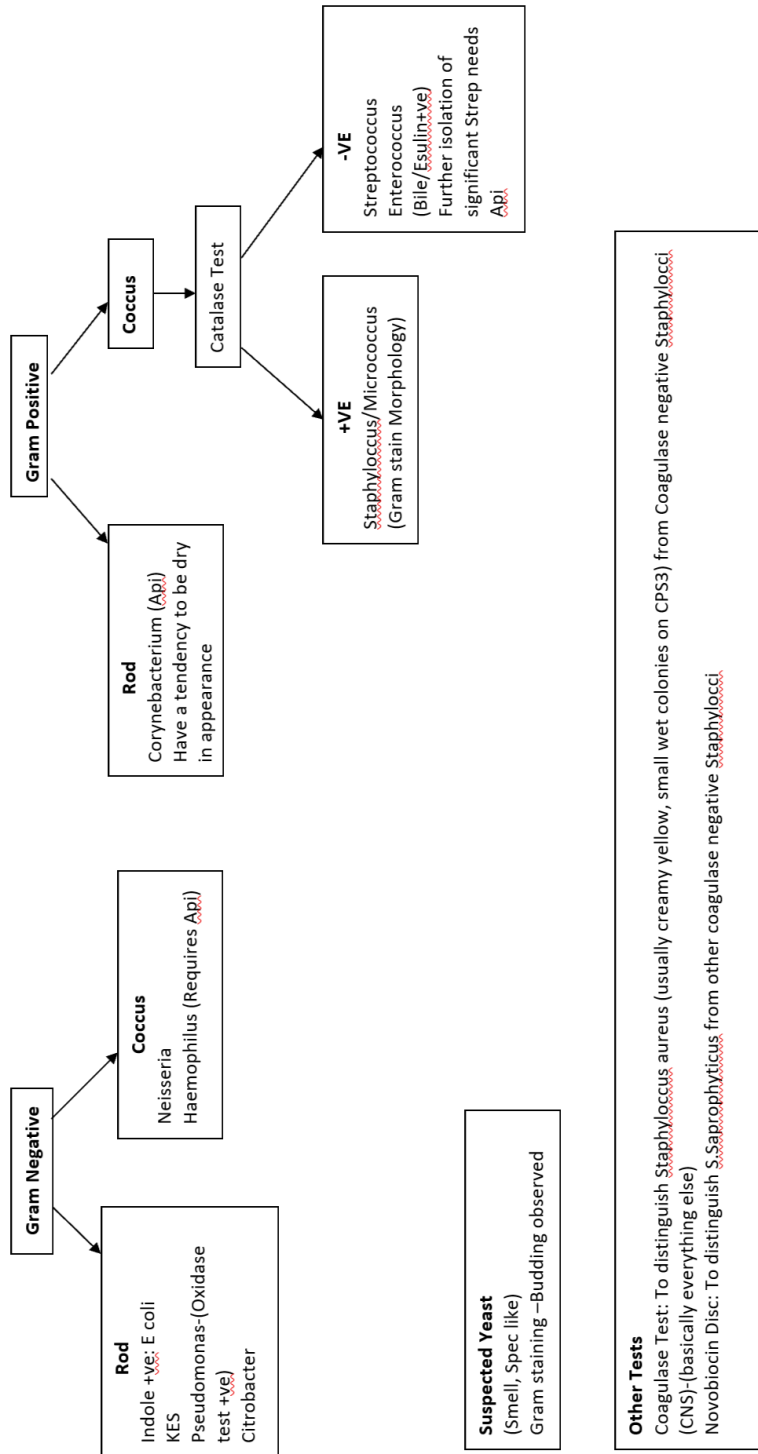
1. Crystal Violet –flood slide for 30 seconds then Rinse slide with stream of water
2. Gram's Iodine –flood slide for 30 seconds then Rinse slide with stream of water
3. Acetone –flood slide for 1-2 seconds then Rinse slide with stream of water

Dilute Carbol fuchsin – flood slide for 30 seconds then Rinse slide with stream of water

Leave slide to dry on slide burner (5-10 min)

Add one drop of immersion oil and view under light microscope at x 100 (oil immersion lens)

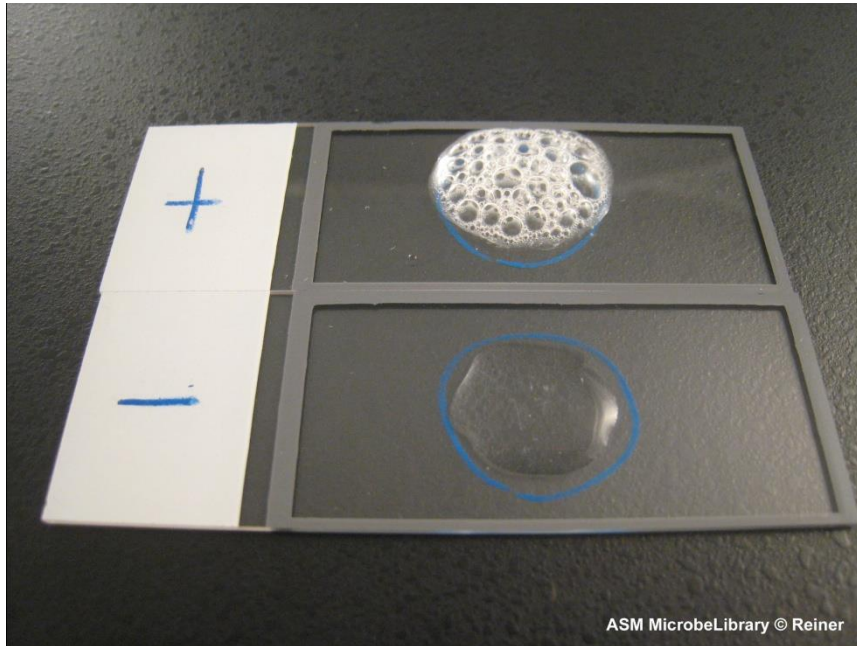
Gram Stain



Bench Top Tests:

1. Indole test for Gram negative Rod – Ecoli will be positive, KES, Proteus and Pseudomonas will be negative
2. Oxidase test for Gram Negative Rod – Positive for Pseudomonas only
3. Catalase Test

Using a sterile inoculating loop or wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place it onto the microscope slide. Be careful not to pick up any agar. This is particularly important if the colony isolate was grown on agar containing red blood cells. Carryover of red blood cells into the test may result in a false-positive reaction. Using a dropper or Pasteur pipette, place 1 drop of 3% H_2O_2 onto the organism on the microscope slide. Do not mix. Immediately cover the petri dish with a lid to limit aerosols and observe for immediate bubble formation ($\text{O}_2 + \text{water} = \text{bubbles}$). Observing for the formation of bubbles against a dark background enhances readability.



Appendix 8 DAPI epifluorescent cytology protocol

1. Prepare 4 % formaldehyde in Phosphate buffered saline (PBS) – dilute 16% formaldehyde (16% formaldehyde 1 ml ampules, Thermo Scientific, from Fisher Scientific) 1:4 in PBS solution ie) 25 μ l 16% formaldehyde into 75 μ l PBS. Keep away from light.
2. Prepare combined Wheat germ agglutinin (WGA) and DAPI stain. Wheat germ agglutinin (WGA) is conjugated to alexa fluor-488 (Invitrogen Ltd, Paisley, UK) and is used to label the cell membrane to aid cellular identification. DAPI (4',6-diamidino-2-phenylindole) (Sigma-Aldrich) is used for fluorescent counterstaining of host and pathogen DNA. Using the stock WGA (orange in colour and labeled WGA) and DAPI (green in colour and labeled DAPI) aliquots stored at -20°C, prepare a WGA 1 in 200 dilution and DAPI 1 in 100 dilution in Hanks balance salt solution (HBSS) ie) add 1 μ l WGA and 2 μ l DAPI to 197 μ l HBSS.
3. Pre label a Thermo Scientific, Superfrost Ultra Plus microscope glass slide and approximately mark field area for cells using filter card (Fisher Scientific) as guide.
4. Assemble cuvette holder, slide, filter card and Shandon single funnel cuvette.
5. Position assembled cuvette in cytocentrifuge (Shandon cytospin 2 cytocentrifuge) and balance.
6. Add 80 μ l of the human urine to single funnel cuvette assembly.
7. Spin at high acceleration, at 800rpm (\approx 75g RCF) for 5 minutes to result in a visible disc of urinary particulate, approximately 5mm in diameter (\approx 20 mm²), deposited on the slide.
8. Promptly remove from cytocentrifuge and carefully remove slide without dislodging cellular deposit.
9. Circumscribe area with a hydrophobic barrier pen (ImmEdge pen, Vector Laboratories) to avoid wastage and to act as a visual aid.
10. Fix cells by adding 80 μ l of 4% formaldehyde over cellular deposit. Ensure cells are fully covered. Cover slides with foil try to shield from light.
11. Leave for 20 minutes at room temperature.
12. Aspirate the 80 μ l of 4% formaldehyde and wash with 80 μ l PBS three times
13. Add 80 μ l of the earlier prepared WGA/DAPI stain. Ensure cells are covered. Cover slides with foil try to shield from light.
14. Aspirate WGA/DAPI stain and with 80 μ l of PBS four times.
15. Ensure no excess fluid is left on slide.
16. Immediately mount the slide with a drop of FluorSave mounting reagent (Calbiochem).
17. Gently place on a cover slip ensuring minimal or no air bubbles. If there is any excess mounting fluid gently aspirate. Secure cover slip with clear nail varnish at each corner. Avoid any movement of the coverslip to avoid any disruption or smudging of cells.
18. Cover slide with foil tray to prevent exposure to light and allow slide to dry for at least 1 hour.
19. Once dry store slide at 4°C in a light protective box.
20. View slide using fluorescent microscope to perform clue cell count. Alexa fluor 488 excites at a wavelength of 495nm and emits at 519nm, appearing green

under fluorescence. DAPI excites at a wavelength of 360nm and emits at 460nm giving mammalian nuclei and bacteria a blue appearance under fluorescence. It is capable of penetrating cellular membranes, hence the ability to label intracellular and adherent pathogens without the need for permeabilisation.

Appendix 9 Whittington pain score questionnaire

Pathophysiological signals in lower urinary tract symptoms Sponsor Protocol ID 11/0017

Whittington Pain Score (Chaliha et al 2009)

Study number:

Initials:

Visit:

Your research doctor will fill in the 'study number', 'initials' and 'visit'.

Whittington Pain Score

Please enter date of completion: **dd-mmm-yy**

We should be grateful if you would answer the following questions, thinking about how you are **at the moment**. Please put a circle around your response for each question.

Do you experience pain or discomfort on bladder filling?	Yes	No
Do you experience pain or discomfort in or over the pubic area?	Yes	No
Do you experience burning or pain when passing urine?	Yes	No
Do you experience pain in your back over your kidneys?	Yes	No
Do you experience pain in the genitals?	Yes	No
Do you experience pain on the left or right side of the lower abdomen?	Yes	No
Do you experience general abdominal pain or discomfort?	Yes	No
Do you experience pain radiating down your legs?	Yes	No

Total score	For Doctor
--------------------	------------

Appendix 10 Whittington Urgency score questionnaire

Pathophysiological signals in lower urinary tract symptoms Sponsor Protocol ID 11/0017

Whittington Urgency Score (Al Buheissi et. al. 2008)

Study number: Initials: Visit:

|

Your research doctor will fill in the 'study number', 'initials' and 'visit'.

Whittington Urgency Score

Please enter date of completion: **dd-mmm-yy**

We should be grateful if you would answer the following questions, thinking about how you are **at the moment**. Please put a circle around your response for each question.

Do you experience urgency? <i>That is having to hurry in order to pass urine</i>	None	Some	Much
Do you experience urge incontinence? <i>That is hurrying to pass urine and not making it in time</i>	None	Some	Much
Does cold weather make your bladder symptoms worse?	None	Some	Much
Do you find that running water from a tap causes urinary urgency?	None	Some	Much
Do you find that running water from a tap causes incontinence?	None	Some	Much
Do you find that putting a key in the front door when returning home causes urinary urgency?	None	Some	Much
Do you find that putting a key in the front door when returning home causes urinary incontinence?	None	Some	Much
Do you find that on getting up from bed in the morning you experience urgency?	None	Some	Much
Do you find that on getting up from bed in the morning you experience urge incontinence?	None	Some	Much
Does anxiety or fatigue make your symptoms worse?	None	Some	Much

Total score	For Doctor
--------------------	------------

TURN OVER PLEASE...

Version 1.0 14-Jun-11

Appendix 11 ICIQ FLUTS questionnaire

Pathophysiological signals in lower urinary tract symptoms Sponsor Protocol ID 11/0017

ICIQ-FLUTS Long Form Copyright © "ICIQ Group" 2004

Study number: Initials: Visit:

Your research doctor will fill in the 'study number', 'initials' and 'visit'.

Urinary Symptoms Questionnaire

Please enter date of completion: **dd-mmm-yy**

We should be grateful if you would answer the following questions, thinking about how you have been, on average, over the **PAST FOUR WEEKS**.

1a. **How often do you pass urine during the day?**

One to six times	<input type="checkbox"/>	0
Seven to eight times	<input type="checkbox"/>	1
Nine to ten times	<input type="checkbox"/>	2
Eleven to twelve times	<input type="checkbox"/>	3
Thirteen times or more	<input type="checkbox"/>	4

1b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

2a. **During the night, how many times do you have to get up to urinate, on average?**

None	<input type="checkbox"/>	0
One	<input type="checkbox"/>	1
Two	<input type="checkbox"/>	2
Three	<input type="checkbox"/>	3
Four or more	<input type="checkbox"/>	4

2b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

Total score	For Doctor
--------------------	------------

Version 1.0 21-Jun-11

Study number: Initials: Visit:

3a. **Do you have a sudden need to rush to the toilet to urinate?**

Never	<input type="checkbox"/>	0
Occasionally	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Most of the time	<input type="checkbox"/>	3
All of the time	<input type="checkbox"/>	4

3b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all **0** **1** **2** **3** **4** **5** **6** **7** **8** **9** **10** *Great deal*

4a. **Does urine leak before you can get to the toilet?**

Never	<input type="checkbox"/>	0
Occasionally	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Most of the time	<input type="checkbox"/>	3
All of the time	<input type="checkbox"/>	4

4b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all **0** **1** **2** **3** **4** **5** **6** **7** **8** **9** **10** *Great deal*

5a. **Do you have pain in your bladder?**

Never	<input type="checkbox"/>	0
Occasionally	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Most of the time	<input type="checkbox"/>	3
All of the time	<input type="checkbox"/>	4

5b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all **0** **1** **2** **3** **4** **5** **6** **7** **8** **9** **10** *Great deal*

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6a. **How often do you leak urine?**

Never	<input type="checkbox"/>	0
Once or less per week	<input type="checkbox"/>	1
Two or three times a week	<input type="checkbox"/>	2
Once per day	<input type="checkbox"/>	3
Several times a day	<input type="checkbox"/>	4

6b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

7a. **Does urine leak when you are physically active, exert yourself, cough or sneeze?**

Never	<input type="checkbox"/>	0
Occasionally	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Most of the time	<input type="checkbox"/>	3
All of the time	<input type="checkbox"/>	4

7b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

8a. **Do you ever leak for no obvious reason and without feeling that you want to go?**

Never	<input type="checkbox"/>	0
Occasionally	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Most of the time	<input type="checkbox"/>	3
All of the time	<input type="checkbox"/>	4

8b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

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9. **How much urinary leakage occurs?**

No leakage	<input type="text"/>	0
Drops/pants damp	<input type="text"/>	1
Dribble/pants wet	<input type="text"/>	2
Floods/soaking through to outer clothing	<input type="text"/>	3
Floods/running down legs or onto floor	<input type="text"/>	4

10a. **Is there a delay before you can start to urinate?**

Never	<input type="text"/>	0
Occasionally	<input type="text"/>	1
Sometimes	<input type="text"/>	2
Most of the time	<input type="text"/>	3
All of the time	<input type="text"/>	4

10b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all **0** **1** **2** **3** **4** **5** **6** **7** **8** **9** **10** *Great deal*

11a. **Do you have to strain to start urinating?**

Never	<input type="text"/>	0
Occasionally	<input type="text"/>	1
Sometimes	<input type="text"/>	2
Most of the time	<input type="text"/>	3
All of the time	<input type="text"/>	4

11b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all **0** **1** **2** **3** **4** **5** **6** **7** **8** **9** **10** *Great deal*

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12a. **Do you stop and start more than once while you urinate?**

Never	<input type="text"/>	0
Occasionally	<input type="text"/>	1
Sometimes	<input type="text"/>	2
Most of the time	<input type="text"/>	3
All of the time	<input type="text"/>	4

12b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

13a. **Do you leak urine when you are asleep?**

Never	<input type="text"/>	0
Occasionally	<input type="text"/>	1
Sometimes	<input type="text"/>	2
Most of the time	<input type="text"/>	3
All of the time	<input type="text"/>	4

13b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

14a. **Would you say the strength of your urinary stream is...**

Not reduced	<input type="text"/>	0
Reduced a little	<input type="text"/>	1
Quite reduced	<input type="text"/>	2
Reduced a great deal	<input type="text"/>	3
No stream	<input type="text"/>	4

14b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

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Initials:

Visit:

15. **Have you ever blocked up completely, so that you could not urinate at all, and had to use a catheter to drain the bladder?**

- | | | |
|----------------------|--------------------------|---|
| Never | <input type="checkbox"/> | 0 |
| Yes, once | <input type="checkbox"/> | 1 |
| Yes, twice | <input type="checkbox"/> | 2 |
| Yes, more than twice | <input type="checkbox"/> | 3 |

16a. **Do you have a burning feeling when you urinate?**

- | | | |
|------------------|--------------------------|---|
| Never | <input type="checkbox"/> | 0 |
| Occasionally | <input type="checkbox"/> | 1 |
| Sometimes | <input type="checkbox"/> | 2 |
| Most of the time | <input type="checkbox"/> | 3 |
| All of the time | <input type="checkbox"/> | 4 |

16b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all **0** **1** **2** **3** **4** **5** **6** **7** **8** **9** **10** *Great deal*

17a. **How often do you feel that your bladder has not emptied properly after you have urinated?**

- | | | |
|------------------|--------------------------|---|
| Never | <input type="checkbox"/> | 0 |
| Occasionally | <input type="checkbox"/> | 1 |
| Sometimes | <input type="checkbox"/> | 2 |
| Most of the time | <input type="checkbox"/> | 3 |
| All of the time | <input type="checkbox"/> | 4 |

17b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all **0** **1** **2** **3** **4** **5** **6** **7** **8** **9** **10** *Great deal*

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18. **Can you stop the flow of urine when you are urinating?**

Yes easily

	0
--	---

Yes, with difficulty

	1
--	---

No, cannot stop it flowing

	2
--	---

Thank you very much for answering these questions.

Appendix 12 ICIQ-LUTSqol questionnaire

Pathophysiological signals in lower urinary tract symptoms Sponsor Protocol ID 11/0017

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Study number:

Initials:

Visit:

Your research doctor will fill in the 'study number', 'initials' and 'visit'

Urinary Symptoms Questionnaire

Please enter date of completion: **dd-mmm-yy**

We should be grateful if you would answer the following questions, thinking about how you have been, on average, over the **PAST FOUR WEEKS**.

- 1a. **To what extent does your urinary problem affect your household tasks (e.g. cleaning, shopping, etc.)**

Not at all	<input type="text"/>	1
Slightly	<input type="text"/>	2
Moderately	<input type="text"/>	3
A lot	<input type="text"/>	4

- 1b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 *Great deal*

- 2a. **Does your urinary problem affect your job, or your normal daily activities outside the home?**

Not at all	<input type="text"/>	1
Slightly	<input type="text"/>	2
Moderately	<input type="text"/>	3
A lot	<input type="text"/>	4

- 2b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 *Great deal*

Total score	For Doctor
--------------------	------------

Version 1.0 21-Jun-11

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Study number:

Initials:

Visit:

3a. Does your urinary problem affect your physical activities (e.g. going for a walk, run, sport, gym, etc.)?

Not at all	<input type="checkbox"/>	1
Slightly	<input type="checkbox"/>	2
Moderately	<input type="checkbox"/>	3
A lot	<input type="checkbox"/>	4

3b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 *Great deal*

4a. Does your urinary problem affect your ability to travel?

Not at all	<input type="checkbox"/>	1
Slightly	<input type="checkbox"/>	2
Moderately	<input type="checkbox"/>	3
A lot	<input type="checkbox"/>	4

4b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 *Great deal*

5a. Does your urinary problem limit your social life?

Not at all	<input type="checkbox"/>	1
Slightly	<input type="checkbox"/>	2
Moderately	<input type="checkbox"/>	3
A lot	<input type="checkbox"/>	4

5b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 *Great deal*

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Visit:

6a. Does your urinary problem limit your ability to see/visit friends?

Not at all	<input type="checkbox"/>	1
Slightly	<input type="checkbox"/>	2
Moderately	<input type="checkbox"/>	3
A lot	<input type="checkbox"/>	4

6b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

7a. Does your urinary problem affect your relationship with your partner?

Not applicable	<input type="checkbox"/>	0
Not at all	<input type="checkbox"/>	1
Slightly	<input type="checkbox"/>	2
Moderately	<input type="checkbox"/>	3
A lot	<input type="checkbox"/>	4

7b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

8a. Does your urinary problem affect your sex life?

Not applicable	<input type="checkbox"/>	0
Not at all	<input type="checkbox"/>	1
Slightly	<input type="checkbox"/>	2
Moderately	<input type="checkbox"/>	3
A lot	<input type="checkbox"/>	4

8b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

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Study number: Initials: Visit:
 9a. **Does your urinary problem affect your family life?**

Not applicable	<input type="text"/>	0
Not at all	<input type="text"/>	1
Slightly	<input type="text"/>	2
Moderately	<input type="text"/>	3
A lot	<input type="text"/>	4

9b. **How much does this bother you?**
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

10a. **Does your urinary problem make you feel depressed?**

Not at all	<input type="text"/>	1
Slightly	<input type="text"/>	2
Moderately	<input type="text"/>	3
Very much	<input type="text"/>	4

10b. **How much does this bother you?**
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

11a. **Does your urinary problem make you feel anxious or nervous?**

Not at all	<input type="text"/>	1
Slightly	<input type="text"/>	2
Moderately	<input type="text"/>	3
Very much	<input type="text"/>	4

11b. **How much does this bother you?**
Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

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Study number:

Initials:

Visit:

12a. Does your urinary problem make you feel bad about yourself?

Not at all	<input type="checkbox"/>	1
Slightly	<input type="checkbox"/>	2
Moderately	<input type="checkbox"/>	3
Very much	<input type="checkbox"/>	4

12b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

13a. Does your urinary problem affect your sleep?

Never	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Often	<input type="checkbox"/>	3
All the time	<input type="checkbox"/>	4

13b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

14a. Do you feel worn out/tired?

Never	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Often	<input type="checkbox"/>	3
All the time	<input type="checkbox"/>	4

14b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

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Study number: Initials: Visit:

Do you do any of the following? If so, how much?

15a. **Wear pads to keep dry?**

Never	<input type="text"/>	1
Sometimes	<input type="text"/>	2
Often	<input type="text"/>	3
All the time	<input type="text"/>	4

15b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

16a. **Be careful how much fluid you drink?**

Never	<input type="text"/>	1
Sometimes	<input type="text"/>	2
Often	<input type="text"/>	3
All the time	<input type="text"/>	4

16b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

17a. **Change your underclothes when they get wet?**

Never	<input type="text"/>	1
Sometimes	<input type="text"/>	2
Often	<input type="text"/>	3
All the time	<input type="text"/>	4

17b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

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Study number: Initials: Visit:

18a. **Worry in case you smell?**

Never	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Often	<input type="checkbox"/>	3
All the time	<input type="checkbox"/>	4

18b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

19a. **Get embarrassed because of your urinary problem?**

Never	<input type="checkbox"/>	1
Sometimes	<input type="checkbox"/>	2
Often	<input type="checkbox"/>	3
All the time	<input type="checkbox"/>	4

19b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

20. **Overall, how much do urinary symptoms interfere with your everyday life?**

Please ring a number between 0 (not at all) and 10 (a great deal)

Not at all 0 1 2 3 4 5 6 7 8 9 10 Great deal

Thank you very much for answering these questions.

Appendix 13 Protocol for management of patients

Protocol for management of patients with chronic lower urinary tract symptoms with clinical evidence of urinary tract infection – Whittington Lower Urinary Tract Symptoms Clinic Revised January 2018

The evidence for the practices described in this protocol have been reviewed briefly in a separate accompanying paper.

Typical patient This protocol covers the management of patients with lower urinary tract symptoms likely to include recurrent urinary infections, chronic bladder pain, interstitial cystitis and chronic cystitis. Their symptoms have been present for an average of five years. The average age of the patients is 50 (95% CI 48 to 51), 80% female 20% male. Most will describe a history of multiple tests and consultations in secondary and/or tertiary care. The story of symptoms despite numerous normal urinalyses is common and most patients believe that doctors think there is nothing wrong with them. The typical investigations that have been used include blood tests, renal tract and pelvic ultrasound scans, CT scans, MRI scans, and urodynamics. Most patients will have undergone cystoscopy, with or without urethral dilation or cystodistension. Bladder biopsies will have revealed various manifestations of chronic cystitis. It is common to report multiple cystoscopies. Other than bladder biopsies, these various investigations will usually have proved negative. A variety of bladder infusion treatments may have been attempted without benefit.

Outcomes Using symptoms scores [1, 2] and analyses of urinary white blood cell excretion [3, 4] and urothelial cell shedding [5, 6] we have been able to measure the outcomes to the treatment regimes covered in this paper. The evidence gleaned implies that the treatments are successful in resolving these symptoms [7].

Abbreviations Urinary tract infection (UTI) Lower Urinary Tract Symptoms (LUTS) White blood cells (wbc) Urinary epithelial cells (epc) USp – simple void into bowl and collecting first part of stream [8] Extended spectrum beta-lactamase – (ESBL)

Communication These notes are commensurate with the scripts that we use to explain our management methods which are used to communicate with the GPs and the patients. Both parties receive identical information.

Tools to assist diagnosis Symptoms

- **Voiding symptoms**
- **Pain symptoms**
- **Urgency symptoms**
- **Stress incontinence symptoms**
- **History of recurrence and the story of management**

Table 1
The question inventory in the order that the questions are asked

Storage symptoms	Stress symptoms	Voiding symptoms	Pain symptoms
1.Urgency	12.Cough sneeze incontinence	20.Hesitancy	27.Suprapubic pain
2.Urge incontinence	13.Exercise incontinence	21.Reduced stream	28.Filling bladder pain
3.Latchkey urgency	14.Laughing incontinence	22.Intermittent stream	29.Voiding bladder pain
4.Latchkey urge incontinence	15.Passive Incontinence	23.Straining to void	30.Post void bladder pain
5.Waking urgency	16.Bending incontinence	24.Terminal dribbling	31.Pain relieved by voiding
6.Waking urge incontinence	17.Standing incontinence	25.Postvoid dribbling	32.Partially voiding relief
7.Running water urgency	18.Lifting incontinence	26.Double voiding	33.No voiding relief
8.Running water urge incontinence	19.Pre-cough preparation		34.Loin pain
9.Cold urgency			35.Iliac fossa pain
10.Anxiety urgency			36.Pain radiation to genitals
11.Premenstrual aggravation			37.Pain radiation to legs
			38.Dysuria
			39.Urethral pain

Signs

- Suprapubic tenderness
- Loin tenderness
- Urethral tenderness
- Prostate tenderness

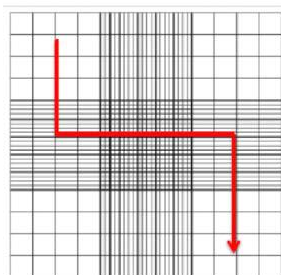
Testing

- **Urinary pyuria >0 wbc μl^{-1} on microscopy of an immediately fresh unspun properly collected urine sample (US) [3, 9]**
- **Urinary epithelial cell count >0 epc μl^{-1} on microscopy of an immediately fresh unspun properly collected urine sample (US) [6]**

Immediate fresh urine microscopy for interpretation of lower urinary tract symptoms

A fresh, unspun aliquot of urine was examined by microscopy. Urine (1 μl) is loaded into a clean Neubauer haemocytometer counting chamber [10]. This preparation is examined using a x20 objective with a x10 eyepiece (magnification x200). The leukocyte count (wbc μl^{-1}) are enumerated by counting cells in 5 large squares out of 9 and multiplying the result by 2 because the volume of the whole chamber was 0.9 μl . If a cell overlaps a dividing line it is counted if the line runs along the top or right side and ignored if the line runs along the bottom or left side. The same method is used to count the epithelial cells. We require the white cell count and the epithelial cell counts and it is helpful to plot these results on a time axis.

Neubauer haemocytometer counting chamber



The red arrow shows the pattern used to search for and count the cells in the urine sample.

- USp to laboratory for routine culture
- Lung function tests (If patients are taking Nitrofurantoin at each visit)
- Liver function tests (Given antibiotic exposure twice yearly)
- U&E & Creatinine (Given antibiotic exposure twice yearly)
- FBC (On clinical judgement)
- Inflammatory markers (On clinical judgement)

Monitoring disease progression and resolution

We generate plots of symptoms, pyuria and epithelial cells on a time axis. These are expected to show a damped oscillation featuring a series of peaks falling slowly towards full resolution (Figure 1). The last curves to settle are likely to be the symptoms plots. The symptoms must dictate the treatment decisions, nevertheless the wbc and epc counts are accurate markers of disease activity. A number of patients will struggle to respond will show more disordered graphs (Figure 2) but these should not dismay, they can be brought under control, but a damped oscillation will be important to establish. A small number of patients will show plots that fall to the baseline without oscillations on the way (Figure 3).

Physical signs can be helpful. The patients may demonstrate suprapubic tenderness and specific loin tenderness.

Problems to be confronted

These are commonly long-term chronic infections that have been exposed to partial treatment strategies. The infections are more likely to be mixed than not. There are problems with microbes forming biofilms on the urothelial cell surfaces, and parasitising the interior of the urothelial cells. These properties are associated with very little microbial division [11]. Thus the barriers presented by the cells and biofilms combined with the lack of dividing renders the microbes resistant to antibiotic attack. Higher concentrations are likely to be necessary to penetrate the barriers and brief courses of treatment are unlikely to have much of an effect [12]. We are extremely suspicious of the presence of non-culturable, obligate intracellular, fastidious microbes [13, 14].

The cell-associated properties of these microbes make them extremely resistant to antibiotic attack. Penetration of the cells and biofilms is very difficult and demanding of high doses of antibiotic to achieve adequate urine level. There is less of a problem with resistance [15, 16].

Many patients believe that they have not been listened to and their problems dismissed. They may mistrust clinical staff, feel angry and can be unusually assertive. All this is understandable and in many cases appropriate. The clinicians must do their best to be kind, patient and sympathetic, always ensuring that the story and symptoms are acknowledged and recorded.

To control and clear these infections we have to use long-term antibiotic treatment. This is not welcomed in our current culture and effort must go into reassurance and explanation of the reasons and motivation for such treatment regimes.

Urine cultures

One of the problems that troubles us the most is knowing what microbes are causing the symptoms. In nature, some seeds, such as nasturtiums, are easy to grow, others, like the Tibetan poppy are difficult. The same applies with microbes. Just because you detect the presence of an organism though culture of the urinary sediment it does not mean that it is a culprit, it might be that it is just easy to grow. This is becoming all too evident from our current laboratory work. There appears to be no reliable method for implicating an isolate in the aetiology of the symptoms. The normal bladder is not sterile and has a polymicrobial microbiome of well over 450 different species [17]. We can still see presumed pathogenic species in the urine of patients who have recovered and in our normal control specimens. For years it has been widely assumed that if you detect a microbe in the urine, obtained from someone with lower urinary tract symptoms, then it must be the cause of the symptoms. It is difficult to accept this given modern evidence.

This is why we put so much emphasis on the symptoms and the plots of the urinary urothelial cell counts and pyuria. We see patients who have sent urine to the USA and elsewhere, seeking special cultures. All too often, the data obtained do not help matters but do encourage people to focus on specific bugs without necessarily knowing whether they are relevant [18, 19].

Antibiotic options

In all cases we must use a full therapeutic dose. Note that some patients can notice a resurgence of symptoms after a single missed dose, this is not imagined. The use of the medication in full dose, usually twice daily is very important. Make your initial antibiotic choice on the history of past tolerances and response.

First line

(1) Nitrofurantoin Macrocrystals CR 100 mg bid – If the CR is not available you must spread the ordinary Nitrofurantoin Macrocrystals over a four dose schedule so that the 24-hours are covered.

(2) Cephalexin 1 gm bid (Cephalexin has one of the lowest C.Diff rates)

(3) Trimethoprim 200 mg bid

Methenamine 1 gm bid should be added into the first-line regime once an effective and tolerated treatment has been established. This urinary antiseptic appears to act synergistically and has enabled us to realise effective first-generation regimes where previously these were unachievable. Eventually, we hope to get the patient off the antibiotic regime and onto methenamine alone.

Second line

(1) Amoxicillin 500 mg bid to tid

Second line when clinical presentation points to obligate intracellular microbe

(2) Azithromycin 500 mg daily for three days and then thrice weekly (Particularly in the presence of urethral pain and male LUTS with urethral and prostate pain).

Some patients will describe trough effects; a falling off of efficacy between the bi-weekly doses. This should prompt a rise in the dose to 500 mg daily.

(3) Doxycycline 100 mg bid (Particularly in the presence of urethral pain and male LUTS with urethral and prostate pain)

A useful pointer to patients likely to benefit from a macrolide or tetracycline is the description of pain centred on the urethra with a comparative low urinary pyuria with a urothelial cell count in excess of the pyuria count. We have identified this as a clinical cluster that favours these antimicrobials as the eventual treatment selection.

Response to candida infections identified by urinary yeasts

(1) Fluconazole 100 mg daily for seven to 14 days

(2) Itraconazole 100 mg daily for seven to 14 days

(3) Candida can be resistant so be ready to culture and seek microbiological advice

(4) Vaginal candida is best treated topically with Clotrimazole vaginal pessary used thrice weekly– listen carefully and ascertain whether bladder symptoms are part of the clinical picture

Candida should always be considered when there is a particularly precipitous rise in pyuria without commensurate symptoms.

Often the problem with candida is that it needs a much more aggressive approach to treatment than many assume. Rarely we have to give as much as 800 mg of fluconazole daily to get a grip on a troublesome infection. Whilst this is unusual it serves to illustrate that a heavier hand may be all that is needed.

Third line

Pivmecillinam 200 mg bid to 400 mg bid

Fosfomycin 3 gm thrice weekly (only when combined with another agent and advised by Prof JML).

Fourth line

Ciprofloxacin 500 mg bid (we seek to avoid this if at all possible because of the C.Diif threat)

Fifth Line

If patients claim penicillin sensitivity check this with skin tests.

Nowadays the fifth-line treatment regimes must be preceded by a sediment culture with sensitivity analysis.

Ertapenem 1 gm IV over 30 minutes daily for five days

If penicillin intolerant

Gentamicin 7 mg / kg once daily IV for five days.

Nowadays we avoid IV treatment regimes as much as possible - Courses of IV antibiotic fall far short of expectations when treating a chronic urinary tract infection. They can have an immediate dramatic effect on the symptoms such that we can be persuaded that we have cracked the problem. That is a mistake we have made too often in the past.

The core problem is that the microbes persist inside the cells and in niches where they are protected by biofilms. As soon as the IV regime is withdrawn these sleepers break out into the spaces that have been vacated and the symptom cycle starts all over again sometimes with a dramatic resurgence. We used to use IV regimes in the past but we have now largely abandoned them.

The secret of effective care is to identify an oral regime that can be tolerated and which shows signs of efficacy in the urinalysis and / or symptoms. We should then stick at it with some tenacity expecting there to be fluctuations on the way. The flares that happen are a usual part of the recovery process and should not easily persuade us to a change of treatment.

How to manage the regimes:

We do not put a time limit on the treatment regime instead we adopt a staged approach to bring the disease under control.

Patients presenting with symptoms but without microscopic pyuria.

An antibiotic regime should not be initiated unless there is a pyuria signal. Many patients will attend with the appropriate symptoms but their urinalysis by microscopy proves negative. The commonest reason is that they have been advised to over imbibe and the urine is dilute. In these circumstances it is better to review and defer treatment until there is a urinary cellular signal to plot. If the symptoms are clear, Methenamine may be used on its own during the interregnum.

Patients presenting with symptoms and microscopic pyuria

(1) Establish a first generation antibiotic regime that is tolerated and shown to be effective because of an improvement in the symptoms score and in the urine microscopy without significant side effects.

If we can spot a symptom response, no matter how incomplete, then we tend to advise persistence with the current regime so as to allow this to grow into something more substantial. The symptoms tend to clear gradually one feature after another with some alterations in quality along the way.

We have made some serious errors in the past when patients have felt pressure to achieve an effective response because of a pending deadline. The failure of an early response has prompted an early change of prescription with detrimental results. It is more often the case that the original prescription was correct and that the shuffling of antibiotics under pressure proved harmful.

(2) Once the first line regime has been established, add Methenamine 1 gm twice daily and sustain this with the selected antibiotic.

(3) If the patient describes trough symptoms, consider increasing the dose of the antibiotic.

(4) We seek to achieve a damped oscillation in the symptoms and urine microscopy results and the dose and antibiotic may have to be manipulated to achieve this.

(5) Flares are to be expected and when they occur we should be reluctant to change the regime and instead respond by dose increase.

Symptom flares tend to pepper the history of treatment of these patients. A good response can be interrupted by an eruption of unpleasant symptoms. In addition, if the patient is convalescent the symptoms can feel more severe than at the outset despite the disease activity being less. Many of these flares settle without there being a need to do anything different. The recovery involves a series of oscillations of decreasing amplitude so that we expect these ups and downs. If treatment is necessary we usually respond by doubling the dose of one (or more) of the agents that are being used to treat the chronic infection. If the patient has been on an effective regime we should be reluctant to change it because the results tend to prove adverse. We should not normally consider changing the prescription without doing a urinalysis and identifying a change in the urinary inflammatory signal. The majority of the time the wisest course is to wait for the flare to settle of its own accord, recognising that it is most likely to be an inconvenient but natural fluctuation in the expected course of the disease and not a cause for alarm. In short we are extremely reluctant to alter the prescription without compelling reasons because a history of response is associated with persisting efficacy. Thus a flare should usually be managed by loyalty to the current regimen through dose manipulation and some patience. Do not assume resistance, it is a very rare occurrence.

(6) Once a damped oscillation has been achieved follow this down to a flat baseline and then commence a series of trials without antibiotic until a successful withdrawal be achieved. These episodes of treatment cessation to see what happens are an essential element in the management programme and we should be considering whether to initiate them at each consultation

(7) If a symptom response proves partial with a lingering sense of something going on in the background the antibiotic should be taken up to the highest permitted and tolerated dose. If that fails the efficacy of the antibiotic should be checked by a short-lived stop/start experiment. If this proves positive the antibiotic should be reintroduced at a standard dose and a suitable companion antibiotic added in. This occurs most frequently with suspected obligate intracellular pathology and a combined macrolide/tetracycline regimen evolves. As to other cases, combined treatment is less frequently needed and usually involves patients with extremely complex problems. We use this approach much less than in the past

The key guidance comes from the graphs of the urinalysis and symptoms. If there is a downward trend in these graphs then you should be reassured that the treatment is appropriate and stick with it despite the fluctuations.

Intolerance is by far the commonest reason for altering the prescription and in some patients this can require a considerable amount of shuffling to find an effective tolerated regime. This can lead to considerable multiplicity at the outset.

Efficacy failure is a valid reason for changing. Some patients will forget their medication for some reason. The record of symptom resurgence in such circumstances is a valuable indicator of therapeutic efficacy. If no such opportunity has arisen in an established regime this must be validated by precipitating an attenuated resurgence through a temporary suspension of treatment.

Because of the mixed nature of these infections combination therapies may be required. These should be identified on evidence. Typically the patient responds to an antibiotic that they are comfortable with. If an acute exacerbation occurs this should be managed with a dose increase. Failing that the regime should be changed. On many occasions the symptoms will deteriorate on the transfer from the first antibiotic. This implies that the original antibiotic was working on some part of the pathology and that there is another microbe to address as well. In such circumstances keep the first going and work out the companion antibiotic using the trial and error approach seeking a response and tolerance.

A pointer to a mixed infection requiring combination therapy is an evident response to the first antibiotic but this is noted to be incomplete. Patients will describe benefit but a lingering sense that things are not quite right and that something is going on under the surface.

A mixed infection requiring combined therapy can sometimes be spotted because the symptom fluctuations differ in quality. A patient may describe a response followed by an exacerbation but she is able to discern a difference in the quality of the symptoms. Thus, listening carefully to what the patient says is extremely important.

At every consultation it is imperative that the patient and GP be provided with a written analysis of the whys and wherefores of the treatments that are being prescribed. This can be achieved by use of the extensive library on Artemis.

Tolerance

We ask the patients about tolerance and adverse reactions at every consultation. "Are you tolerating this medication; is it causing any troubles?" There are specific questions to be asked in relation to Nitrofurantoin, Azithromycin and Co-amoxyclav. The email service is available for us to address side effects as a priority and the patients should expect a response within 24-hours. The availability of the service and the correct contact address should be impressed on the patient at each attendance. In addition at each attendance we should remind the patients that we are practising outside of guidelines and off license so that the risks of treatment are comparatively greater and that monitoring for side effects must be taken seriously.

If nitrofurantoin proves necessary for more than six months, the case should be presented to the MDT. Any cough or shortness of breath experienced whilst taking nitrofurantoin, whatever the cause, should result in immediate cessation.

Any experience of diarrhoea should result in immediate suspension of antibiotic treatment until the diarrhoea has stopped. The event should be used to collect stop/start efficacy information.

Dealing with complications, failures and adverse events

We must be available to respond to exacerbations, treatment intolerance and response failures. It is not right to expect patients to wait for their next appointment. We have tried several approaches to the provision of extraordinary access between clinic appointments. The email service has proved to be the most successful by a long margin. Telephone requests of emergency contacts and presentation at reception failed under the pressure of demand.

We seek to support patients who deteriorate, those who notice no change for the better in their symptoms and suspected adverse drug reactions.

Figure 1

This is an example of a damped oscillation seen in plots of the symptoms and of the pyuria. This pattern will show up in many different forms but the trend of recurring peaks of decreasing amplitude is often easy to discern. Sometimes the time frame can be quite protracted.

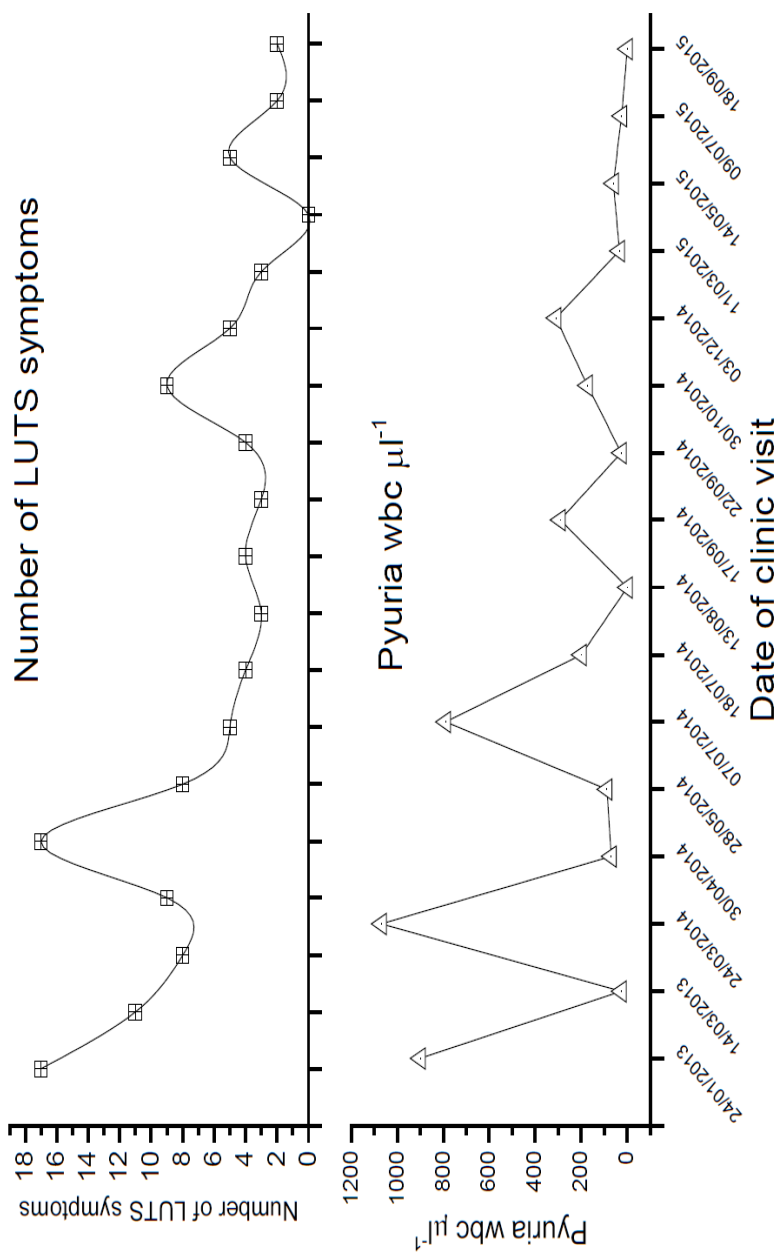


Figure 2

This is a plot of the pyuria and of the urothelial cells. They are correlated. The first part of the graph shows a period of struggle whilst we tried to establish a stable damped oscillation. This was eventually achieved and once evident the treatment regime should be kept constant other than the use of increased doses during the flares.

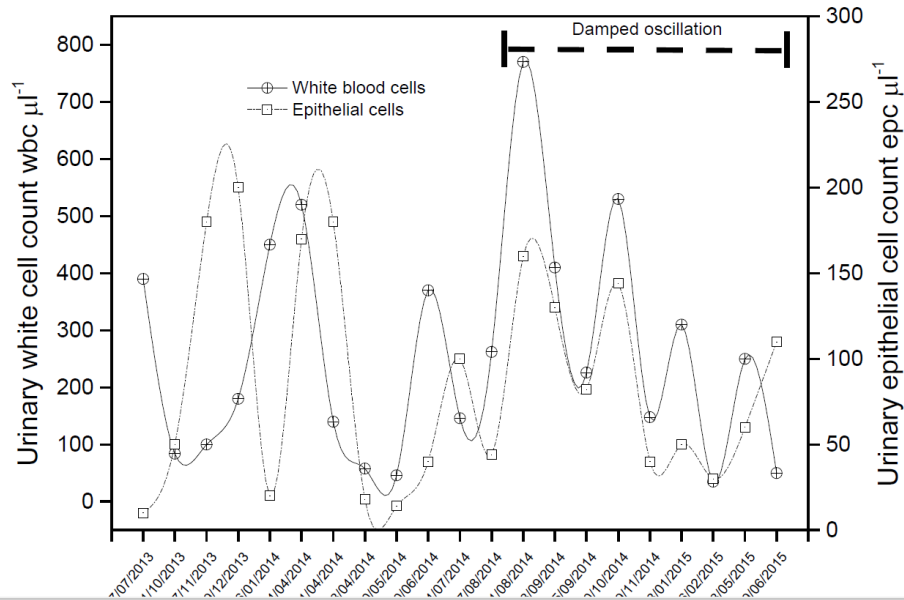
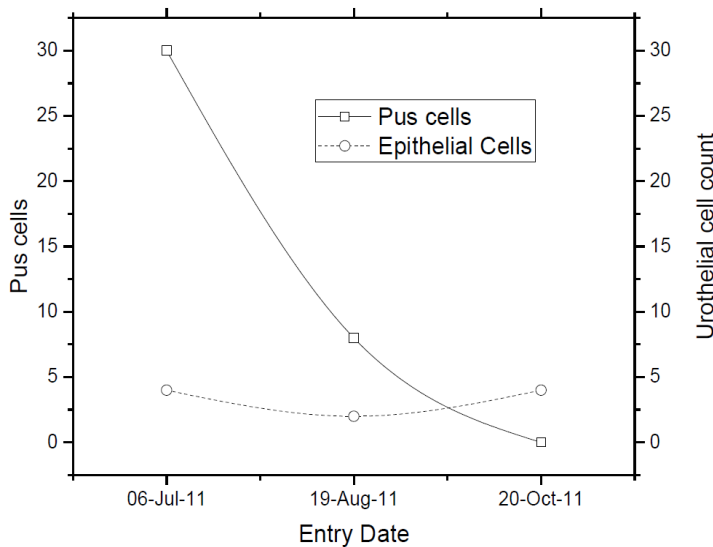


Figure 3

This is a critically damped oscillation in the pus cells. There is an unremitting fall in the pus cells over the course of the treatment period. Such plots are very welcome but unfortunately not common. The expectation should be that the symptoms and urinalysis will oscillate up and down during a gradual, protracted resolution.



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Note: These are not linked to thesis references

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Appendix 14 List of publications as a result of this thesis

1. Fallacies and misconceptions in the diagnosis of UTI; Future Medicine 2014
2. Recalcitrant chronic bladder pain and recurrent cystitis but negative urinalysis: What should we do? doi.org/10.1007/s00192-018-3569-7
3. Cross-over data supporting long-term antibiotic treatment in patients with painful lower urinary tract symptoms, pyuria and negative urinalysis. Int Urogynecol J. 2019 Mar;30(3):409-414. doi: 10.1007/s00192-018-3846-5. Epub 2018 Dec 18
4. How can we improve investigation, prevention and treatment for recurrent urinary tract infections-ICI-RS 2018; Neurourol Urodyn. 2019 Dec;38 Suppl 5:S90-S97.doi: 10.1002/nau.24021

Appendix 15 List of abstracts as a result of this thesis

1. Observational study reporting on antimicrobial resistance patterns in MSU cultures of Chronic UTI (CUTI) patients on long term antibiotic treatment compared to ED attenders with acute UTI.
2. Swamy S, Sathiananthamoorthy. S, Khasriya R, Malone-Lee J
3. Quantitative microbiology is unhelpful in distinguishing female chronic LUTS patients from controls even with enhanced cultures
4. The imposition of a hospital antimicrobial guideline on patients with chronic, recalcitrant UTI and LUTS - The consequences for the patients – A cautionary tale
5. S Swamy, D Dharmasena, J Malone-Lee
6. Study of the pathophysiological signals in the urine of female patients with recalcitrant LUTS presenting with acute flare while on long term antibiotic treatment
7. S Swamy, Guy Aldous, Nicole Braham, S Sathiananthamoorthy, J Malone-Lee
8. Long-term antibiotic treatment can resolve the recalcitrant lower urinary tract symptoms associated with chronic infection
9. Swamy S, Gill K, Kupelian A, Sathiananthamoorthy S, Horsley H, Collins.L, Malone-Lee J
10. A blinded cross-sectional study comparing the pathophysiological signals in the urine of diabetics from LUTS clinic, routine outpatient diabetic clinic and controls
11. S Swamy, S Sathiananthamoorthy, M Tuz, K Gill, H Horsley, J Malone-Lee, University College London

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