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Are empirical antibiotics currently prescribed for patients presenting to the emergency department with uncomplicated cystitis appropriate?

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ARE EMPIRICAL ANTIBIOTICS CURRENTLY PRESCRIBED FOR PATIENTS PRESENTING TO THE EMERGENCY DEPARTMENT WITH UNCOMPLICATED CYSTITIS APPROPRIATE?

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of
Masters of Science in Medicine in Emergency Medicine.

Johannesburg, 2013

DECLARATION

I, Jennifer Frankel, declare that this research report is my own work. It is being submitted for the degree of Master of Science in Medicine (Emergency Medicine) to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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DEDICATION

This work is dedicated to my little son - Robert Tyler Hurly

ABSTRACT

Aim: To determine the types of uropathogens encountered in patients presenting to a busy private emergency department in Johannesburg and compare sensitivity patterns of the bacteria identified with current antimicrobial prescribing patterns.

Design and methods: We conducted a retrospective single institution analysis of all adult female patient data and laboratory reports for culture positive urine specimens submitted for investigation over a one year period (January 2008 – January 2009). Isolates were examined in a single laboratory for standardisation of data.

Setting: Patient data collected from an emergency department in a private hospital in Johannesburg

Main Results: A total of 213 specimens (N=213) were analysed. The majority of culture positive specimens demonstrated a bimodal age variation. The most common isolate was *Escherichia Coli* (83%), followed by *Proteus Mirabilis* (5%) and *Enterococcus Faecalis* (5%). There was no statistical significance between the frequency of symptoms and the type of uropathogen cultured. Overall antimicrobial sensitivities for the majority of bacteria cultured displayed high levels of resistance to Ampicillin/ Amoxycillin (70.1%) and Co-trimoxazole (53.4%). *Escherichia Coli* specifically, demonstrated significant resistance to Ampicillin/ Amoxycillin (78.6%), Amoxycillin – Clavulanic Acid (42.9%) but a lower resistance to Co-trimoxazole than the overall figures (28.6%).In our study, the utilisation of the urine dipstick for on-site testing revealed a consistent dipstick and laboratory analysis result, especially when assessing the four major organisms identified. It therefore forms a solid basis for therapeutic decision making.

Conclusion: *Escherichia Coli* is the most common aetiological agent identified and in the context of South African emergency departments, still remains susceptible to Cephalosporins, Nitrofurantoin and Quinolones. Resistance levels to Co-Trimoxazole and Penicillin based *B*-Lactams illustrate that they should not be employed as empiric therapy in current practice guidelines.

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NOMENCLATURE

Abbreviations

UTI	Urinary Tract Infection
ED	Emergency Department
E. Coli	*Escherichia Coli*

Definitions

Acute Uncomplicated Cystitis	Simple infection of the lower urinary tract
Uncomplicated Urinary Tract Infection	Simple infection of the lower urinary tract

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1. INTRODUCTION

1.1 Motivation and rationale for this research

Uncomplicated cystitis remains a significantly common infection in women and a true challenge for those who treat them. Uncomplicated urinary tract infections account for one of the top five presenting diagnoses in our South African emergency departments daily. Although international guidelines are available and should be adhered to, more knowledge of our local community and geographical resistance patterns would prove beneficial. The lack of surveillance studies and antimicrobial guidelines specific to Southern Africa makes implementation of empiric therapy difficult.

Therefore the intention of this study is to investigate whether empirical antimicrobial agents currently prescribed for patients presenting to the emergency department are appropriate.

1.2. Aim and objectives

1.2.1 Study aim

To compare sensitivity patterns of uropathogens identified with antimicrobial prescribing patterns currently employed in a private emergency department.

1.2.2 Study objectives

- To determine the types of uropathogens encountered in a private emergency department located in a middle/ upper class area of Johannesburg, South Africa, currently managing over sixteen hundred patients per month;
- To identify antibiotic resistance and sensitivity patterns related to the uropathogens isolated;

- To compare these patterns to current prescribing practices;
- To acquire a foundation of information for subsequent study that will allow for the development of clinical protocol guidelines for the improved management of uncomplicated urinary tract infections.

2. LITERATURE REVIEW

2.1 Introduction

Urinary Tract Infections (UTIs) constitute a large proportion of the infectious burden encountered by emergency physicians in emergency departments. Presentations vary in disease severity, from uncomplicated cystitis to urosepsis in a broad spectrum of patients. The majority of patients can be managed with minimal diagnostic investigations in an outpatient setting with the use of empirical antibiotic therapy. [1]

2.2 History of UTI's

UTIs have been represented in literature for over 5000 years; the first known description was published in 3000BC by Pen Tsa whose initial works suggested numerous herbal remedies for the management of UTIs. Subsequently, contributions to UTI management were made by Hippocrates, Galen, Rhazes, Pasteur, Lister, Koch and Osler. [2]

2.3 Epidemiology

Although there is scant data to identify the burden on healthcare in Southern Africa, in either the public or private sector, Tambekar *et al* stated that UTIs are "the most common infection in the human being." [3] UTIs are among the most prevalent infectious diseases affecting women. Studies have shown that 25 – 50% of all women will experience a UTI in their lifetime, 33% before 24 years and 50% by 35 years. [2, 4]

In the United States of America, UTIs account for over eight million ambulatory appointments per year and 1.1 million visits to emergency departments annually. [5, 6]. UTIs are the second most common presenting infection in community practice and account for the second most common

admitting diagnosis, surpassed only by Pneumonia. They are however the most common source of hospital acquired infections. [2, 7, 8]

UTIs place a substantial burden on both the patient and healthcare system, physically and financially. In studies on patients with uncomplicated cystitis, although easily managed as outpatients, they accounted for significant morbidity.

Patients experienced anguishing symptoms (pain, dysuria and generalised lethargy) for an average of 6.1 days, and they stayed in bed for 0.4 days, and miss approximately 1.2 days of work or school. [2, 9]

In a South African study conducted by Habit *et al*, they concluded that urinary tract infections incurred the highest healthcare cost of any of the urological diseases in RSA. The cost burden of UTIs exceeded that of renal failure, dialysis and eventual renal transplantation. [10]

Perfetto *et al* estimated direct costs of UTI management in the United States of America to exceed 1 billion Dollars per year. Indirect costs were not quantified but included loss of productivity and diminished quality of life. [11]

2.4 Definition and Classification of UTIs

Norris and Young defined a UTI as “an inflammatory response to any of the cells lining the urinary tract to micro-organisms. It may involve the upper tract, lower tract or both.” [12]

UTIs can be classified according to site of infection, where Pyelonephritis describes an infection in the kidneys and ureters, (Upper tract / Nephropathies), and Cystitis denotes an infection of the bladder and urethra (Lower tract/ Uropathies). They can further be classified into symptomatic or asymptomatic and complicated or uncomplicated conditions. Despite a clear classification, there can be substantial overlap in the emergency department.

“Urinary Tract Infection” is a universal term describing an “inflammatory response of urogential cells to a pathogenic organism. It encompasses the ureters, bladder and kidneys, or a combination thereof. It is imperative to diagnose the location and severity of the disease to ascertain the type of antimicrobial required and the duration of the treatment needed. [1, 12, 13]

Uncomplicated Cystitis is generally defined as an infection occurring in healthy, pre-menopausal, non-pregnant female patients, who lack features of a complicated infection. The structure and function of the urinary tract are preserved. It is a symptomatic inflammation of the bladder in response to infection and is, in most cases, monobacterial.
Acute cystitis can be characterised by the presence of dysuria, frequency, urgency, haematuria and occasionally suprapubic tenderness. [1, 8, 12, 14, 16]

Miller and Tang differentiated between complicated and uncomplicated infections as a longer duration of therapy required and the choice of therapeutic agent more complex. [15]

Complicated infections are described as occurring in male patients, extremes of age, pregnant women;unusual organisms and in cases of recurrent infection. Other factors contributing to a complicated infection include structural or functional abnormalities, co-morbidities, urolithiasis, immunosuppression or the presence of an indwelling catheter. [1, 15]

2.5 Pathophysiology of the disease process

The pathophysiology of UTIs is well described as an ascending infection, due to bacterial colonisation of the peri-urethral tissue, entering via the urethra and extending upwards to the bladder. The faecal-perineal-urethral hypothesis has also been widely recognised as a sound justification for site infection. The pathogens colonising the rectal flora are adept at ascending, inhabiting and resulting in infection. The uropathogens’ resultant infection is not due to their presence, but the manifestation of the virulence properties of the micro-

organism which activates their adherence to the perineum and urethral walls. [12, 17, 18, 19]

The female is more prone to acute cystitis due to an anatomically shorter distance and comparative proximity of the urethral opening to the vagina and peri-anal area. [2]

The postulated host defence is the passing of sterile urine through the urethra, destabilising the bacteria and reducing the bacterial burden, thus limiting the likelihood of infection. The urethral lining is adept at entrapping bacteria, these host cells are then discarded in the urine, removing the micro-organism from the urinary tract. [12, 1 9]

There is colonisation in the bladder and healthy vaginal mucosa of protective organisms, by example, *Lactobacillus* that exists in a low pH environment and contributes to the prevention of adherence. It is important to recognise that colonisation represents a form of protection from the virulent, pathogenic organisms [19]

2.6 Pathogenesis of UTIs

Uropathogens causing disease have remained highly consistent throughout the years, with bacterial pathogens being far more prevalent than other fungal or viral micro-organisms. The microbial spectrum showed little geographic variability, with enteric bacteria being the most frequent culprit, specifically *Escherichia Coli*. [2, 5]

In the majority of patients with uncomplicated infections, cultures demonstrated monobacterial infections with *E. Coli*; while polymicrobial growth was implicated in complicated cystitis. [1]

The ARESC (Antimicrobial Resistance Epidemiological Survey on Cystitis) Study which represented 9 European countries in an epidemiological survey

demonstrated that *E. Coli* accounted for 76.7% of the causative agents responsible for uncomplicated infection. [4]

Zhanel *et al*. reinforced these findings in the NAUTICA trial conducted across 41 medical centres in the United States of America and Canada. Their findings demonstrated that *E. Coli* represented 57.5% of infections, followed by *Klebsiella Pneumonia* (12%), *Enterococcus* (6.6%) and finally *Proteus* (5.4%). [23]

In a study that utilised data extracted from the Surveillance Network Database (a database representing microbiological findings from 9 different geographic regions in the USA), *E. Coli* and *Staphylococcus Saprophyticus* accounted for 74% of all uropathogens isolated from female adult patients presenting with uncomplicated cystitis.[20]

Chung expressed that *E. Coli* represented 95% of bacterial pathogens causing in UTIs in Australia. [19]

A cross sectional survey conducted over five years found that *E. Coli* and *Staphylococcus Saprophyticus* represented 90% of the bacteria isolated. [11]

Marco and Parker in a cross sectional study demonstrated that *E. Coli* was responsible for 61% of cases of uncomplicated cystitis. This was reinforced by Norris and Young, confirming that 75 – 95% of cultures they identified, implicated *E. Coli*. The remainder of the cultures were variable, isolating *Klebsiella Pneumonia*, *Proteus Mirabilis*, *Enterococcus, Enterobacter* Species and *Pseudomonas Aeruginosa*. [12, 21]

Habte *et al* conducted a retrospective analysis in Pretoria, South Africa, where findings indicated that the most common pathogen was *E. Coli* (39%), followed by *Klebsiella Pneumonia* (20%) and *Enterococcus Faecalis* (8.2%).

Bahagon *et al* conducted a 5 month study in an emergency department in Jerusalem, Israel, and isolated *E. Coli* in 58% of bacteraemic patients with no other statistically significant associations.

2.6 Signs and Symptoms of UTI's

The symptomatology of acute cystitis is variable, often presenting with one or more non-specific symptoms ,including frequency of urination, urgency, burning on micturition (dysuria), nocturia, suprapubic tenderness and in a quarter of cases, haematuria. There is often the presence of a fever. [1, 6, 15, 22]

The most frequent clinical presentation that drives patients to seek medical assistance is painful micturition. It is important to note that the symptoms are so distinctive that most women are highly reliable at self –diagnosing the condition. [11, 26]

Relying on symptomatology alone for diagnosis is problematic as these symptoms can be observed in a variety of other genitourinary conditions including vaginitis, sexually transmitted infections as well as allergic and traumatic events [1].

2.7 Diagnostic Approach to UTIs

A systematic review conducted by Schmiemann *et al* demonstrated an error rate of 33% when relying on symptoms in isolation for diagnostic purposes. In an evidence based review conducted later by Abrahamian *et al* in an ED, they showed that if a patient presented with one or more symptoms, the likelihood of a UTI was 50%, however if a patient presented with the triad of urgency, frequency and no vaginal indicators, the probability of acute cystitis was increased to 96%.[1,27]

There are a number of diagnostic algorhythms that have been trialled where both symptom indicators and urine dipstick testing have been .incorporated.

Schmiemann *et al* illustrated that a thorough medical history may increase or decrease the probability of acute cystitis:

Dysuria, Frequency, Nocturia, Polyuria	Increase
Macrohaematuria	Increase
Suprapubic tenderness	Increase
Offensive odour/ turbid urine	Increase
Previous history of UTI	Increase
Altered /New discharge	Decrease

Table 2 Acute cystitis probability algorithm

[27]

McIsaac based his algorithm on 3 criteria:

1. A burning sensation or discomfort when passing urine
2. The detection of leucocytes in the urine
3. The detection of nitrites in the urine

In the presence of at least two of these criteria, he concluded a sensitivity of 80% but a specificity of 54%, showing a high rate of false positives due to the low specificity. [27]

Schmiemann *et al* also analysed the point system, which demonstrated a sensitivity of 76% and a specificity of 74%:

Presence of Nitrites	2
Presence of Leucocytes	2
Haematuria	1
Dysuria	1
Nocturia	0.5

Table 3: Point System Algorithm

[27]

The collection of urine for investigation can be done through multiple methods. To avoid specimen contamination from colonising bacteria of the distal urethra, suprapubic aspiration is the procedure of choice, it is however, seldom indicated. Suprapubic aspiration is a painful and invasive process which requires excessive time and resources to be practical. [5]

Insertion of a single catheter via the *straight catheter technique* is the next best manner in which to obtain a specimen with negligible contamination. This is also not indicated in most patients due to time, cost and discomfort of the invasive procedure. [5]

Most urine specimens are collected from adult patients utilising the midstream, clean catch collection. This method has a number of advantages over any of the other techniques. It is non-invasive, simple, cost effective and painless. It can be performed in any clinical setting with no risk of introducing bacteria into the genitourinary tract. Bacterial colony counts compare very well with those collected from suprapubic aspirates; however the sample runs a risk of contamination with bacteria residing in the distal urethra. [5, 15]

Diagnosis of a urinary tract infection through qualitative laboratory culture of a properly collected urine specimen is the gold standard. This method of testing identifies the micro-organism responsible for the infection and provides comprehensive information regarding the susceptibility of antibiotics for the management of the disease. [1, 2]

A positive culture is generally defined as "10^5 or more, colony forming units (CFU's) of bacteria per millilitre of urine". Isolation of three or more pathogens is considered a contaminated specimen in the uncomplicated patient. [12]

Wilson and Galdo discussed urine specimens submitted for culture that accounted for 25 – 40 % of hospital based laboratories' workload, of which about 80% were submitted from patients not admitted to the hospital. [5]

Despite being a reference standard, the Infectious Disease Society of America (IDSA) only recommend cultures be performed on complicated urinary tract infections and uncomplicated infections where empiric therapy has failed.
The rationale for not performing routine cultures is the collective disadvantages of the culture process. It is slow (48 – 72 hours), costly and has not shown to predict the therapeutic outcome. Cultures are collected prior to antimicrobial administration to maximise the diagnostic yield, but any delay in therapy until results are available can prolong patient symptoms, increase overall morbidity and ultimately the cost of therapy. Performing routine cultures, although not cost effective, enables surveillance of aetiology and susceptibility patterns. [1, 2, 15]

In ED or outpatient settings, a UTI is often diagnosed on history and examination with the addition of side room urine dipstick analysis. A study conducted by Miller and Tang confirmed dipstick results to have reasonably good sensitivity and specificity, in some cases exceeding 90%. [15]

Leucocyte esterase is an enzyme released from white blood cells in the urine and nitrites are converted from urinary nitrates in the presence of certain bacteria. Multistix are the dipsticks most often utilised by physicians, detecting the presence of leucocyte esterase, nitrites, protein and blood. [12, 28]

Schmiemann and Knell revealed that while there was a high probability of a urinary tract infection if nitrites were detected, the sensitivity of nitrites in isolation was low. The discovery of leucocytes increased the probability of acute cystitis, but to a lesser degree. Blood was found to be extremely sensitive but conveyed very little specificity. [27]

Leman reinforced the findings in a study on the validity of urinalysis in an ED. Focusing on nitrites as a screening test in isolation; nitrites were shown to be poorly sensitive with only average specificity. When combined with leucocyte

analysis, the study revealed a positive predictive value of 100%. The discovery of a positive leucocyte and nitrite test yielded 100% specificity. [5, 28] Deville and colleagues examined the negative predictability of the dipstick and established that if leucocytes and nitrites were negative, it can exclude the presence of a urinary tract infection across all populations. [2]

Abrahamian *et al* investigated the presence of pyuria in specimens and found a sensitivity of 95% and specificity of 71%. Norris and Young showed a nitrite sensitivity of 81% and leucocyte sensitivity of 77%. [1, 12]

Lammers *et al* investigated the over treatment and under treatment of acute cystitis based on dipstick findings and found that in the event of a positive leucocyte and nitrite finding, over treatment was 47% and under treatment was 13 %. No other studies were identified to substantiate this finding. [12]

The urine dipstick had been well documented throughout the literature and remains the key investigation in EDs. It remains a reasonable alternative to urine culture although the sensitivities and specificities are variable. It has been demonstrated to be of assistance in excluding the presence of an acute infection but is not a substitute for culture. It is therefore imperative that physicians understand its usefulness, strengths and limitations in the diagnosis of UTI. [5]

2.8 Management Strategies

The concept of empiric therapy has been widely employed by EDs worldwide, as the delay in awaiting diagnostic urine culture impedes immediate physician management of the patient. The rationale is that therapeutic management instituted prior to the results being made available, reduces the severity, duration and worsening clinical course of the disease. However, multiple studies now reflect a sharp rise in antibiotic resistance rates demonstrating an increased risk for treatment failure and poor clinical outcomes. [1, 12]

2.8.1 Resistance Patterns

There has been a substantial rising trend since 1990, demonstrating increasing resistance to commonly recommended agents. Co-trimoxazole has shown an increase in resistance from 5%- 7% to 16% in 2001, while. Fluoroquinolones showed a similar drift from 0% to 3.7% by 1999. [2]

Foster claimed that although the spectrum of urinary pathogens has remained largely unaffected, their ability to elude antimicrobial therapy is developing and mutating constantly. [2]

There is clear substantiation of data that confirms the potential of antibiotic resistance being transmitted from animal sources that are fed antibiotics to promote their growth, with particular reference to *E.Coli*. Studies have demonstrated however, that the overuse of antimicrobials in humans is the true explanation of the abnormally increasing resistance rates over time. [14]

It is thus critical that physicians across all specialities who encounter and manage patients with acute uncomplicated UTIs, practice what Nicolle *et al* proposed as “Antimicrobial Stewardship” which emphasises the correct and applicable utilisation of antibiotic agents that assists in limiting the expansion and spread of resistance. [26]

The continual increase in antibiotic resistance leads to a variety of negative outcomes including a rise in morbidity and a substantial increase in cost due to the need for re-evaluation, reassessment and re-treatment with broad spectrum antimicrobials.[6]

The Surveillance Network Database extracted study found that *E. Coli* resistance to Co-trimoxazole in 16 – 18 % of isolates, in contrast to a 1% resistance to Nitrofurantoin. The study went further to claim 91% sensitivity across all uropathogens to Nitrofurantoin. [20]

The European ARESC Study completed in 2009 of nine European countries, compared its findings to the ECOSENS Study conducted in 2000 over 17 European countries, confirmed an increased resistance to ampicillin, ciprofloxacin and co-trimoxazole. In the ARESC study, 48.3% of uropathogens were resistant to ampicillin and 29.4% to co-trimoxazole.

The study showed reasonable sensitivity to nitrofurantoin(95.2%), ciprofloxacin(91.7%) and amoxycillin/clavulanic Acid(82%). Resistance demonstrated a high variability between countries and regions. It outlines that the most important factor in resistance was the overuse of antibiotics. The studies also reflected overall resistance to be lower in the Nordic countries and highest in the Mediterranean. [4, 6, 13]

Zhanel *et al* conducted the NAUTICA Study involving medical centres in the United States of America and Canada, it proved resistance rates were higher in the USA, but overall resistance rates concurred that resistance to ampicillin was the highest(45.9%), followed by co-trimoxazole (20.4%), nitrofurantoin (14.3%) and ciprofloxacin(9.7%).[23]

A study by Marco and Parker corresponded to the previous findings and showed resistance to ampicillin was the most significant, then nitrofurantoin, trimethoprim and ciprofloxacin. They concluded higher levels of resistance than had previously been identified. [21]

Kalpana and Gupta reviewed evidence on in-vitro susceptibility patterns among uropathogens and agreed that there has been a substantial decrease in the vulnerability of bacteria over the last decade, however, patterns showed a wide variability among geographic locations and institutions. The emergence of resistance will therefore further complicate the management of UTIs over the next 10 years. [29]

In 2010, the Infectious Disease Society of America (IDSA) reviewed 4 large studies on in-vitro susceptibility in North America and Europe. The analysis concluded substantial geographic discrepancies in the resistance patterns. [30]

Miller and Tang agreed that although there was substantial geographic variability, trends of resistance patterns emerged when analysing 16 European countries and Canada. Ampicillin demonstrated the highest resistance (18 – 54%), followed by trimethoprim (9 – 27%) and sulfamethoxazole (16 – 44%). Resistance to nitrofurantoin was generally less than 3%. [15]

Antimicrobial	Spain	Greece	Hong Kong	USA	Canada	Iran	Korea
Ampicillin	52.1	25.8	52.8/60.1	47.3	41.5	85	65.5
Amoxicillin/Clavulanate	2	5.2	1.8/<3				
Cefuroxime	2.4	1.7	9.2				
Ceftriaxone						28	0.5
Amikacin/Gentamicin			17			20	0.9
Fluoroquinolones	18.2	2.2	12.9/22.1	10.6	7.1	31	14.3
TMP-SMX	25.9	19.2	29/34	22.2	14.8	54	37.9
Nitrofurantoin	6.2	10.7	6.6/1.5	15.3	11.5	0.05	
	(14)	(16)	(31,32)	(23)	(23)	(7,24)	(25,33)

Table 4 Geographic variation of antimicrobial sensitivities in percentages (%)

In the ARESC Study, Schito *et al* demonstrated another danger in antimicrobial resistance, that of multi-drug resistance. In their review, more than ten percent of *E.Coli* was resistant to more than one antibiotic agent. [4]

A national survey of urinary isolates in the USA showed that 7.1% of uropathogens were resistant to three or more antimicrobial, with; 12 % resistant to 2 or more. The survey was conducted in 2000 on outpatient cultures with acute uncomplicated cystitis. The majority of resistance was to ampicillin, cephalothin and co-Trimoxazole, then ciprofloxacin. [29]

A Spanish study conducted by Gobernado, showed that resistance to 2 or more drugs was seen in 20.4% of isolates. Ampicillin was implicated in 94% of strains, with co-trimoxazole and ciprofloxacin in 73.2% and 32.7% strains respectively[14]

The global reality of the increasing resistance rates across all classes of antibiotic families is that the medical profession is creating future, untreatable diseases for which we may still have therapy for. If the preservation of the antimicrobial classes that are required for more significant and serious disease was practised, then future resistance too much needed therapies can be prevented. [26]

The need for susceptibility is underscored when considering that isolates are showing cross-resistance to some antibiotic agents. Uropathgens resistant to one drug are more likely to be resistant to others. Ho *et al* illustrated that 23.1% of strains that were resistant to ampicillin, were also resistant to ciprofloxacin, and that 52.4% of ampicillin resistant isolates were also resistant to co-trimoxazole. Of the urinary pathogens resistant to ciprofloxacin, a number were also resistant to ampicillin (94.3%) and co-trimoxazole (68.6%). [32]

In many of the studies, co- trimoxazole has been the most associated with simultaneous resistance to other commonly utilised urinary antibiotics. *E.Coli* resistant to co-trimoxazole are 14 times more likely to be resistant to ciprofloxacin and four times more likely to be resistant to nitrofurantoin. [34]

Karlowsky *et al* demonstrated the expected cross resistance between ciprofloxacin and citrofurantoin. 10.4% of ciprofloxacin resistant strains were resistant to nitrofurantoin and 29.8% of nitrofurantoin resistant isolates were resistant to ciprofloxacin. [34]

The evidence exemplifies that an increased use of any of the suggested urinary antibiotic agents may select for resistance to more than one agent.

2.8.2 The History of UTI Management

The first victorious documentation in history of the management of patients with UTIs was in 1937, with the introduction of antibiotics to manage acute cystitis. Dr Hernholz reported to have successfully treated a patient with a urinary tract infection with sulphonamides. Other scientists continued to explore different avenues due to the high side effect profile of those particular agents (severe gastrointestinal disturbances and respiratory distress with cyanosis). [2,20]

Nitrofurantoin (in 1953) was the first widely accepted, effective and well tolerated antimicrobial therapy available for clinical utilisation in acute cystitis. Despite its continued successful management of the disease and relatively low resistance profile, it has been largely replaced by newer agents. Late into the 1980s, fluoroquinolones became available in the pharmaceutical market and by the mid 1990's had largely replaced all other antibiotics used for genitourinary infection. [2]

2.8.3 Empiric Therapy

Aypak *et al* described the concept of empiric therapy as "a superior chance in microbiological terms to eradicate infection". The value of empiric therapy depends on the continual reassessment of resistance patterns to bacterial isolates. The choice of empiric agent is dependent on the type of patient; in terms of age, gender, history, the nature of infection (site, complicated or uncomplicated); local microbiologic aetiology and susceptibility patterns; severity of infection and the cost effectivity analysis and selection of resistant strains. [1, 6, 35]

The selection of empiric therapy is further guided by susceptibility patterns shown to initiate distinct clinical archetypes. The foundation for empiric

treatment is then based on the predictable spectrum of urinary pathogens and the characteristic and distressing features of UTI indicators. [16]

Although the causative micro-organisms have remained largely unchanged, their ability to elude antimicrobial therapy is constantly improving. In an ED, physicians rely on empiric therapy and therefore require extensive knowledge of the common organisms and their associated resistance patterns specific to their region to accurately and appropriately treat patients. Empiric treatment is advocated in the ED for the management of UTIs due to the narrow and predictable aetiological spectrum. [2, 14]

It is therefore crucial that goal directed therapy be instituted to include the ”narrowest-spectrum” appropriate agent. The employment of broad spectrum antibiotics in a well understood disease with a limited aetiological profile would be contrary to the principle of “Antimicrobial Stewardship”. [26]

The first guidelines for the empirical management of UTIs were published in 1999 by the Infectious Disease Society of America (IDSA) in conjunction with the European Society for Microbiology and Infectious Diseases. The IDSA specified that the management guidelines were limited to apply only to non-pregnant, pre-menopausal women with no structural urological abnormalities and no co-morbidities. [30]

IDSA recommended that patients presenting with uncomplicated UTIs be managed with co-Trimoxazole, unless the resistance rates in the local area exceeded 10 – 20 %. A three day course was encouraged. In the presence of high resistance, the provider should revert to fluoroquinolones for treatment. Since the guidelines published in 1999, newer agents have become available, antibiotic resistance has increased markedly and different durations and concentrations of therapies have been investigated. With new evidence and growing concerns of treatment failure and adverse effects of the advocated medication, the recommendations were seldom adhered to. [1, 9, 30]

The rationale for the 10 – 20% resistance “cut off” appeared to be a combination of both clinical and cost considerations. Miller and Tang substantiated these findings when assessing the clinical outcome of patients treated with the co-trimoxazole regimen in an area of high resistance. The combination yielded a cure rate of less than 60%. In the implementation of empirical therapy practices, it is still likely that some women will be managed with an antimicrobial that does not exhibit in-vitro susceptibility to the uropathogen (empiric therapy as approved in the absence of an available culture). It is therefore imperative to continually assess the local resistance patterns, if there is a rising resistance in a particular patient population to a specific antibiotic, the likelihood of failure outweighs the benefit of its use. [30, 34]

The IDSA recommended that “Performance Measures” be activated to assist physicians to gauge the benefits and disadvantages of employing the proposed guidelines. Digressions from the parameters were expected in a percentage of cases and 80 – 95 % compliance was an expected range.

The following measures were identified by the IDSA:

1. In Uncomplicated Cystitis, the use of the recommended antibiotic is advocated, in the absence of absolute or relative contraindications or complications e.g. allergy, recent use, unavailability of the suggested agent.
2. The use of Fluoroquinolones only to be utilized in uncomplicated UTIs when the first –line agent cannot be applied. [30]

Lee and Miller conducted a cost minimisation and sensitivity analysis to support the proposed IDSA guidelines. They assessed the level of resistance in a specific population that should prompt the use of fluoroquinolones in place of co-trimoxazole. The study concluded that when the resistance reaches 22%, the use of fluoroquinolones as a first line therapy becomes more cost effective. The analysis included complications of treatment failure

including yeast colonisation, hospitalisation for worsening infection, re-treatment and follow up consultations. [36]

Prior to the release of updated guidelines in 2010, many studies were conducted to assess the prescribing patterns of physicians managing acute uncomplicated cystitis and the adherence to the initial guidelines. Very few studies consider the prescribing principles used in an ED and of those available, few follow infectious disease directives. [1]

Marco and Parker confirmed in a survey of emergency physicians that there was a wide variation among emergency departments as to whether cultures were submitted and to the choice of antibiotics used. A study conducted in an ED exhibited that of 100 patients who were prescribed fluoroquinolones for the management of acute cystitis, 81% received unsuitable prescriptions. [20,21]

Aypak *et al* presented evidence that fluoroquinolones were the most utilised antimicrobials for UTIs. The increase in prescriptions in general in the past decade had lead to an increased resistance in most communities. [6, 29]

A survey conducted in Spain by Martinez *et al* established acceptable first and second line agents. The enquiry concluded that a large number of second-line agents were employed and also demonstrated a high variability among doctors and hospitals. [37]

McIsaac *et al* assessed prescribing habits of 2000 family physicians across Canada and ascertained that 40.8% of doctors prescribed co-trimoxazole as a first line agent for empiric therapy. Fluoroquinolones and nitrofurantoin were almost equitable as the second most commonly prescribed agents (27.4 % and 26.6% respectively).This was the first national study conducted on uncomplicated UTIs in Canada. [38]

Although national and international guidelines advise against the unethical use of antimicrobials for uncomplicated infections, most studies identify that the warnings are largely ignored. Most of the studies conducted and reviewed, have illustrated that worldwide, physicians are hesitant or reluctant to adhere to or follow guidelines. [27, 39]

Lugtenberg *et al* investigated prescribing principles in general practice among a representative sample of general practitioners in the Netherlands, They concluded that despite the availability of evidence based guidelines, adherence was less than 42%, and the level of conformity also showed a wide variability. The reasons for the lack of adherence were not well understood. [40]

Kuehlein *et al* analysed the prescribing habits and perceptions of general practitioners and confirmed that the principles outlined in the guidelines were seldom employed. The barriers identified included that personalities of doctors felt threatened by “higher powers” that dictated their prescriptions. Some felt the guiding principles necessitated a change in their current practice that was currently successful and therefore saw no need to alter it. [40, 41]

Other barriers identified, included: a lack of applicability to the resistance patterns identified in their particular communities; a lack of availability of the prescribed dosages at local pharmacies; and some antimicrobials require multiple daily dosing which is inconvenient and their patients become non-compliant. [40, 41]

The result of lack of adherence is the subsequent inability to practice evidence based medicine and the implementation of best practice principles and thus the best possible patient outcomes are compromised.

Empiric therapy of symptomatic women presenting with uncomplicated urinary tract infections has proven to be effective, however continuous evidence based strategies need to be assessed and modernised. There are multiple

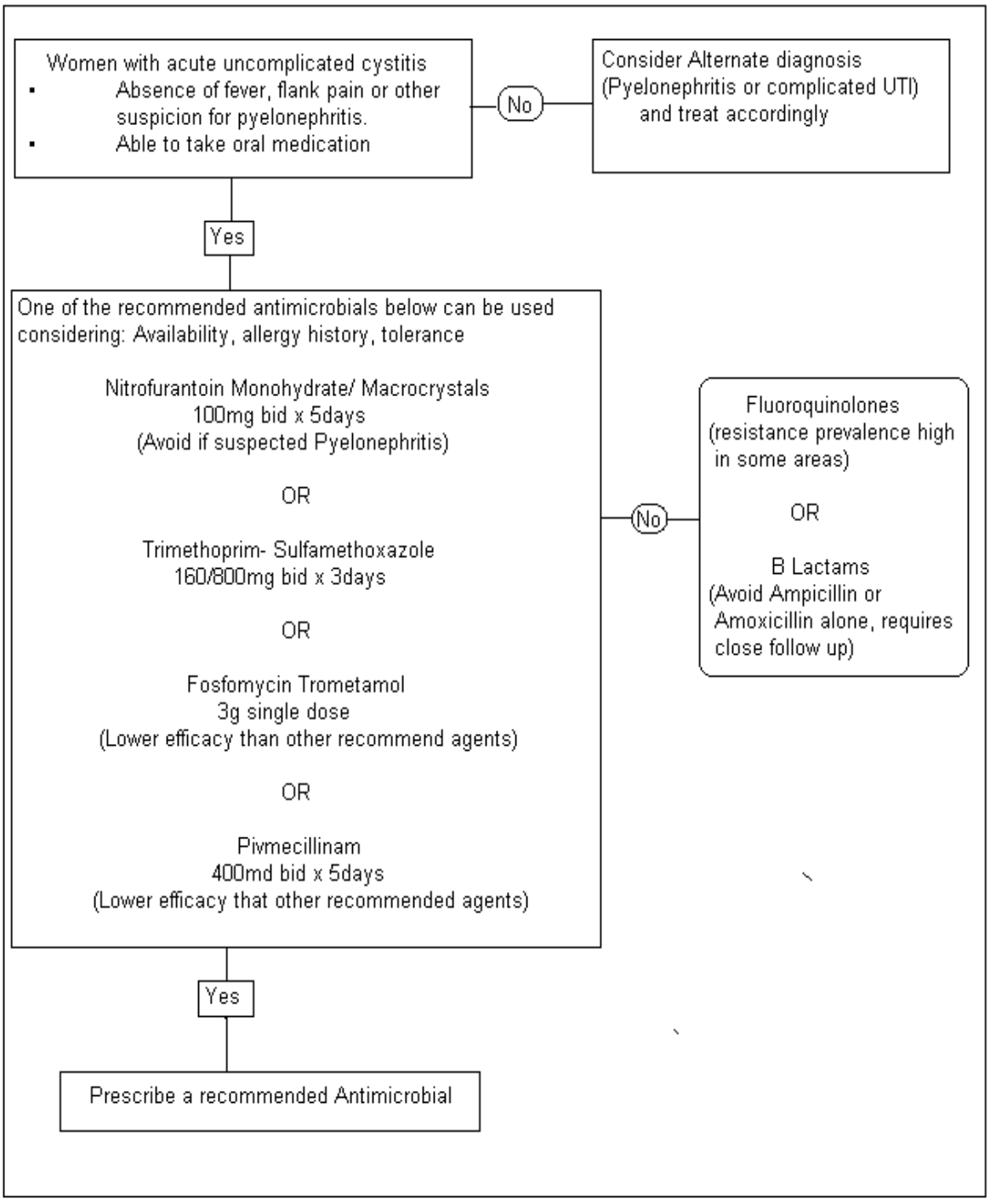
sources of country specific guidelines proposed and published for physicians to follow and the employment of guidelines should be encouraged to support the objective of antimicrobial stewardship. [26]

We could not uncover any evidence of medical boards, societies or government organisations exerting any pressure on physicians or any other means of encouragement to adhere to current guidelines.

The role of antibiotic therapy has been proven to shorten duration of illness and prevent worsening of the disease process. Falagas *et al* conducted a meta-analysis of 5 randomised controlled trials of patients treated with an antibiotic regimen versus a placebo. The analysis concluded that the clinical cure rate was much higher in women who received antibiotics when compared to their placebo counterparts. [13]

In 2010 the IDSA published updated guidelines for the management of Uncomplicated Cystitis in women. [30, 35]

Figure 1 IDSA Guidelines for the management of uncomplicated cystitis in women (2010)



2.8.3.1 Nitrofurantoin

Nitrofurantoin is the oldest antimicrobial agent documented for the urinary tract and remains in use today. The latest data suggests that resistance to nitrofurantoin remains low and is an effective agent against *E. Coli* isolates with resistance data across all studies regardless of site or year, falling between one and three percent. [23, 29]

Habte *et al* in a South African study confirmed that *E. Coli* isolates remain susceptible to nitrofurantoin, this finding was in keeping with other studies conducted in the context of developing countries. They agreed with the IDSA recommendations for nitrofurantoin to be utilised as a first line agent for uncomplicated cystitis. [10]

Although not well understood, the reason that nitrofurantoin may have maintained its efficacy could be related to the several mechanisms of action, whereby organisms are required to develop multiple mutations to develop resistance. Many cellular processes are activated by the reduction of Nitrofurantoin by bacterial flavoproteins, leading to the inhibition of protein synthesis, DNA synthesis, RNA synthesis and cell-wall synthesis. [20,29]

Another postulation for nitrofurantoin maintaining its low resistance profile is that it has limited use elsewhere and therefore the use has been fairly restricted to urinary tract infections, it has limited systemic absorption which prevents its usefulness in other systems. [26]

Nitrofurantoin can be maintained as a first- line agent (recommended by IDSA 2010), used in an endeavour to construct regimens where fluoroquinolone use is minimised. It must be noted however that prescriptions require multiple daily dosing regimens and a moderate side effect profile which may include gastrointestinal upset and acute or chronic pulmonary problems. Although early formulations of nitrofurantoin were associated with severe gastrointestinal distress, newer macrocrystalline formulations have resulted in

a marked decrease in adverse effects and displays an improved tolerance. [16, 26, 30, 31, 32]

The other major drawback in its use; is that *Proteus* species are inherently resistant to nitrofurantoin and *Klebsiella* also shows a high resistance pattern. The efficacy is therefore limited to other uropathogens, and infections of the lower tract as there is no evidence to support its efficiency in upper urinary tract infections. [12, 16]

Norris and Young claimed limited evidence to support the use of a 5 day course as guidelines recommend, suggesting a minimum of 7 days duration of therapy. This may result in poor compliance when combined with a four times per day dosing. [12]

A placebo- controlled, double blind study confirmed an 88% cure rate in 78 adult female patients presenting with uncomplicated UTIs, who received a 7 day course of nitrofurantoin. [12]

2.8.3.2 Co- Trimoxazole

Co-trimoxazole has remained the agent of choice over the last decade for the management of uncomplicated urinary tract infections, often prescribed empirically, in the absence of microbiological culture and sensitivity analysis to guide the antimicrobial selection. This was reinforced in the IDSA guidelines published in 1999. [29, 30]

It is important to note that although the side-effect profile is limited in most patients, there is documented evidence of rare but severe adverse events, including Lyell Syndrome (Toxic epidermal necrolysis) , a life-threatening dermatological emergency. [13]

Studies conducted assessing the resistance profile of co-trimoxazole consistently demonstrated the lack of effectiveness when the resistance

prevalence threshold exceeded 20%, where it is no longer advocated for use in acute cystitis. [29, 30]

Hooten and Bester disputed the findings suggesting that the evidence of co-trimoxazole resistance has been inflated and clinicians are needlessly replacing an effective agent. [34]

Norris and Young showed that prior to 1990, *E. Coli* resistance to co-trimoxazole was between 0 and 5%. By the mid 1990s they demonstrated a consistent increase to 7- 18%, and in their last analysis in 2001 this had increased to 36%. [12]

A case control analysis undertaken in an emergency department by Wright *et al* revealed certain risk factors for patient resistance. These included previous therapy on co-trimoxazole in the preceding three months (5 x increased risk) and previous therapy in the preceding two weeks (17x increased risk).Other variables included international travel, intestinal carriage of co-trimoxazole resistant *E. Coli*, secondary resistance obtained through contact with family members and close confined residents or children in day care on antibiotic treatment. The hazards were expanded upon by Miller and Tang who added recent hospitalisation, diabetes mellitus, recurrent urinary tract infections, oral contraceptive agents and oestrogen replacement medications. Some of the variables would place the patients in the category of complicated infections and therefore the agent would not have been recommended. [15, 29]

2.8.3.3 Fluoroquinolones

Fluoroquinolones as a class, have the only proven efficacy rates equivalent to Co –Trimoxazole when prescribed for three days. It is therefore often used in cases where resistance or other factors precludes the use of other first line agents. [29]

The selection of which fluoroquinolone is less important as they have very similar efficacies when managing acute cystitis. The decision of agent relies on tolerance, compliance and required duration of therapy. [13]

In the IDSA 2010 review, fluoroquinolone resistance remained below 10%, but there is comprehensive evidence of an increasing resistance when compared to previous years. [30]

Kalpana and Gupta agreed with the findings in the USA and Canada, in that the resistance levels remain well below the 20% sub- minimum, but that there has been a consistent rise in resistance over the past few years. [29]

Karlowsky *et al* noted that ciprofloxacin is the only agent that has demonstrated a three- fold increase in resistance in five years (1996 – 2001). [14]

In a worldwide study conducted on the susceptibility of norfloxacin, a 25 % increase in resistance was identified in the short duration of its availability for use in UTIs. [20]

Khawcharoenporn *et al* examined fluoroquinolone resistance rates in the ED and highlighted the importance of monitoring local resistance patterns and determining appropriate empiric antibiotic selection. [42]

It is important to note that fluoroquinolones are a critical and vital class of antimicrobial agents, especially for the management of food borne infections, sexually transmitted infections and nosocomial disease. Although they maintain a crucial role in the treatment of genitourinary infection, they should not be utilised as first line agents, as resistance to fluoroquinolones becomes a significant danger to public health, and should therefore only be employed discriminately to preserve their efficacy. [29, 34]

In the South African climate, the conservation of fluoroquinolone use is especially prudent. Ciprofloxacin is one of the only agents currently available for the management of Multi-Drug Resistant Tuberculosis (MDR TB). It is therefore extremely important to limit its use. [10]

Ciprofloxacin has a low side effect profile and excellent effectiveness in the management of uncomplicated UTIs and is therefore tempting to any physician. The use of the agent is not discouraged for its lack of efficacy but for the potential for increased resistance which will restrict the use in more difficult cases. [12, 16]

2.8.3.4 *B*- Lactams

B Lactams are no longer recommended for the management of uncomplicated cystitis. Amoxicillin and ampicillin are no longer a viable option in most communities as resistance rates across most studies exceed 25 %. Resistance in most regions of North America now exceeds 50%. [26, 29, 43]

Between 1970 and the late 1980's, amoxicillin was regarded as the empiric antibiotic of choice for the management of uncomplicated cystitis, however its indiscriminate use for diverse infections has rendered the agent ineffectual. [20]

Cephalosporins have been not been well investigated in the management of UTIs. They do play a role in managing infections especially in pregnant women. Third generation agents are generally superior in eradicating cystitis, but longer courses than other classes of antimicrobials are advocated. [13]

Uropathogen resistance to *B* Lactams has been demonstrated from the early 1990s and very little further analysis on the drug class has been investigated. There are only a few studies that have assessed the response of urinary tract pathogens to amoxicillin/ clavulanic Acid or more advanced generations. They

are being utilised in complicated infections especially pregnant women in whom other drugs are not considered safe for the foetus. [16 ,29]

In the same South African study referred to earlier, Habte *et al* proved that *E. Coli* and *Enterobacteriacae* are less susceptible to ampicillin and amoxicillin than other antimicrobial agents. *Proteus* species were more susceptible than *E. Coli* and *Klebsiella* are intrinsically resistant to ampicillin. As a class, *B*-Lactams need to be prescribed for a minimum of 7 days and still demonstrated suboptimal cure rates when compared with their non-*B*-Lactam counterparts. [10, 16]

2.9 Antibiograms

Hospital antibiograms are maintained by local microbiologists and often utilised by emergency physicians practising in their emergency department. Antibiotic selection is often largely based on their conclusions. [1, 26]

It is essential to take into account that most antibiograms overestimate the resistance patterns of a community when considering uncomplicated cystitis. Most laboratory surveys do not perform cultures for true acute UTIs; most cultures are submitted from inpatients or emergency departments following treatment failure or recurrent UTI cases. It is therefore likely that antibiograms will detect pathogens with a greater probability of resistance. [1, 26, 29]

Grover *et al* demonstrated that culture antibiotic sensitivity patterns differed significantly between outpatients and those admitted to the hospital. In an analysis of *E. Coli* sensitivity to co-trimoxazole, 94% of outpatient cases were sensitive, however only 71% of inpatients proved susceptibility to the antimicrobial. Their study suggested that the use of hospital antibiograms as a guide for empiric outpatient therapy was inappropriate. [9]

An investigation conducted in Singapore by Miller and Tang analysed a prospective survey of 2 outpatient clinics' data submitted to the district

hospital micro-laboratory. 46% of *E. Coli* was resistant to co-trimoxazole, but only 21% of the cases obtained from outpatients demonstrated resistance. [15]

Norris and Young pointed out the selection bias obtained from hospital specimens, illustrating that antibiograms tend to reflect the spectrum of uropathogens generally isolated from in-hospital specimens. These uropathogens are captured from complicated patients or those with nosocomially acquired infection. It therefore follows that the antibiogram will overestimate the overall resistance in the surrounding community and the scope of atypical uropathogens cultures. [12]

Antibiograms should not be discounted entirely but rather used as a guide to provide insight into local aetiology and susceptibility patterns. The decision of which antimicrobial might be utilised should not be based on them alone. [1, 12]

2.10 Adjuvant therapy

The role of adjuvant therapy must not be discounted either as symptomatic control with urinary alkalinisers and non steroidal anti-inflammatory agents may successfully alleviate the dysuric symptoms. “Flushing” the tract with increased water intake may shorten the duration of illness. There are many studies that discuss the role of cranberry and other natural products in both treatment and prevention but this topic is beyond the scope of this study. [19]

Prevention guidance can be given to patients, physicians should promote good bathroom hygiene, anterior to posterior wiping habits, post – coital voiding and cotton underwear as a few useful methods of preventing disease recurrence. [19]

Gobernado *et al* have stressed the importance of controlling antibiotic prescribing principles in an effort to reduce and retard the extension of resistance. This can only be done by continual surveillance and improved

comprehension of the determinants of resistance and the optimisation of antibiotic utilisation for bacterial infections across the board. [14, 30]

3. MATERIALS AND METHODS

3.1 Ethics

This research was approved by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of the Witwatersrand (protocol approval number M110433 - see Appendix 2).

3.2 Study Design

The study is a retrospective evaluation of data collected from patients attending the Life Fourways Hospital Trauma and Emergency department with laboratory confirmed urinary tract infections between January 2008 and January 2009.

3.3 Study Setting and Population

The study included all female patients who presented to the ED with symptomatic uncomplicated cystitis based on the analysis of a "*ComboStix 10*" urine dipstick. Urine microscopy, culture and sensitivity results were analysed. A positive culture was defined as 10^5 colonies/ ml

The urine was collected and analysed by a single laboratory to ensure uniformity, from all symptomatic patients presenting to the EDComplicated patients were removed retrospectively from the study group. The data was used to identify pathogens responsible for uncomplicated UTIs and their sensitivity to antimicrobial agents. Resistance to uropathogen isolates were established and antimicrobial sensitivity patterns elicited. The data was readily available to the researcher.

There is no bias with regard to antimicrobial therapies as there was no pharmaceutical company involvement and no compulsory hospital formulary. Although medical practitioner prescribing habits were recorded, they will not influence the laboratory sensitivity data as the culture was obtained prior to the commencement of antimicrobial therapy.

There is limited cost as the study is retrospective and the data easily available.

Inclusion criteria:

Demographic data will include the patient's age.

Exclusion criteria:

No data will be excluded from the study.

3.4 Study Protocol

3.4.1 Data collection

A spreadsheet was specifically designed to accommodate the data from the patient files and laboratory records. Data collected included patients' ages, presenting symptoms and urine dipstick analysis findings. Where possible, the antimicrobial prescribed for the patient by the doctor on duty was documented. Laboratory findings documented included microscopic identification of white blood cells, red blood cells and pus cells and chemical detection of blood, nitrites and leucocytes. Recognition of organism growth and susceptibility testing was also extracted from laboratory records. (See Appendix 1)

3.4.2 Outcome Measures

Outcome measures included:

1. Age analysis and infection pattern differences in bimodal age peaks.
2. “Side room” urine dipstick testing findings compared to formal laboratory testing- reliability of screening.
3. Organism culture and sensitivity patterns.

3.4.3 Sample Size Estimation

In this study, a minimum sample size required for confidence levels to exceed 95% ($p<0.05$) was 100 patients. 213 patients have been included in this study. The sample was found to be sufficient for execution of normal population sampling.

3.4.4 Data Analysis

Data was analysed utilising the following:

1. Descriptive Statistics for interval and categorical data.
2. Comparative Statistics e.g. age vs. organism cultured.
3. Correlations e.g. symptoms experienced relative to organism cultured.
4. Chi- Square test with contingency tables for “Goodness of fit”.
5. T – Tests for difference of means.

3.4.5 Significance level

A $p < 0.05$ was considered to be significant for all statistical tests.

3.5 Software

All data was entered and stored in a Microsoft Excel® (Microsoft Office 2007, Microsoft Corporation) spreadsheet.

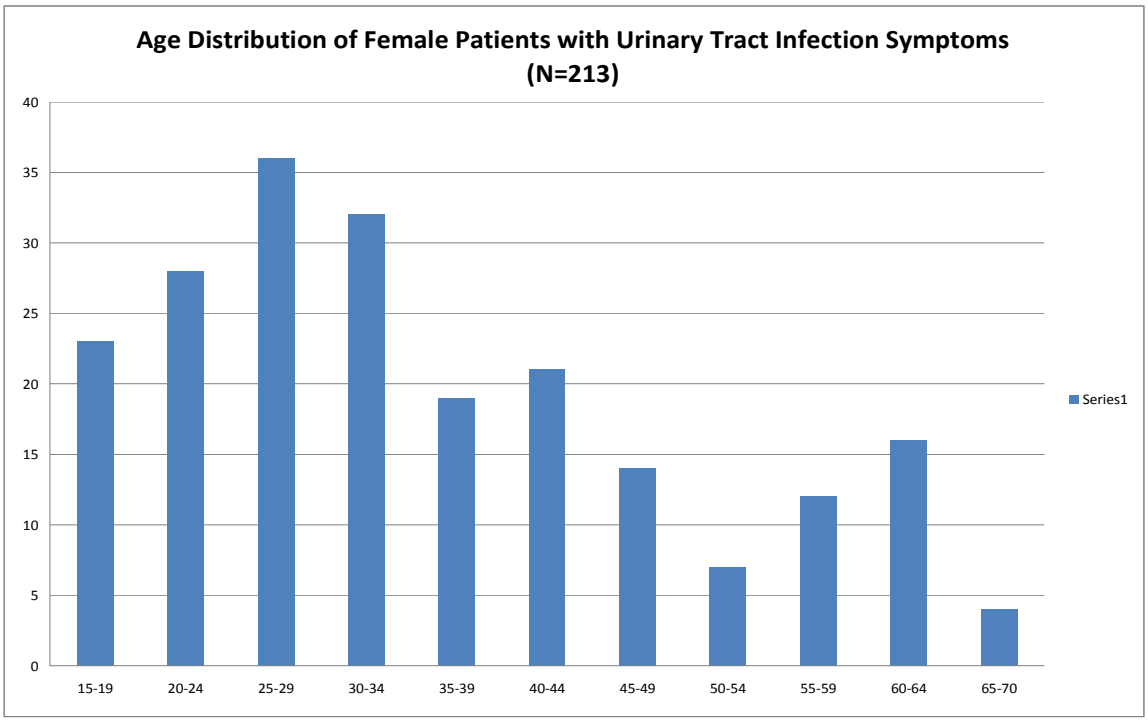
3.6 Methodological limitations of this study

- There was a time limit of one year placed on this study. Although it included the presenting population within a 1 year period, data cannot be extrapolated had the study gone on for longer, as to emerging patterns;
- This study did not document follow- up visits or symptom resolution;
- The study was limited to a single institution.

4. RESULTS

4.1 Age Distribution

Figure 2 Age distribution analysis of female patients presenting with UTI



Analysis of the age distribution demonstrated an overall mean age of 36.54 years. There was also clear evidence of a bimodal age variation with the first peak incidence at 25 – 29 years and the second at 60 – 64 years of age.

4.2 Age Distribution of female patients by cultured organism

	Total Sample	E.Coli	Proteus Mirabilis	E. Faecalis	Grp B Strep	E. Cloacae	Klebs. Pneum.	Pseudo. Aerug.	Staph Spp.
n (%)	213 (100%)	174 (81.7%)	9 (4.2%)	8 (3.8%)	7 (3.3%)	3 (1.4%)	3 (1.4%)	3 (1.4%)	2 (0.9%)
Mean Age (yrs)	37	36	49	41	30	26	44	50	21
Age Range (yrs)	15-69	16-69	20-61	27-61	22-35	16-42	32-65	29-61	17-21
Std Deviation	14	13.4	16.7	10.9	4.8	n/a	n/a	n/a	n/a
Median age (yrs)	34	33	58	41	30	n/a	n/a	n/a	n/a
Mode (yrs)	20	20	61		34	n/a	n/a	n/a	n/a

Table 6 Age distribution of female patients with Urinary Tract Infection - By Cultured Organism (N=213)

4.3 Uropathogenic Aetiology

Causative Organism	NO.	%
Escherichia Coli	174	83
Proteus Mirabilis	9	5
Enterococcus Faecalis	8	5
Group B Streptococci	7	3
Enterobacter Cloacae	3	1
Klebsiella Pneumoniae	3	1
Pseudomonnas Aeruginosa	3	1
Citrobacter Freundii	2	1
Enterobacter Agglomerans	1	0
Staphylococcus Aureus	1	0
Staphylococcus Saprophyticus	1	0
Acinetobacter Baumanni	1	0

Table 7 Causative aetiology responsible for UTIs in women

The study demonstrated that E Coli represented over 80 percent of the bacteria responsible for uncomplicated urinary tract infections.

4.4 Presenting Symptoms

SYMPTOMS	No	%
Dysuria	136	31
Abdominal Pain	132	31
Frequency	65	15
Lower back pain	55	13
Urgency	44	10

Table 8 Common presenting symptoms of UTIs in women

4.5 Frequency of Symptoms caused by specific bacteria

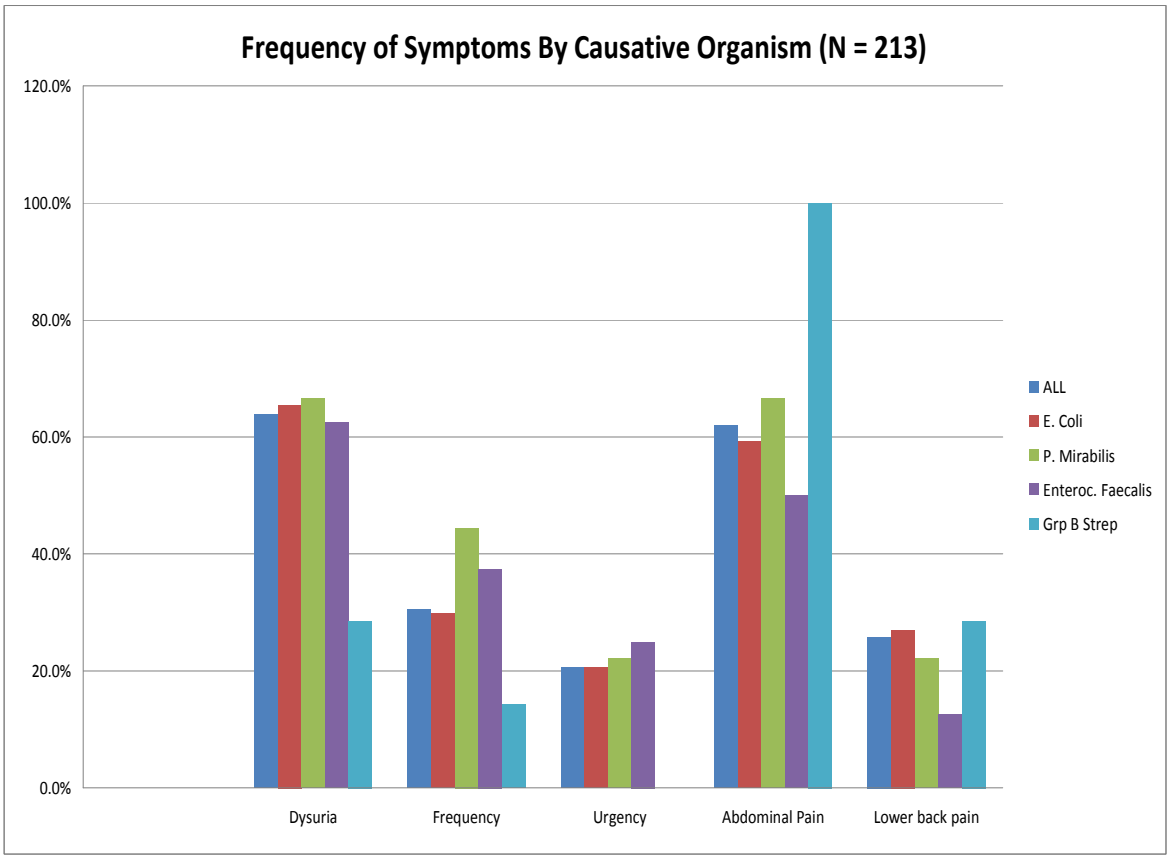
	Dysuria	Frequency	Urgency	Lower Abdominal Pain	Lower Back Pain
ALL	136	65	44	132	55
E. Coli	114	52	36	103	47
P. Mirabilis	6	4	2	6	2
E. Faecalis	5	3	2	4	1
Group B Strep	2	1	0	7	2

Table 9 Frequency of symptoms related to aetiology (N)

As % of N	Dysuria	Frequency	Urgency	Lower Abdominal Pain	Lower back pain
ALL	63.8%	30.5%	20.7%	62.0%	25.8%
E. Coli	65.5%	29.9%	20.7%	59.2%	27.0%
P. Mirabilis	66.7%	44.4%	22.2%	66.7%	22.2%
Enteroc. Faecalis	62.5%	37.5%	25.0%	50.0%	12.5%
Grp B Strep	28.6%	14.3%	0.0%	100.0%	28.6%

Table 10 Frequency of symptoms related to aetiology (%)

Figure 4 Frequency of symptoms related to aetiological agent



4.6 Validity of Side-room Screening investigations – Urine Dipstick Analysis

4.6.1. White Blood Cells

		Laboratory		
		YES	**NO**	
Dipstick	**YES**	205	0	**206**
	NO	0	1	**1**
		206	**1**	**207**

Table 11 Validity of leucocytes in urine dipstick analysis

It is Important to note that in seven of the patients, the dipstick findings were not documented or not performed as no evidence of the procedure was recorded in the doctors notes.

Specificity = 1/1 = 100%

Sensitivity = 205/206 = 99.5%

4.6.2. Blood

	Laboratory			
		YES	**NO**	
Dipstick	**YES**	177	10	**187**
	NO	10	19	**29**
		187	**29**	**216**

Table 12 Validity of blood in urine dipstick analysis

Sensitivity= 177/187 = 94.6%

Specificity = 19/29 = 65.6%

4.6.3. Nitrites

	Laboratory			
		YES	NO	
Dipstick	YES	81	15	**96**
	NO	14	110	**124**
		95	**125**	**220**

Table 13 Validity of Nitrites in urine dipstick analysis

Sensitivity = 81/ 95 = 85.3%

Specificity = 110/125 = 88%

4.7. Sensitivity Analysis

	Ampicillin/ Amoxycillin	Amoxycillin + Clavulanate	Pip/Taz	Cefuroxime	Ceftriaxone	Aminoglycosides	Quinolones	Co Trimoxazole	Nitrofurantoin
Resistant	126	20	10	8	8	8	19	95	16
Sensitive	64	170	180	182	182	182	171	95	174
% Sensitive	33.7%	89.5%	94.7%	95.8%	95.8%	95.8%	90.0%	50.0%	91.6%

Table 14 Sensitivity data of commonly presenting uropathogens

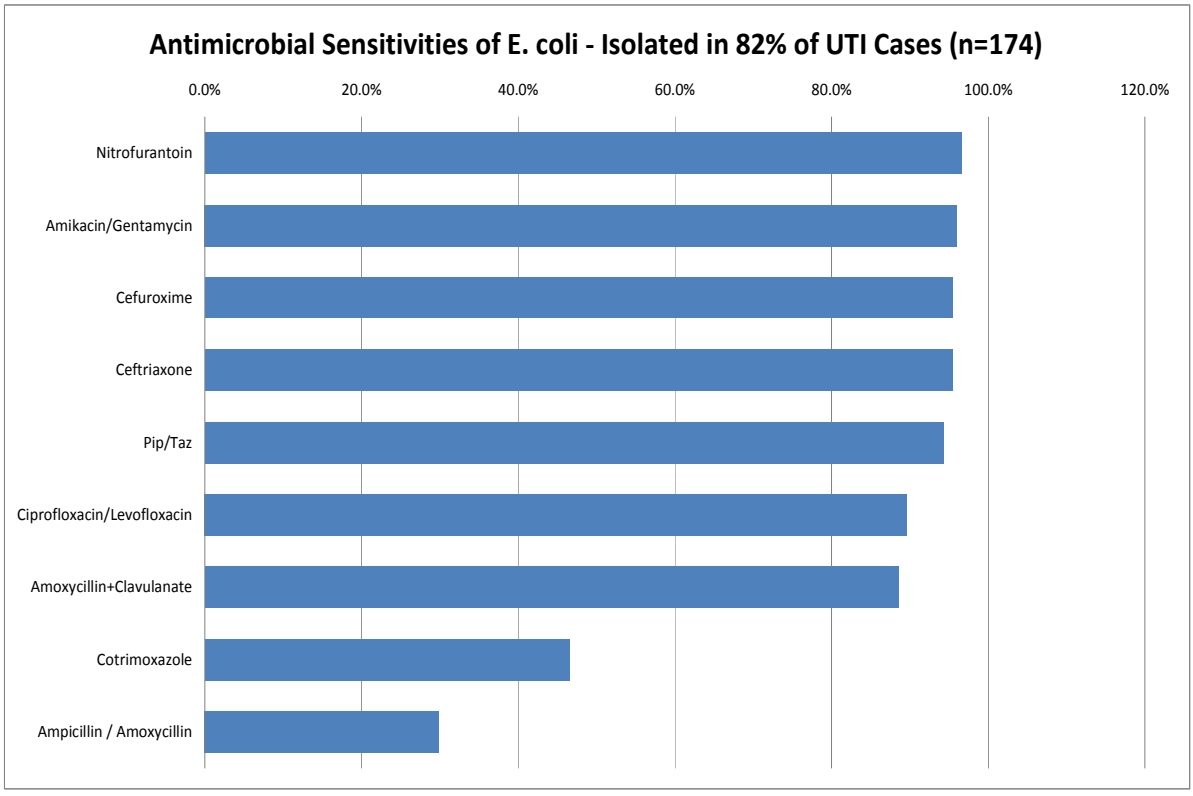


Figure 6 Antimicrobial sensitivities for commonly presenting uropathgens

	Ampicillin/ Amoxycillin	Amoxycillin + Clavulanate	Pip/Taz	Cefuroxime	Ceftriaxone	Aminoglycosides	Quinolones	Co Trimoxazole	Nitrofurantoin
Resistant	11	6	2	3	2	2	2	4	3
Sensitive	3	8	12	11	12	12	12	10	11
Total	14	14	14	14	14	14	14	14	14
% Sensitive	21.4%	57.1%	85.7%	78.6%	85.7%	85.7%	85.7%	71.4%	78.6%

Table 16 Antimicrobial sensitivities of less common causes of uncomplicated cystitis

5. DISCUSSION

5.1. Age Distribution

The age distribution curve identifies a bimodal incidence peak in uncomplicated infections. The initial peak at 25 – 29 years is consistent with other studies and may be attributed to young pre-menopausal non – pregnant females who are sexually active. Behrooozi et al described sexual intercourse in the preceding 2 weeks as a major risk factor for uncomplicated infection.(7)

The second peak identified was between 60 and 64 years. It demonstrated a lower peak and maybe consistent with mucosal and anatomical changes associated with menopause. Age may also be a factor in early identification (or lack thereof) of complicated infection and should therefore be monitored more closely.

5.2. Uropathogenic Aetiology

There are four main organisms responsible for over ninety percent of the isolates and this should be considered when selecting an empirical antimicrobial agent suitable for these types of infections. The findings were consistent with findings of other developing countries e.g. Greece and Korea. (See table 1)

5.3. Presenting Symptoms

The symptoms are fairly consistent in uncomplicated infections and often patients in early infection only present with one complaint. The significance of the table of presenting symptoms outlines the importance of excluding UTIs in all patients who present with lower abdominal pain or lower back pain, even in the absence of dysuria, urgency, frequency or other symptoms.

5.4. Frequency of Symptoms by Causative Organism

The study demonstrated no statistical significance in the symptoms defined by organism; thus reinforcing the utilisation of empiric therapy prior to laboratory diagnosis.

5.5. Validity of Urine Dipstick analysis

It has been well documented that the gold standard for urine analysis remains laboratory microscopy, culture and sensitivity. There are however delays and significant cost implications from both an investigation in isolation perspective, as well as loss of productivity of the patient and a higher complication rate.

In this study, blood presence on urinalysis was found to be fairly sensitive (94.6%) but non-specific (65.6%) and should therefore not be used in isolation to identify or diagnose uncomplicated cystitis. The dipstick, when utilising leucocyte esterase and nitrites, was 90% reliable for detection and diagnosis. This supports the argument that empiric treatment of patients can be initiated on the results of a urine dipstick.

The dipstick result forms a good basis for therapeutic decision making, especially in conjunction with 4 major organisms responsible for over 90% of the infections and a consistent dipstick result.

There was no statistically significant correlation between each of white blood cells, presence of blood and nitrites with the micro-organism subsequently cultured.

5.6. Sensitivity Data Evaluation

The sensitivity data was found to be in keeping with other developing countries. Co-trimoxazole is no longer a viable option for treatment, as within international guidelines, it demonstrates above 20% resistance across most uropathogens (50% resistance across common uropathogens). It is important to note that amoxycillin and ampicillin demonstrate particularly high resistance rates as well (66.3% resistance across common uropathogens). There is no confidence in prescribing Penicillins for uncomplicated cystitis and patients should be followed closely if this is required.

The sensitivity to Nitrofurantoin was also in keeping with other international studies, demonstrating less than 6 % resistance across all isolates, despite being one of the oldest antimicrobial agents. It is also important to note that from a pharmaco-economic standpoint it could allow for significant cost savings as the agent is one of the least expensive antibiotics. Nitrofurantoin must be administered in four times daily dosing which is one of the negative aspects that should be taken into consideration when deciding on the appropriateness of the medication.

Significant resistance is already being demonstrated to the quinolone family, with over 10% resistance to ciprofloxacin and levofloxacin. This has a significant impact on the South African patient community, especially when anti-tuberculous medications for drug resistant strains are taken in to account. The use of these drugs should be curbed immediately to prevent further resistance. A small number of older patients demonstrated less common aetiologies and it is only in these patients where ciprofloxacin may be prescribed as other antibiotics in this range require intravenous administration.

Another significant discovery is the low resistance rates to cephalosporins as a class. These are extremely useful agents as they can be administered in complicated infections as well. They can be considered for first line

administration due to their low resistance rates, easy accessibility, good tolerability and daily to twice daily dosing schedule which allows for improved compliance relative to Nitrofurantoin

5.7. Limitations of the Study

- A limitation of positive culture defined excludes patients that do not meet the criteria. This may exclude patients with sub-clinical or asymptomatic infections and patients who meet other criteria for UTI;
- All complicated urinary tract infections (as defined), male and paediatric, were excluded from the study;
- There was not 100% compliance of urine samples sent for culture, as some symptomatic patients samples were not sent for culture;
- Specimens may be contaminated or incorrectly collected despite explanation;
- Some data was collected from patient chart review and this relies on the emergency doctor for completeness of information;
- The study does not address the clinical significance of the antimicrobial resistance as clinical follow up was not obtained. It therefore cannot be established whether the resistance identified complicated the use of empirical treatment and led to treatment failure. Clinical and microbiological outcomes with regard to antibiotic resistance was not evaluated, but could well be the basis for further study.

6. CONCLUSION

The study outlined the high resistance rates of uropathogens to conventional antimicrobial agents and highlighted the haphazard or indiscriminate prescribing patterns of many practitioners managing such infections. The study outlined the wide variation of prescribing patterns in the absence of formalised protocols.

We have again demonstrated the need for robust empirical guidelines with evidence based answers to be employed in all settings where patients seek acute care.

7. RECOMMENDATIONS

Further research should be undertaken in the South African primary and emergency care arena to assess prevalence and local differences as this will yield a better understanding of the impact of antimicrobial utilisation and resistance in uncomplicated UTIs.

As there is no statistical significance demonstrated in the antibiotic resistance levels in the four most susceptible antimicrobials, cost considerations and antibiotic stewardship become more important. In the South African climate, financial considerations are substantial and therefore Nitrofurantoin would be our most recommended agent, followed by generic Cephalosporins. Nitrofurantoin should in fact be employed as a first line agent and Quinolone – sparing regimens should be introduced into all emergency departments.

Of particular interest is that during the process of this review, it became evident that Nitrofurantoin has been withdrawn from the South African market and is no longer available in private or government sectors. Nitrofurantoin still features in WHO Essential Drug List, and should seriously be considered for reintroduction in the wake of the National Health Insurance programme.

Ongoing surveillance of resistance trends is imperative in conjunction with the review and updating of evidence based guidelines.

Although our data was in keeping with other developing countries, emerging pattern variations are constant and should be detected early.

8. REFERENCES

(1) Abrahamian FM, Moran GJ, Talan DA. Urinary tract infections in the emergency department. Infect Dis Clin North Am 2008 Mar; 22(1):73-87, vi.

(2) Foster RT S. Uncomplicated urinary tract infections in women. Obstet Gynecol Clin North Am 2008 Jun;35(2):235-48, viii.

(3) Tambekar DH, Dhanorkar DV, Gulhabe SR, Khandelwal VK, Dudhane MN. Antibacterial susceptibility of some urinary tract pathogens to commonly used antibiotics. Afr J Biotechnol 2006;5(17):1562-1565.

(4) Schito GC, Naber KG, Botto H, Palou J, Mazzei T, Gualco L, et al. The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. Int J Antimicrob Agents 2009 Nov;34(5):407-413.

(5) Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis 2004 Apr 15;38(8):1150-1158.

(6) Aypak C, Altunsoy A, Duzgun N. Empiric antibiotic therapy in acute uncomplicated urinary tract infections and fluoroquinolone resistance: a prospective observational study. Ann Clin Microbiol Antimicrob 2009 Oct 24;8:27.

(7) Behrooozi A, Rahbar M, Yousefi JV. A survey on the epidemiology of urinary tract infections and resistance pattern of uropathogens in an Iranian 1000-bed tertiary care hospital. Afr J Microbiol Res 2010 May;4(9):753-756.

(8) Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann Clin Microbiol Antimicrob 2007 Mar 23;6:4.

(9) Grover ML, Bracamonte JD, Kanodia AK, Bryan MJ, Donahue SP, Warner AM, et al. Assessing adherence to evidence-based guidelines for the diagnosis and management of uncomplicated urinary tract infection. Mayo Clin Proc 2007 Feb;82(2):181-185.

(10) Habte TM, Dube S, Ismail N, Hoosen AA. Hospital and community isolates of uropathogens at a tertiary hospital in South Africa. S Afr Med J 2009 Aug;99(8):584-587.

(11) Perfetto EM, Keating K, Merchant S, Nichols BR. Acute uncomplicated UTI and E. coli resistance: implications for first-line empirical antibiotic therapy. J Manag Care Pharm 2004 Jan-Feb;10(1):17-25.

(12) Norris DL,2nd, Young JD. Urinary tract infections: diagnosis and management in the emergency department. Emerg Med Clin North Am 2008 May;26(2):413-30, ix.

(13) Wagenlehner FM, Weidner W, Naber KG. An update on uncomplicated urinary tract infections in women. Curr Opin Urol 2009 Jul;19(4):368-374.

(14) Gobernado M, Valdes L, Alos JI, Garcia-Rey C, Dal-Re R, Garcia-de-Lomas J, et al. Antimicrobial susceptibility of clinical Escherichia coli isolates from uncomplicated cystitis in women over a 1-year period in Spain. Rev Esp Quimioter 2007 Mar;20(1):68-76.

(15) Miller LG, Tang AW. Treatment of uncomplicated urinary tract infections in an era of increasing antimicrobial resistance. Mayo Clin Proc 2004 Aug;79(8):1048-53; quiz 1053-4.

(16) Katsarolis I, Poulakou G, Athanasia S, Kourea-Kremastinou J, Lambri N, Karaiskos E, et al. Acute uncomplicated cystitis: from surveillance data to a rationale for empirical treatment. Int J Antimicrob Agents 2010 Jan;35(1):62-67.

(17) Matute AJ, Hak E, Schurink CA, McArthur A, Alonso E, Paniagua M, et al. Resistance of uropathogens in symptomatic urinary tract infections in Leon, Nicaragua. Int J Antimicrob Agents 2004 May;23(5):506-509.

(18) Yamamoto S, Higuchi Y, Nojima M. Current therapy of acute uncomplicated cystitis. Int J Urol 2010 May;17(5):450-456.

(19) Chung A, Arianayagam M, Rashid P. Bacterial cystitis in women. Aust Fam Physician 2010 May;39(5):295-298.

(20) Kashanian J, Hakimian P, Blute M,Jr, Wong J, Khanna H, Wise G, et al. Nitrofurantoin: the return of an old friend in the wake of growing resistance. BJU Int 2008 Dec;102(11):1634-1637.

(21) Marco CA, Parker K. Antimicrobial resistance among organisms causing urinary tract infections. Acad Emerg Med 1997 Feb;4(2):159-160.

(22) Bahagon Y, Raveh D, Schlesinger Y, Rudensky B, Yinnon AM. Prevalence and predictive features of bacteremic urinary tract infection in emergency department patients. Eur J Clin Microbiol Infect Dis 2007 May;26(5):349-352.

(23) Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Palatnik LP, et al. Antibiotic resistance in outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int J Antimicrob Agents 2005 Nov;26(5):380-388.

(24) Farajnia S, Alikhani MY, Ghotaslou R, Naghili B, Nakhlband A. Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. Int J Infect Dis 2009 Mar;13(2):140-144.

(25) Lee SJ, Lee SD, Cho IR, Sim BS, Lee JG, Kim CS, et al. Antimicrobial susceptibility of uropathogens causing acute uncomplicated cystitis in female outpatients in South Korea: a multicentre study in 2002. Int J Antimicrob Agents 2004 Sep;24 Suppl 1:S61-4.

(26) Nicolle L, Anderson PA, Conly J, Mainprize TC, Meuser J, Nickel JC, et al. Uncomplicated urinary tract infection in women. Current practice and the effect of antibiotic resistance on empiric treatment. Can Fam Physician 2006 May;52:612-618.

(27) Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. The diagnosis of urinary tract infection: a systematic review. Dtsch Arztebl Int 2010 May;107(21):361-367.

(28) Leman P. Validity of urinalysis and microscopy for detecting urinary tract infection in the emergency department. Eur J Emerg Med 2002 Jun;9(2):141-147.

(29) Gupta K. Emerging antibiotic resistance in urinary tract pathogens. Infect Dis Clin North Am 2003 Jun;17(2):243-259.

(30) Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011 Mar;52(5):e103-20.

(31) Ho PL, Wong RC, Yip KS, Loke SL, Leung MS, Mak GC, et al. Antimicrobial resistance in Escherichia coli outpatient urinary isolates from women: emerging multidrug resistance phenotypes. Diagn Microbiol Infect Dis 2007 Dec;59(4):439-445.

(32) Ho PL, Yip KS, Chow KH, Lo JY, Que TL, Yuen KY. Antimicrobial resistance among uropathogens that cause acute uncomplicated cystitis in women in Hong Kong: a prospective multicenter study in 2006 to 2008. Diagn Microbiol Infect Dis 2010 Jan;66(1):87-93.

(33) Lee G, Cho YH, Shim BS, Lee SD. Risk factors for antimicrobial resistance among the Escherichia coli strains isolated from Korean patients

with acute uncomplicated cystitis: a prospective and nationwide study. J Korean Med Sci 2010 Aug;25(8):1205-1209.

(34) Hooton TM, Besser R, Foxman B, Fritsche TR, Nicolle LE. Acute uncomplicated cystitis in an era of increasing antibiotic resistance: a proposed approach to empirical therapy. Clin Infect Dis 2004 Jul 1;39(1):75-80.

(35) Araujo SM, Mourao TC, Oliveira JL, Melo IF, Araujo CA, Araujo NA, et al. Antimicrobial resistance of uropathogens in women with acute uncomplicated cystitis from primary care settings. Int Urol Nephrol 2010 Jun 18.

(36) Le TP, Miller LG. Empirical therapy for uncomplicated urinary tract infections in an era of increasing antimicrobial resistance: a decision and cost analysis. Clin Infect Dis 2001 Sep 1;33(5):615-621.

(37) Martinez MA, Inglada L, Ochoa C, Villagrasa JR, Spanish Study Group On Antibiotic Treatments. Assessment of antibiotic prescription in acute urinary tract infections in adults. J Infect 2007 Mar;54(3):235-244.

(38) McIsaac WJ, Prakash P, Ross S. The management of acute uncomplicated cystitis in adult women by family physicians in Canada. Can J Infect Dis Med Microbiol 2008 Jul;19(4):287-293.

(39) Bojic-Milicevic G, Mikov M, Dautovic R. Common errors in diagnosis and management of urinary tract infections--microbiological aspects]. Med Pregl 2005 Jul-Aug;58(7-8):380-387.

(40) Lugtenberg M, Burgers JS, Zegers-van Schaick JM, Westert GP. Guidelines on uncomplicated urinary tract infections are difficult to follow: perceived barriers and suggested interventions. BMC Fam Pract 2010 Jun 28;11(1):51.

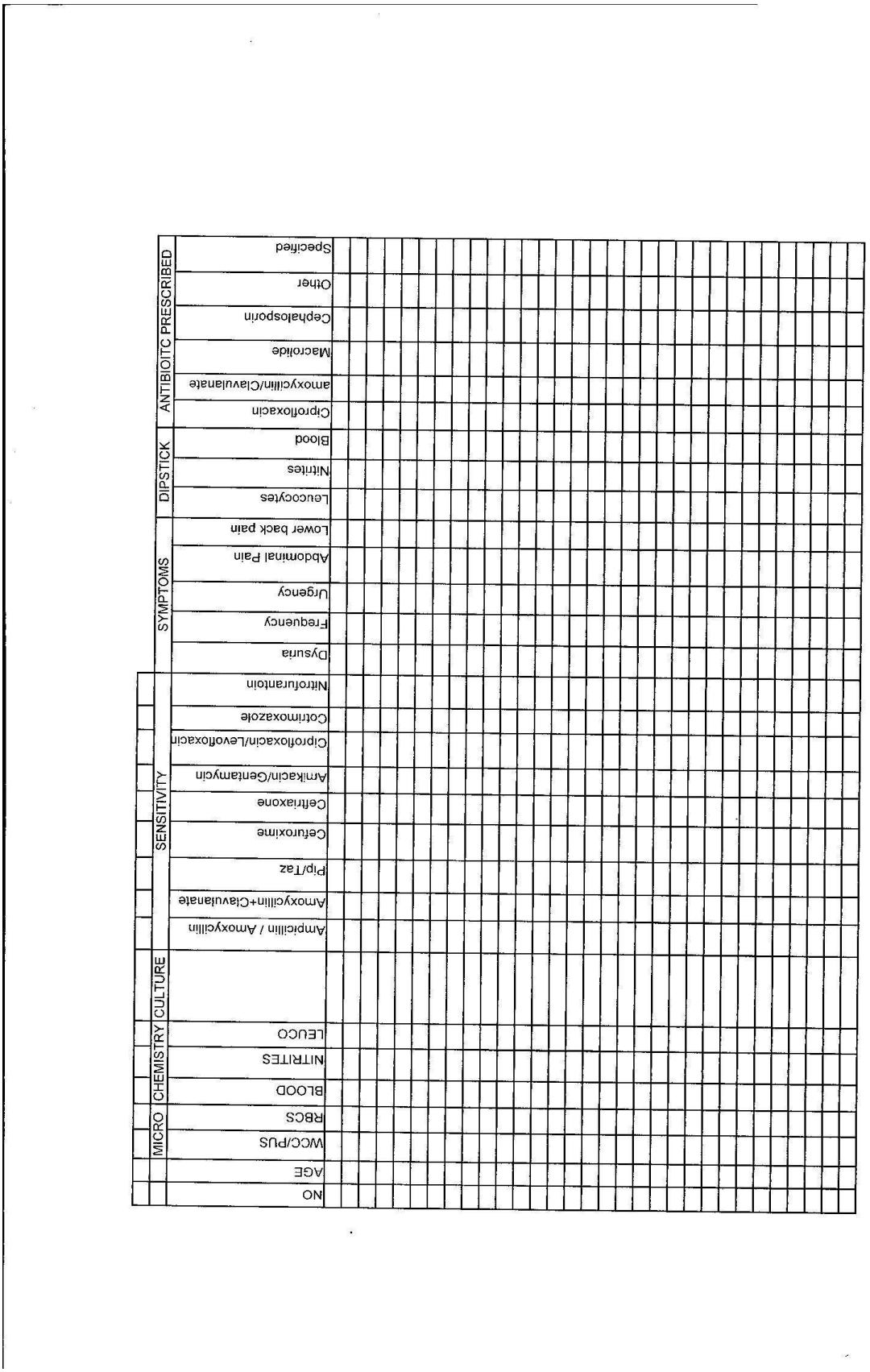
(41) Kuehlein T, Goetz K, Laux G, Gutscher A, Szecsenyi J, Joos S. Antibiotics in urinary-tract infections. Sustained change in prescribing habits

by practice test and self-reflection: a mixed methods before-after study. BMJ Qual Saf 2011 Jan 24.

(42) Khawcharoenporn T, Vasoo S, Ward E, Singh K. High rates of quinolone resistance among urinary tract infections in the ED. Am J Emerg Med 2010 Nov 12.

(43) Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. Ann Intern Med 2001 Jul 3;135(1):41-50.

APPENDIX 1: Data collection spreadsheet



APPENDIX 2: Human Research Ethics Committee clearance

M110433

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Jennifer Frankel

CLEARANCE CERTIFICATE	**M110433**
PROJECT	Are Empirical Antimicrobial agents Currently Prescribed for Adult Patients Presenting to the Emergency Unit with Uncomplicated Cystitis Appropriate?
INVESTIGATORS	Dr Jennifer Frankel.
DEPARTMENT	Department of Emergency Medicine
DATE CONSIDERED	06/05/2011
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 06/05/2011 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Dr Charl van Loggerenberg

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...