FOSFOMYCIN: AN ORAL TREATMENT OPTION FOR MULTI DRUG RESISTANT UROPATHOGENS

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DECLARATION

I, Mrs Alicia Naidoo, student number 200005216, hereby declare that the dissertation entitled:

Fosfomycin: an oral treatment option for multi drug resistant uropathogens

is the result of my own original work and has not been submitted in any other form to the University of KwaZulu Natal or any other tertiary institution for the purposes of obtaining an academic qualification, whether by myself or any other party. Where the work of others has been used, it has been duly noted and acknowledged in the text.

All routine and experimental work carried out in this dissertation was performed by me under the supervision of Dr Khine Swe Swe Han and Dr Nomonde Mvelase.

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DEDICATION

This dissertation is dedicated to my family, who have always encouraged and supported me through all my endeavours.

PRESENTATIONS

Oral Presentation: African Society for Laboratory Medicine (ASLM) Conference 2021

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LIST OF ACRONYMS

AMC: Amoxicillin Clavulanic Acid

AST: Antimicrobial Susceptibility Testing

ATCC: American Type Culture Collection

CFU: Colony Forming Unit

CLSI: Clinical Laboratory Standards Institute

ESBL: Extended Spectrum Beta Lactamase

EUCAST: European Committee on Antimicrobial Susceptibility Testing

MDR: Multi Drug Resistant

ME: Major Error

mE: Minor Error

MRSA: Methicillin Resistant *Staphylococcus aureus*

MSU: Mid-stream Urine

Mur A: N-acetyl glucosamine enolpyruvltransferase

NHLS: National Health Laboratory Services

PEP: Phosphoenolpyruvate

SA: South Africa

USA: United States of America

UTI: Urinary tract infection

VME: Very Major Error

VRE: Vancomycin Resistant *Enterococcus*

ABSTRACT

Introduction

Urinary tract infections (UTIs) are commonplace in both the community and hospital environment where it is accepted clinical practice to treat empirically. Consequently, this has led to an alarming increase in antimicrobial resistance in frequently prescribed oral treatment options. In light of the global shortage of newly discovered antibiotics, there is a need to relook at older antimicrobial agents, such as fosfomycin, to fill the gap in therapeutic guidelines.

Purpose of the Study

It was the purpose of this study to ascertain the extent of antimicrobial resistance in commonly isolated urinary pathogens to frequently prescribed antibiotics. This was done with the intention of bringing to light the severity of the situation. We also looked at fosfomycin as a possible alternative to the currently recommended treatment options most especially in multi drug resistant (MDR) infections.

Method

A retrospective analysis of antimicrobial susceptibility data of positive urine specimens collected during 2018 – 2020 was performed. Additionally, fosfomycin susceptibility testing was performed in 178 stored MDR uropathogenic isolates using the gold standard agar dilution method. We also compared agar dilution (reference method) to disk diffusion and E test as they are less labour intensive. All data and isolates were obtained from RK Khan Laboratory located in KwaZulu Natal, South Africa. The Clinical and Laboratory Standards Institute guidelines was utilised as the guiding document.

Results

While conducting the laboratory information system (LIS) based review, it was determined that within the study time frame, 3044 common urinary pathogens were isolated, with *Escherichia coli* being the most frequent cause of UTI (1603: 53%), followed by *Klebsiella* spp (437: 14%). Both organisms showed high rates of resistance to amoxicillin clavulanic acid (AMC) (29.8% and 42.3%) and ciprofloxacin (37.7% and 30.4%) which are popular treatment options.

Our study on fosfomycin susceptibility in MDR uropathogenic isolates revealed that of the 178 isolates, *E. coli* was the most prevalent isolate (97: 55%), followed by *Klebsiella* spp (55: 30.9%). *E. coli* had a susceptibility rate of 93.8% to fosfomycin, while *Klebsiella* spp had susceptibility rate of 78%. Categorical agreement was achieved between agar dilution and disk diffusion at

91%, although there was a high rate of false susceptibility at 42.9%. Categorical agreement between agar dilution and E tests at only 89% did not meet CLSI guideline.

Conclusion

While *E. coli* remains the most commonly isolated uropathogen, resistance rates for both *E. coli* and *Klebsiella* spp to frequently prescribed oral treatment options are alarmingly high, leaving clinicians very little in the way of viable treatment options. There is a need to keep abreast of current antimicrobial resistance trends as well as look at alternative treatment options.

To that end, the use of fosfomycin as an alternative oral treatment option in a UTI is a viable solution, most especially when the infection is caused by a MDR *E. coli*. Lastly, though laborious, agar dilution remains the most reliable method in establishing antimicrobial susceptibility in fosfomycin.

CHAPTER 1

Introduction and Literature review

Although the urinary tract is sterile, the prevalence of urinary tract infections (UTI) is quite high with approximately 95% of all UTIs occurring because of periurethral contamination by enteric uropathogens. [1]

These pathogens colonise the urethra where they subsequently ascend into the bladder, invading the uroepithelium and producing toxins and proteases that cause host cell damage. By multiplying and evading the host's immune defence system, the bacteria consequently ascend to the kidneys causing tissue damage. [2]

This has led UTIs to be one of the most common bacterial infections, accounting for up to 3% of all doctor's consultation with the prevalence of UTIs being greater in women than men. [3] The estimated frequency of UTIs is almost 1 infection/ person / year in sexually active women with approximately 50% of all women having at least one occurrence of a UTI in their lifetime. [4]

Generally, UTIs are classified as either a complicated or an uncomplicated UTI. Uncomplicated UTIs mostly affect people who are otherwise healthy and have no structural or neurological urinary tract abnormalities. Complicated UTIs are associated with factors that compromise the urinary tract such as urinary obstruction or retention. [2] Clinically, it is important to correctly classify a UTI, as complicated UTIs carry a higher risk of treatment failure and require more attention.

UTIs can be caused by many organisms, including both Gram positive and Gram-negative bacteria, as well as certain fungi, but by far the most common cause of both complicated and uncomplicated UTI is uropathogenic *Escherichia coli*, accounting for up to 95% of all cases. [1,2] Other enteric bacteria, especially *Proteus* spp. and *Klebsiella* spp., as well as Gram positive bacteria such as *Staphylococcus saprophyticus* and *Enterococcus* spp. account for a smaller percentage of uncomplicated UTIs. [5]

A South African study, done in the province of Gauteng by Lewis *et al* 2013, demonstrated that of 204 urinary pathogens isolated, 89.2% were Gram-negative bacilli and 10.8% were Gram-positive cocci. *Escherichia coli* was the most predominant organism at 79.6% and *Enterococcus faecalis* was the principal Gram-positive cocci isolated at 4.0%. [6] Similarly, another local study conducted in the obstetric departments of six public sector hospitals in Durban, KwaZulu-Natal, South Africa, where data was collected over a six-year period (2011-2016)

E. coli was the most common uropathogen at 54.2%, followed by *Klebsiella pneumoniae* at 12.9%. *Enterococcus faecalis* was the most prevalent Gram-positive cocci at 4.2%. [7]

The diagnosis of uncomplicated UTIs is made in patients who have the clinical signs and symptoms of a UTI together with laboratory findings of pyuria and /or bacteriuria. The decision to treat is often made solely on clinical findings as laboratory results are generally unavailable at the time of consultation. Conversely, urine cultures with susceptibility data are definitely recommended in patients with risk factors for a complicated UTI as there is an increase in the emergence of antimicrobial resistance, especially in the drugs of choice. [8] This was reported by Bosch *et al* in 2011, where antimicrobial resistance was higher in cases of complicated UTIs compared to uncomplicated cases. [9]

Globally, treatment options for UTIs have been well established, with ciprofloxacin long being considered the drug of choice when treating uncomplicated UTIs. Amoxicillin clavulanic acid (AMC), nitrofurantoin, fosfomycin as well as cephalosporins are also regarded as possible treatment options for UTIs. [8,10]

However, the rampant misuse of most of these drugs has led to an overwhelming increase in antimicrobial resistance. This has been seen in multiple studies around the world. A Canadian study done by Ou *et al,* 2016, noted that of the 106 Extended Spectrum Beta Lactamase (ESBL) producing organisms isolated, only 14% were susceptible to ciprofloxacin, while 96% was susceptible to fosfomycin. It was also noted in Vancomycin Resistant Enterococci (VRE) 81% was susceptible to fosfomycin while, as expected, a very low percentage was susceptible to ampicillin, ciprofloxacin and nitrofurantoin (6%,0% and 0% respectively). [11] In India, a study done by Banerjee *et a.,* in 2016, showed that *E. coli* had a high rate of resistance to cephalosporins, AMC and ciprofloxacin (64.97%, 67.59% and 58.8% respectively). [12]

Lewis *et al,* noted that the resistance rate of ciprofloxacin, in Gauteng Province, South Africa, had increased from 2.8% to 5.9% in a span of 10 years. It was also noted that cephalosporin's together with amoxicillin clavulanic acid have shown resistance rates of 5% and 17% respectively. [6] Another South African study, done by Bosch *et al*, 2011, showed that ciprofloxacin had a susceptibility rate of 89% in uncomplicated UTIs versus 59% in complicated UTIs. It was also noted in the same study, that ciprofloxacin was prescribed most often at 35% in complicated UTIs. [9]

It is also important to note that, *Enterobacterales*, the main causative agents in UTIs have shown high rates of antimicrobial resistances with the global spread of ESBL producing and carbapenem resistant *Enterobacterales* (CRE) increasing. [13] This can be seen in a study done in the United Kingdom, by Williams *et al,* 2016, where of the 69% of UTIs caused by *E. coli*, 59% of were

ESBL producing. In North America, the Study for Monitoring Antimicrobial Resistance Trends (SMART) concluded that there was significant increase in ESBL producing *E. coli* from urinary isolates in the United States for the period 2010 to 2014, where figures increased from 7.8% to 18.3%. In the same period, Canada, also showed an increase, but to a lesser degree, moving from 10.4% to 13.0%. [14] Studies done in the developing world presents similar patterns. A study done in India, 2016, yielded 356 significant bacterial isolates. Among these isolates, 64.78% were ESBL producers, 15.97% were CREs and 42.7% of isolates were Multi Drug Resistant *Enterobacterales.* [12]

Regrettably, studies done on UTIs in the developing world, specifically South Africa, are sadly lacking and this dearth of information leaves researchers relying far more heavily on first world studies when assessing the impact of increasing antimicrobial resistance in urine pathogens as well as possible solutions.

As multi drug resistance is far reaching and has a detrimental effect on our health care system, [15] and with our present global shortage of appropriate newly discovered antibiotics, we are forced to repurpose older antimicrobial agents to update empirical therapeutic guidelines. [13]One such agent is fosfomycin, also known as phosphonomycin, which is of the epoxide class of antibiotics. [16] It is a bactericidal antibiotic that was first isolated, in 1969, in the fermentation broths of various *Streptomyces* spp., at the Medina Foundation in Spain. [17]

Fosfomycin is considered to be a safe drug with the most common side effect being mild gastrointestinal distress which is primarily seen with oral administration of fosfomycin. [16,18] Other side effects include, dizziness, headaches, vaginitis as well as respiratory infection. [16]

Structurally fosfomycin is unrelated to any other antimicrobial agent and has a unique mode of action, where it inhibits the initial phase of microbial cell wall synthesis. [18,19] This is achieved by the inactivation of the cytosolic enzyme N-acetyl glucosamine enolpyruvltransferase (MurA), which then prevents the formation of N-acetylmuramic acid from N-acetylglucosamine and phosphoenolpyruvate (PEP), thereby disrupting the initial step in the peptidoglycan chain formation of the bacterial wall. [18]

For these bactericidal activities to occur the drug must first be transported across the bacterial permeability barrier which is achieved by transport systems. Glycerol-3-phosphate (GlpT) is the primary transport system, while the hexase phosphate transport system (UhpT), which is induced in the presence of glucose -6-phosphate, is the alternative. [18]

Both Gram positive and Gram-negative bacteria require the formation of N-acetylmuramic acid for peptidoglycan synthesis [17] , this allows fosfomycin to have a very broad spectrum of activity,

including those varieties of organisms that are MDR. [18] Fosfomycin is active against many common Gram-positive organisms such as *Staphylococcus aureus* (including MRSA), majority of coagulase negative *Staphylococcus* spp., *Enterococcus faecalis* and vancomycin resistant *Enterococcus faecium* (VRE) as well as, various *Streptococcus* spp. [18]

It has also shown excellent activity against most Gram-negative bacteria namely *E. coli*, *Klebsiella* spp., *Proteus mirablis* as well as other *Enterobacterales*. Most importantly fosfomycin has shown activity against MDR organisms as well as ESBL and carbapenemase producing *Enterobacterales*. [18]Whereas *Pseudomonas aeruginosa* has shown variable susceptibility and *Acinetobacter* spp. and *Morganella morganii* is seen as resistant, fosfomycin could be considered in the context of combination therapy. [18,19]

There are many key resistance mechanisms to fosfomycin. These include modification or reduced expression of functional transporters, modification or over expression of MurA or the production of fosfomycin modifying enzymes. The first two mechanisms are chromosomal whereas the last one can be either chromosomal or placid mediated. [18]

The more common mechanism of resistance is the mutation or inactivation of one or both of the chromosomally – encoded transport genes (glpT and/or uhpT) or the regulatory genes *uhpA*, *uhpB* and *uhpC* of the UhpT system which lead to loss of function of these transporters, thereby reducing permeability and causing resistance to fosfomycin. [18] (Sastry *et al*, 2016).

Though not common, modification, as well as over expression of the drug's target, MurA, can also create fosfomycin resistance. For instance, in *E. coli*, the drug covalently binds with cysteine in position 115 of MurA, if aspartate is substituted in this active site, resistance to fosfomycin is seen.[18]

In the last mechanism of resistance, there are four groups of fosfomycin modifying enzymes that create resistance to the drug. This is done in two different ways. Enzyme groups FosA, FosB and FosX function by nucleophilic attack on carbon atom one of the drug, opening the epoxide ring and rendering the drug inactive. The 4th group, a plasmid mediated fosfomycin modifying enzyme FosC, uses ATP to add a phosphate group to the drug altering its properties and making it inactive. [18] Generally, FosA and FosX enzymes are produced by Gram-negative bacteria and the FosB enzyme is produced by Gram-positive bacteria. [18]

When looking at the rate of resistance for fosfomycin, it is important to keep in mind that antimicrobial susceptibility patterns vary according to time and geographical location. Currently there are only two published South African studies on the susceptibility of fosfomycin among

urinary pathogens, both of which were carried out in Gauteng province. Lewis *et al*., used E tests to determine fosfomycin susceptibility, but they did not indicate if their isolates consisted of MDR pathogens. Their results showed fosfomycin susceptibility of 95.5%. [6] The second study was a chart-based study conducted at the Charlotte Maxeke Johannesburg Academic Hospital, which noted that fosfomycin had an overall susceptibility rate of 95.7% to *Enterobacterales,* with a >90% susceptibility to MDR *Enterobacterales*. The fosfomycin susceptibility method used in this study was the disk diffusion method. [20]

However, the use of fosfomycin is more common in European countries, where more statistical data is available. ECO SENS, a study conducted in the year 2000, by various European countries, looked at prevalence and susceptibility of pathogens causing community acquired UTIs. It was noted that fosfomycin resistance rates were very low in all investigated countries, the highest being in Greece (1.5%) There were no differences in countries that did not use fosfomycin like Norway (1.2%) and countries that had a long history of use like France (1.0%). [21] During the study period of 2007 - 2008, ECO SENS was revisited, the data collected showed little increase in the fosfomycin resistance rate, with most countries being <2%, with the exception of Greece at 2.9%. [22]

In a study done in the UK, in 2018, it was reported that fosfomycin had an overall susceptibility rate of 95% among ESBL producing *Enterobacterales*. Of the 889 *E. coli* strains isolated, 98% were susceptible to fosfomycin, this was followed by 62% of *Klebsiella* spp. and 85% of other *Enterobacterales.* [15] A study done in India, in 2017, noted that 95.93% of the MDR isolates were found to be susceptible to fosfomycin, *E. coli*, *Klebsiella pneumnoniae* and *Enterococcus* spp. were 98.14%, 95.52% and 97.72% susceptible to fosfomycin respectively. [12] Lu et al, 2011, conducted a study in Taiwan, in 2008, on the antimicrobial susceptibilities of common bacteria to fosfomycin. In this study the author used antimicrobial susceptibility testing guidelines from both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as well as their American counterparts, as well as the Clinical and Laboratory Standards Institute (CLSI), as a basis to perform their study. It was reported that 100% of *E. coli* were susceptible using both criteria. For *K. pneumoniae* and *Enterobacter cloacae,* 92% and 85% respectively, were considered susceptible according to CLSI whereas 85% and 72% susceptible were recorded according to EUCAST. *Enterococcus faecalis* showed a 98.8% susceptibility rate using CLSI and 91.3% using EUCAST. Interestingly, there was a vast difference in the susceptibility rate of *Enterococcus faecium* when using the two criteria's, 88.8% were susceptible using CLSI and only 25.0% using EUCAST. [23]

Rationale

Due to the discovery of cephalosporins, fosfomycin was quickly overshadowed, resulting in infrequent international use and therefore low global resistance rates. [13] This, together with its ability to be used as an oral stat dose, and its ability to maintain high urinary concentrations, makes fosfomycin an attractive choice for the treatment of UTIs. [24]

Despite the fact that fosfomycin has very few side effects, readily available and currently on the South African Essential Drug List, it is only limitedly tested in both the private and public sector. This is possibly due to the complexity of the current recommended drug susceptibility test (agar dilution method) for fosfomycin, which is very laborious and not compatible for use in a routine clinical microbiology laboratory. Finding a more practical alternative to the current recommended antimicrobial susceptibility testing method for fosfomycin could possibly encourage fosfomycin to be more widely utilised. Another fact that could influence the use of fosfomycin is the cost, as it is more expensive than the more commonly prescribed oral treatment options.

Whereas the more common antibiotics currently being administered for UTIs, namely amoxicillin clavulanic acid, nitrofurantoin, trimethoprim sulfamethaxole and ciprofloxacin are part of the routine antimicrobial susceptibility testing, the lack of testing of fosfomycin has led to very limited clinical information being available. Being conscience of the fact that UTIs place a considerable burden on our current health care system, determining the susceptibility profiles of uropathogens, as well as looking at fosfomycin susceptibility patterns will assist us in choosing the most appropriate treatment for patients in our region. However, there have been no recent studies conducted in our region of KwaZulu-Natal in order to determine this.

Aim

We therefore aimed to conduct a study in order to evaluate the use of fosfomycin as an alternate empiric treatment for UTIs.

Objectives

1. A retrospective analysis of laboratory data was conducted on all urine samples with positive bacterial growth isolated from specimens that had been sent to NHLS RK Khan Pathology Laboratory between January 2018 to December 2020 to determine the rate of antimicrobial susceptibility to commonly prescribed empiric therapy.

2. Determine the rate of susceptibility of multi drug resistant urinary pathogens to fosfomycin using the gold standard agar dilution method.

3. Assess the suitability of disk diffusion and E tests as an alternative antimicrobial susceptibility testing method.

CHAPTER 2

The following research manuscript will be submitted to the African Journal of Laboratory Medicine for publication.

Antimicrobial susceptibility patterns of common uropathogens during 2018 – 2020 in a regional hospital in KwaZulu-Natal Province, South Africa.

Due to a surge in antimicrobial resistance, there is a greater need for antimicrobial surveillance studies to be conducted regularly, identifying common uropathogens and their antimicrobial susceptibility patterns.

Therefore, this study was undertaken to demonstrate the above in our region of KwaZulu-Natal, South Africa.

Scientific Research Paper 1

Title

Antimicrobial susceptibility patterns of common uropathogens during 2018 – 2020 in a regional hospital in KwaZulu-Natal Province, South Africa.

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Abstract

Background

Urinary tract infection (UTI) is a common bacterial infection, affecting millions worldwide. Treatment options for UTIs are well established, with ciprofloxacin considered one of the main antimicrobial agents of choice. However, the rampant misuse of antibiotics has led to an overwhelming increase in antimicrobial resistance. Although this is seen in multiple studies around the world, there is a dearth of information from the developing world. The objective of this study was to describe the antimicrobial susceptibility patterns of common uropathogens isolated from urine specimens.

Method

A retrospective analysis of antimicrobial susceptibility data of positive urine specimens from RK Khan Hospital, a regional hospital in KwaZulu-Natal, South Africa during 2018 – 2020, collected from the Laboratory Information System. The VITEK® 2 (Biomerieux SA, France) automated system was used to perform identification and susceptibility; results were interpreted using Clinical and Laboratory Standards Institute breakpoints.

Results

Between 2018 and 2020, 3044 common urinary pathogens were isolated. *Escherichia coli* was the most frequent cause of UTI (1603; 53%), followed, by *Klebsiella* spp. (437;14%). Both *E. coli* and *Klebsiella* spp. showed high rates of resistance to amoxicillin clavulanic acid (AMC) (29.8% and 42.3%) and ciprofloxacin (37.7% and 30.4%). Nitrofurantoin resistance was low for *E. coli* at 6.2% but high for *Klebsiella* spp. at 61.3%.

Conclusion

E. coli remains the most commonly isolated uropathogen. Resistance rates for frequently prescribed oral treatment options namely AMC and ciprofloxacin are alarmingly high for both *E. coli* and *Klebsiella* spp. This highlights the importance of regular local antimicrobial surveillance so as to inform appropriate empiric therapy.

Introduction

Although the urinary tract is sterile and has multiple mechanisms in place to keep it so, the prevalence of urinary tract infections (UTIs) is remarkably high with approximately 95% of all UTIs occurring because of periurethral contamination by enteric uropathogens.[1] As a result UTIs are one of the most common bacterial infections, affecting approximately 150 million people worldwide and amounting to more than 6 billion dollars in health care costs.[2] Due to the high incidence of UTIs, and the widespread practice of treating empirically, the rate of antibiotic prescriptions is also considerably high.[3] Unfortunately, this has led to an alarming increase in antimicrobial resistance in frequently isolated urinary pathogens, which further limits treatment options specifically those administered orally.[2,4]

The most typically isolated organisms in the urine are *Escherichia coli* (*E. coli*), *Klebsiella* spp., and *Enterococcus* spp., which are all based on the normal flora of the bowel, with *E. coli* being the most frequently isolated. [5,6]

Treatment options for UTIs have been well established globally with ciprofloxacin, together with amoxicillin clavulanic acid (AMC), nitrofurantoin, fosfomycin as well as cephalosporins, forming part of the empiric treatment. [7,8]

However, studies conducted locally and internationally demonstrate that an alarming increase in the use of most of these drugs, has caused in a surge in antimicrobial resistance and as a result an increase in cases of treatment failures. This exerts a greater pressure on an already overburdened health care system. [2,9] Consequently, there is a greater need for antimicrobial surveillance studies to be conducted regularly. Identifying common uropathogens and their antimicrobial susceptibility patterns can assist to ensure the correct treatment plan is initiated. This study aimed to determine the prevalence of common uropathogens and their antibiotic susceptibility patterns.

Ethics Approval

Ethics approval was granted by Biomedical Research Ethics Committee, University of KwaZulu Natal. (Reference number: BREC/00001578/2020)

Method and Materials

A retrospective analysis of laboratory data was conducted on all urine samples with positive bacterial growth isolated from specimens, of both inpatients and outpatients, that had been sent to NHLS RK Khan Pathology Laboratory between January 2018 to December 2020. This SANAS accredited laboratory is within RK Khan Hospital located in Chatsworth, Durban, and a suburb of KwaZulu Natal, South Africa. RK Khan Hospital has 543 beds, is both a regional and district hospital and serves many internal and external clinics.

Inclusion Criteria:

All pathogenic bacteria isolated from urine specimens cultured from January 2018 to December 2020 was included in the study. The specimens were received from RK Khan Hospital inpatients, both internal and external clinics as well as other district hospitals that are serviced by the RK Khan Pathology Laboratory.

For the purpose of this study, an inpatient was defined as a patient that was admitted to the hospital for medical treatment, whereas an outpatient was defined as a patient who did not require hospitalisation for treatment.

All urine cultures with ≥10 colonies of a single organism, that is $\geq 10^4$ colony forming units/ml (CFU/ml) as well as cultures with 2 different organisms of similar growth was included.

Exclusion Criteria:

All urine cultures with no growth or mixed growth were excluded. Mixed growth was defined as a urine culture with ≥3 organisms cultured.

Urine Cultures with bacterial growth that is $<$ 10 colonies were also excluded has no significant bacterial growth.

Urine Culture:

Urine specimens were processed according to routine standard operating procedure, where a 1µl loop is used to inoculate a Cystine Lactose Electrolyte Deficient (CLED) plate (Diagnostic Media Products, SA). Inoculated plates are incubated at 33º - 37ºC for 18-24 hours in aerobic conditions and then checked for significant bacterial growth. The identification and susceptibility of significant organisms were carried out by the VITEK® 2 (Biomerieux SA, France) automated system. Colonial morphology as well as Gram

stain results guided the selection of the type of VITEK® 2 (Biomerieux SA, France) card utilised.

VITEK® 2 (Biomerieux SA, France) Principle and Method:

The VITEK® 2 (Biomerieux SA, France) is a rapid automated analyser that performs identification and antimicrobial susceptibility testing of microorganisms. The instrument uses advanced colorimetric readings to determine the identification of each organism. The identification is done up to species level, and includes most Gram-negative bacilli, Gram-positive cocci, yeast and organisms within the *Neisseria/Haemophilus* group.

The antimicrobial susceptibility testing (AST) system is based on the minimum inhibition concentration (MIC) method, using a doubling dilution technique. The VITEK® 2 (Biomerieux SA, France) allows for the susceptibility testing of clinically significant Gram-negative bacilli, *Staphylococcus spp.*, *Enterococcus spp.*, and various *Streptococcus spp.*, namely *Streptococcus agalactiae* and *Streptococcus pneumoniae.*

The entire process of bacterial identification and antimicrobial testing was performed from a standardised inoculum prepared from a pure culture which had been incubated at between 33º - 37ºC for 18-24 hours. A purity plate was cultured for each organism to be tested, to ensure that only pure cultures are tested. The results were interpreted using Clinical and Laboratory Standards Institute (CLSI) guidelines.[10]

Data Analysis

The required data was extracted from the laboratory information system (LIS) of the National Health Laboratory Services and captured onto an Excel spread sheet from which the antimicrobial activity of common uropathogens to routinely prescribed urinary antibiotics were calculated. The antibiotic profiles investigated were based on the recommended empiric therapy for a UTI The statistical analysis was carried out using the Chi squared test. A p-value of <0.05 was considered statistically significant.

Results

A total of 4272 urinary pathogens were isolated in the three-year period from 2018 to 2020. Of these, 3044 isolates were considered common urinary bacteria.

As shown in figure 1, *E. coli* was the most common cause of UTI, which was then followed, by *Klebsiella* spp. *Enterobacter* spp., *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and other gram-negative bacilli made up a further 14%. *Enterococcus faecalis* was the most frequent gram-positive cocci isolated.

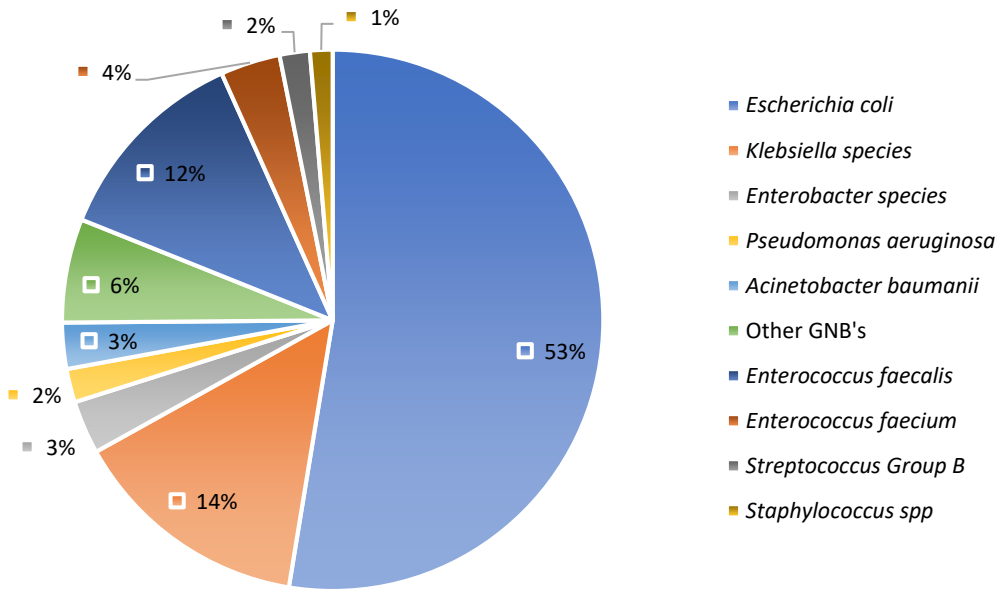

Figure 1: Frequency of UTI pathogens isolated from 2018 to 2020

Figure 2 shows the antimicrobial activity of both *E. coli* and *Klebsiella* spp which were the most prevalent organisms isolated. A statistically significant difference was noted in all the antibiotics tested.

E. coli displayed a susceptibility of <75% to AMC whereas nitrofurantoin displayed a high susceptibility rate of >90%. The more commonly prescribed ciprofloxacin showed a susceptibility rate of 62.3%.

AMC and nitrofurantoin susceptibility were lower in *Klebsiella* spp. While *Klebsiella* spp. susceptibility to ciprofloxacin was somewhat higher than *E. coli.*

Of interest is the percentage of extended spectrum beta lactamase producers, of the 1603 *E. coli* isolates, 26.2 % were ESBL positive and 0.3% are carbapenemase

producing whereas of the 437 *Klebsiella* spp. isolated, 41.8% were ESBL positive and 5% were carbapenemase producing.

An ESBL producer was defined as an organism that is resistant to most beta-lactam antibiotics, including penicillins plus first, second, and third generation cephalosporins.

A carbapenemase producing *Enterobacterales* was defined as any organism that is resistant to carbapenems including ertapenem, imipenem and meropenem.

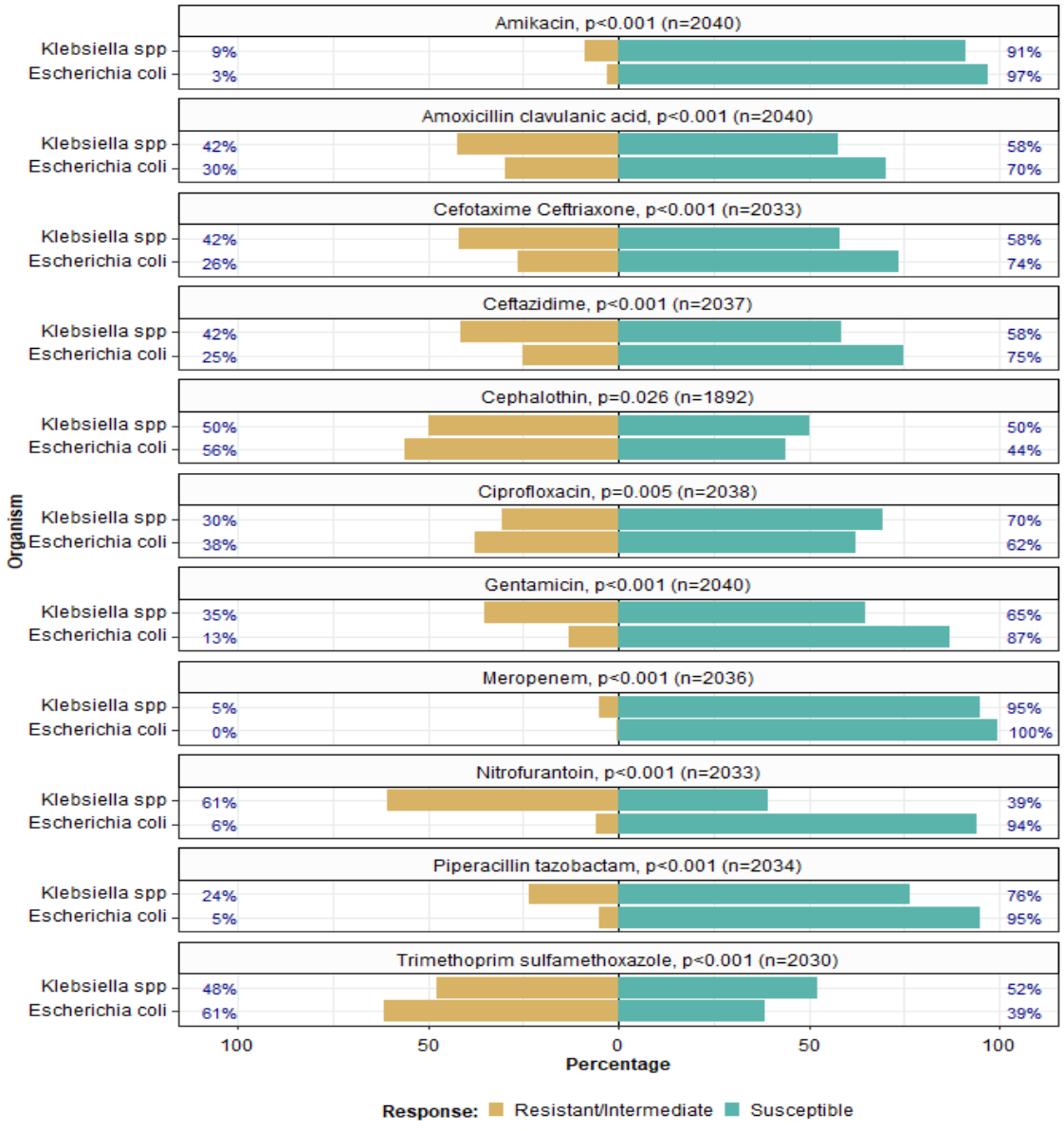

Figure 2. Antimicrobial activity of *E. coli* and *Klebsiella* spp. to commonly used antibiotics.

When comparing the antibiotic activity of *E. coli* in inpatient and outpatient wards, we discovered that while the rate of resistance for AMC, ciprofloxacin and gentamycin are higher than the 10%, there is no statistically significant difference present. Statistically only trimethoprim sulfamethoxazole and the third generation cephalosporins show any significant difference. The distribution of ESBL and CREs, on the other hand, was found to be greater in inpatients at 31.0% and 0.8% as compared to outpatients at 24% and 0.1% respectively.

Table 1. Antimicrobial activity of *E. coli* based on inpatient wards vs outpatient wards

Antibiotic	Inpatient Ward (N=511)	Outpatient Ward (N=1092)	p-value	Overall (N=1603)
Amikacin			Chisq., p = 0.747	
Resistant/Intermediate	16 (3.1%)	31 (2.8%)		47 (2.9%)
Susceptible	495 (96.9%)	1061 (97.2%)		1556 (97.1%)
Amoxicillin clavulanic acid			Chisq., p = 0.067	
Resistant/Intermediate	168 (32.9%)	310 (28.4%)		478 (29.8%)
Susceptible	343 (67.1%)	782 (71.6%)		1125 (70.2%)
Cefotaxime Ceftriaxone			Chisq., p = 0.003	
Resistant/Intermediate	158 (31.0%)	261 (24.0%)	0.007	419 (26.2%)
Susceptible	352 (69.0%)	827 (76.0%)	0.007	1179 (73.8%)
Ceftazidime			Chisq., p = 0.012	
Resistant/Intermediate	149 (29.2%)	254 (23.3%)	0.027	403 (25.2%)
Susceptible	362 (70.8%)	836 (76.7%)	0.027	1198 (74.8%)
Cephalothin			Chisq., p = 0.567	
Resistant/Intermediate	265 (57.1%)	573 (55.5%)		838 (56.0%)
Susceptible	199 (42.9%)	459 (44.5%)		658 (44.0%)
Ciprofloxacin			Chisq., p = 0.095	
Resistant/Intermediate	177 (34.7%)	426 (39.0%)		603 (37.7%)
Susceptible	333 (65.3%)	665 (61.0%)		998 (62.3%)
Gentamicin			Chisq., p = 0.070	
Resistant/Intermediate	78 (15.3%)	131 (12.0%)		209 (13.0%)
Susceptible	433 (84.7%)	961 (88.0%)		1394 (87.0%)
Meropenem			Chisq., p = 0.021	
Resistant/Intermediate	4 (0.8%)	1 (0.1%)		5 (0.3%)
Susceptible	507 (99.2%)	1087 (99.9%)		1594 (99.7%)
Nitrofurantoin			Chisq., p = 0.540	
Resistant/Intermediate	32 (6.3%)	60 (5.5%)		92 (5.8%)
Susceptible	477 (93.7%)	1027 (94.5%)		1504 (94.2%)
Piperacillin tazobactam			Chisq., p = 0.501	
Resistant/Intermediate	28 (5.5%)	51 (4.7%)		79 (5.0%)

Antibiotic	Inpatient Ward (N=511)	Outpatient Ward (N=1092)	p-value	Overall (N=1603)
Susceptible	483 (94.5%)	1035 (95.3%)		1518 (95.1%)
Trimethoprim sulfamethoxazole			Chisq., p<0.001	
Resistant/Intermediate	355 (69.7%)	625 (57.6%)	<0.001	980 (61.4%)
Susceptible	154 (30.3%)	461 (42.4%)	<0.001	615 (38.6%)

N = number; | % and p-values based on non-missing cases | * parametric p-value

Table 2, which details the susceptibility patterns of Gram-positive cocci isolated in urines, we noted high rates of antibiotic susceptibility of *Enterococcus faecalis* compared to that of *Enterococcus faecium*. Vancomycin had high rates of susceptibility for both *E. faecalis* and *E. faecium* as well as *Staphylococcus* spp. (*Staphylococcus aureus* and *Staphylococcus saprophyticus*). *Group B Streptococci* had a high susceptibility rate to both ampicillin and penicillin.

Table 2: Antimicrobial susceptibility of predominately isolated Gram-Positive Cocci to commonly used antibiotics.

Antibiotic	***Enterococcus faecalis* (N=372)**	***Enterococcus faecium* (N=109)**	***Group B Streptococci* (N=55)**	***Staphylococcus* spp. (N=41)**
	N (%)	**N (%)**	**N (%)**	**N (%)**
Ampicillin	369 (99.2)	18 (16.5)	55 (100)	3 (7.3)
Penicillin	363 (97.6)	18 (16.5)	55 (100)	3 (7.3)
Vancomycin	367 (98.7)	106 (97.2)	ND	41 (100)
Cloxacillin	N/A	N/A	N/A	35 (85.3)

(N = number, % = percentage of susceptibility, ND = No Data, N/A = Not Applicable)

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When looking at the susceptibility rate of *E. coli* over the three years, it is evident that amikacin, meropenem, nitrofurantoin and piperacillin/tazobactam show very little change. However, there was a slight increase in susceptibility rates in other antibiotics, which was deemed statistically significant. More investigation is required on the increase of susceptibility as three years of data is not enough to make a clear conclusion on the cause of the increase.

Table 3: Antimicrobial susceptibility of *Escherichia coli* over 3 years

Antibiotic	***E. coli* 2018 (N = 625)**		***E. coli* 2019 (N = 539)**		***E. coli* 2020 (N = 439)**	
	N	**%**	**N**	**%**	**N**	**%**
Amikacin	610	97.6	523	97.0	423	96.4
Amoxicillin Clavulanic acid	398	63.4[a]	385	71.4 [a]	342	77.9 [a]
Ceftriaxone	448	71.7 [a]	389	62.2 [a]	342	77.9 [a]
Ceftazidime	448	71.7 [a]	390	72.4 [a]	360	82.0 [a]
Cephalothin	211	36.1 [a]	243	48.1 [a]	204	50.2 [a]
Ciprofloxacin	357	57.1 [a]	334	62.0	307	69.9[a]
Gentamicin	532	85.1	464	86.1 [a]	398	90.7 [a]
Meropenem	622	99.5	537	99.6	435	99.1
Nitrofurantoin	578	92.5	507	94.1	419	95.4
Piperacillin tazobactam	588	94.1	516	95.7	414	94.3
Trimethoprim sulfamethoxazole	244	39.0	192	35.6	179	40.8

(**a** = p<0.05, N = number, % = percentage of susceptibility)

Discussion

The distribution of common uropathogens isolated from specimens collected at a regional hospital in the South African province of KwaZulu-Natal was investigated in this study. Secondly, we looked at their antimicrobial susceptibility to the most frequently recommended medicines for UTI treatment. We found that *E. coli* was the most frequently isolated uropathogen, and that the resistance rates of commonly identified urinary pathogens to widely administered antibiotics were quite high.

Data analysed from 2018 to 2020 showed that *E. coli* was the most prevalent uropathogen isolated, found in 53% of cases. It was followed by *Klebsiella* spp. at 14% while *E. faecalis* was the most frequently isolated Gram-positive bacteria and the third most commonly isolated bacteria overall. This is consistent with research done both locally and internationally. A study in India showed that *E. coli*, *E. faecalis* and *Klebsiella pneumoniae* were the most prevalent uropathogens isolated at 55.1%, 15.8% and 13.7% respectively. [10] This was also proven in local research

conducted in the obstetric departments of six public sector hospitals in Durban, KwaZulu-Natal, South Africa, where data was collected over a six-year period (2011-2016) reflected that *E. coli* was the most common uropathogen at 54.2%, followed by *Klebsiella pneumoniae* at 12.9%. [11]

In 2010, Infectious Diseases Society of America (IDSA) and the European Society of Microbiology and Infectious Disease (ESCMID), published a clinical practice guideline outlining their recommendations for the treatment of uncomplicated UTIs, with nitrofurantoin, fosfomycin, trimethoprim/sulfamethoxazole and ciprofloxacin considered drugs of choice. [4] Similarly, in the South African Standard Treatment Guideline, 2020, fosfomycin, ciprofloxacin, AMC and nitrofurantoin are also considered as empiric oral treatment options for UTIs. [7]

It was consequently disconcerting to learn in our study that the antimicrobial activity of *E. coli* and *Klebsiella spp*. to all the antibiotics tested were statistically significant as well having a high resistance rate, in some cases. Such as to ciprofloxacin and AMC which was more than 30%. And although *E. coli* was quite susceptible to nitrofurantoin at 93.8%, *Klebsiella* spp. had a susceptible rate of only 38.7%. A nine-year (2011-2019) study conducted in Europe revealed similar outcomes. [12] According to the Hrbacek study, ciprofloxacin had a significant rate of resistance against Gram-negative bacilli (greater than 40%). It was also noted that AMC and nitrofurantoin exhibited better antimicrobial activity against *E. coli* with a resistance rate of approximately 10%, while *Klebsiella* spp. had a resistance rate of more than 50%. These findings are comparable with our study. The high rates of resistance to recommended oral empiric therapy is of major concern as this leads to an increase in treatment failure.

As this was a laboratory-based study, we were unable to distinguish between community acquired and hospital acquired urinary tract infections. We, therefore, examined a snapshot of the antimicrobial patterns of *E. coli* in the inpatient wards versus the outpatient wards, with very little statistical difference being noted. It was noted that the organisms found in the outpatient wards had a higher rate of susceptibility compared to those isolated in the inpatient wards, but this was only statistically significant in the third generation cephalosporins and trimethoprim sulfamethoxazole. It was also noted that there was a higher prevalence of ESBL and carbapenemase producers in the inpatient wards than the outpatient wards. A Japanese study done in 2020, showed that the difference between the two settings was between 5-10%. [13] This is consistent to our

findings when comparing inpatients and outpatients where the difference was between 1-12%.

In a local study by Bhola *et. al*, lower levels of resistance were seen as antimicrobial susceptibility results of pregnant but otherwise healthy individuals were examined. According to the South African public health care system, new patients do not directly present to hospital. Instead, they are initially seen at a local clinic, then if required, referred to hospital. As a result, patients who are seen in hospital are either known hospital patients or patients who have a reason for referral to hospital. Therefore, our study sample had a bias towards patients who were more likely to have resistant organisms. Despite the fact that the antibiotic resistance levels in Bhola's research were lower at 16.9%, they were still high enough that the suggested empiric therapy would fail in a large proportion of afflicted individuals.

Both IDSA and ESCMID recommend that ciprofloxacin should be used as an empiric antibiotic therapy if resistance rate remains less than 10%. [4] The proportion of ciprofloxacin resistance in our study was more than three times this limit. This suggests the possibility that resistance levels are high in both the hospital and the community setting.

While nitrofurantoin is still an option for treating a UTI caused by *E*. *coli*, it is ineffective against the majority of *Klebsiella* spp. infections, leaving the physician with limited empiric oral therapy alternatives. More research is needed to evaluate alternate oral drugs such as fosfomycin, which is not in use in the public sector despite being recommended in the South African guideline.

Limitations

There was a bias towards a more resistant picture as clinicians are unlikely to request susceptibility testing for patients that respond to empiric therapy. Also, due to a lack of clinical information, we were unable to determine the difference between hospital acquired and community acquired infection.

Conclusion

Our study found that *E. coli* remains the most predominate urinary pathogen isolated and antimicrobial resistance levels in commonly isolated uropathogens remain dangerously elevated, especially in frequently prescribed oral antibiotics. Regular, local surveillances are essential to guarantee that suggested empiric therapy is up to date, preventing a rise in treatment failure. This will go a long way in guiding clinicians in making well-informed, and appropriate antibiotic choices for the treatment of UTIs.

We also recommend, further research is done on additional oral treatment options. One such option is fosfomycin, an antibiotic that forms part of the South African Treatment Guidelines but is not routinely prescribed or tested in the South African public health care section.[7]

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Competing interests

The authors declare that they have no financial or personal relationship that may have inappropriately influenced them in writing this article.

Author Contributions

AN: conceptualisation and implementation of the research project, data collection, data analysis, literature review, writing of the first draft, writing, and editing of all subsequent drafts and writing of final draft; AF: Editing first and second draft; NRM:

Conceptualisation of research project, input on all drafts, editing and analysis; KSS-H: input on implementation of the project and editing the first and final draft

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Data Availability

Data sharing is not applicable to this study as no new data was created or all other data is available within the article.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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CHAPTER 3

The following research manuscript will be submitted to the Journal of Clinical Microbiology for publication.

In vitro activity of Fosfomycin in Commonly Isolated Multidrug Resistant Urinary pathogens using three different methods

This study was undertaken to demonstrate the suitability of fosfomycin as a feasible alternative to the current recommended empiric therapy for the treatment of urinary tract infections, as the rate of resistance to frequently prescribed urinary antimicrobial agents is alarmingly high, especially in commonly isolated uropathogens.

Scientific Research Paper 2

In vitro activity of Fosfomycin in Commonly Isolated Multidrug Resistant Urinary pathogens using three different methods

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Abstract

Background

Multi drug resistant (MDR) urinary tract infections (UTIs) are increasingly common, with current empiric therapy considered unreliable as the rate of resistance is too high, thus leaving a gap in treatment options. Fosfomycin, while ideally suited to treat UTIs, remains underutilised with little information on its susceptibility among MDR uropathogens.

Objective

This study was undertaken to determine the susceptibility of MDR uropathogens to fosfomycin, while also demonstrating the suitability of disk diffusion and E tests as an alternative testing method.

Methods

A retrospective analysis of fosfomycin susceptibility to 178 stored MDR uropathogenic isolates obtained from the RK Khan Laboratory was undertaken. Antimicrobial susceptibility was determined using agar dilution, which was compared to disk diffusion and E test.

Results

Of the 178 isolates, MDR *E. coli* was the most prevalent isolate (97: 55%), followed by *Klebsiella* spp. (55: 30.9%), *Enterobacter* spp. (12: 6.7%) and *Enterococcus* spp. (12: 6.7%). MDR *E. coli* had a susceptibility rate of 93.8% to fosfomycin, while MDR *Klebsiella* spp. had susceptibility rate of 78%. Categorical agreement was achieved between agar dilution and disk diffusion at 91%, although the VME rate was quite high at 42.9%. Conversely, categorical agreement between agar dilution and E tests (89%) was not met according to CLSI guidelines.

Conclusion

The inhibitory effect of fosfomycin, though modest on other causes of UTIs, was excellent on *E. coli*, the most frequent cause of UTIs. This, together with a high overall susceptibility to Gram negative bacilli suggests that fosfomycin would be beneficial in the treatment of UTIs specifically MDR infections. Agar dilution, while laborious, remains the most reliable method in establishing antimicrobial susceptibility in fosfomycin.

Introduction

Multi drug resistance (MDR) in urinary tract infections (UTIs) is fast becoming a global problem with far reaching consequences that are often associated with prolonged clinical response, higher treatment costs and increased mortality. [1,2] Recent studies have demonstrated that recommended empiric therapy such as ciprofloxacin and amoxicillin clavulanic acid have resistancerates that should preclude them as treatment options. [3,4,5] This leaves a gap in possible oral treatment options available to the clinician. At present, there is a global shortage

of appropriate newly discovered antibiotics, hence there is a need to repurpose older antimicrobial agents to update empirical therapeutic guidelines. [6]

Fosfomycin, a drug discovered in 1969, that lost its popularity due to the introduction of cephalosporins is such an agent. [6] It is a bactericidal drug, that has a unique mode of action, where it inhibits the initial phase of microbial cell wall synthesis, it also has a wide spectrum of activity as it is active against both Gram positive and Gram-negative organisms. [7] It is an appealing treatment option for UTIs, as it can be used as an oral stat dose, achieves high urinary concentrations and most importantly has shown excellent activity against MDR organisms. [7,8,9]

Even though fosfomycin is ideally suited to treat UTIs and is on the South African Standard Treatment Guidelines, susceptibility testing is not routinely performed in both the private and public sectors due to the need for a more laborious agar dilution method. This has led to a scarcity of studies conducted in our region of KwaZulu-Natal. [10] Currently there are only two published South African studies on the susceptibility of fosfomycin among urinary pathogens, both of which were carried out in Gauteng province. Lewis *et al*., used E tests to determine fosfomycin susceptibility, but they did not indicate if their isolates consisted of MDR pathogens. Their results showed fosfomycin susceptibility of 95.5%. [11] The second study was a chart-based study conducted at the Charlotte Maxeke Johannesburg Academic Hospital, which noted that fosfomycin had an overall susceptibility rate of 95.7% to *Enterobacterales,* with a >90% susceptibility to MDR *Enterobacterales*. The fosfomycin susceptibility method used in this study was the disk diffusion method. [12] Currently, there are no local studies on the antimicrobial activity of fosfomycin using the reference method, agar dilution.

Due to the increase in resistance against the currently used oral drugs and the paucity of research conducted on fosfomycin susceptibility among urine pathogens in South Africa, especially in our province of KwaZulu Natal, we aimed to conduct a study in order to evaluate the susceptibility of MDR urinary pathogens to fosfomycin using the reference method of agar dilution. [13] Furthermore, considering that agar dilution is very laborious, and not routinely done in a diagnostic microbiology laboratory, our study sought to find a feasible alternative method that could be used for routine drug susceptibility testing. Hence, we looked at the recommended agar dilution method and compared it to the disk diffusion as well as the E test methods, both of which are far less labour intensive and more cost effective.

Method

Ethical Considerations:

Ethics approval was granted by the Biomedical Research Ethics Committee, University of KwaZulu Natal. (Reference number: BREC/00001578/2020)

Study design and Setting

This study was a retrospective analysis of fosfomycin susceptibility of the stored multi drug resistant uropathogenic isolates obtained from the RK Khan Laboratory, a SANAS accredited laboratory located in the RK Khan Hospital. RK Khan Hospital is both a regional and district hospital located in Chatsworth, Durban, a suburb of KwaZulu Natal, South Africa. The hospital has 543 beds and serves many internal and external clinics.

Study population/Sampling strategy

Isolates of multi drug resistant urinary pathogens were utilised from clinical microbiology specimens that were routinely identified, collected, and stored. These isolates were consecutively collected and stored from September 2019 to July 2020 until a total number of 200 isolates was reached. Isolates were obtained from both inpatient wards as well as outpatient wards.

Inclusion Criteria:

All urinary cultures that were considered clinically significant with $\geq 10^4$ colony forming units/ml (CFU/ml) of a multi drug resistant organism was included in this study

All *Enterobacterales* that were multi drug resistant including, extended spectrum beta lactamase (ESBL) as well as carbapenemase producing *Enterobacterales.*

A multi drug resistant organism was defined as resistance to at least one agent in three or more antimicrobial classes. [14]

An ESBL producer was defined as an organism that is resistant to most beta-lactam antibiotics, including penicillins plus first, second, and third generation cephalosporins.

A carbapenemase producing *Enterobacterales* was defined as any organism that is resistant to carbapenems including ertapenem, imipenem and meropenem.

All Enterococcus spp. that were resistant to ampicillin as well as *Staphylococcus aureus* that were resistant to oxacillin were included.

Exclusion Criteria:

All urine cultures with no growth or mixed growth were excluded. Mixed growth was defined as a urine culture with ≥3 organisms cultured. Urine Cultures with bacterial growth that is $< 10^4$ CFU/ml were also excluded has no significant bacterial growth. Isolates that were susceptible i.e., those that did not meet the resistance criteria, was also excluded.

It is also important to note that urine pathogens that are intrinsically resistant to fosfomycin, as well as those that require combination antibiotic therapy were excluded from this study, these include: *Pseudomonas* spp*., Acinetobacter* spp*., Stenothrophomonas maltophilia, Burkholderia cepacia, Staphylococcus capitis, Staphylococcus saprophyticus* and *Morganella morganii.*[15]

Laboratory Analysis

Urine samples were handled and processed in accordance with the routine standard operating procedure (SOP). Urine samples were inoculated onto CLED plates (Diagnostic Media Products, SA) and incubated at 33^o - 37^{o}C for 18-24 hours before being examined for clinically significant bacterial growth. The bacterial identification and susceptibility of significant organisms were performed by the VITEK® 2 (Biomerieux SA, France) automated system. All antimicrobial susceptibility results were interpreted using Clinical Laboratory Standards Institute (CLSI) guidelines. [13]

The 200 non duplicated urinary pathogens that fit the inclusion criteria were then sub cultured onto 5% Blood Agar (Diagnostic Media Products, SA) to ensure purity. Isolates were given unique identifiers and then further inoculated into the cryovials the of a microbank™ (Prolab Diagnostics, US) and stored at - 70°C. Of the 200 isolates collected, only 178 isolates remained viable for testing.

For the purpose of this study, CLSI breakpoints were used to determine susceptibility for all isolates. Since the only breakpoints of fosfomycin available from the CLSI are for *E*. *coli* and *E. faecalis,* the breakpoints for the other organisms were extrapolated from these. The current CLSI susceptibility breakpoints are ≤ 64 µg/ml for MIC, which was used for agar dilution method and E test method, and ≥16 mm for disk diffusion. [13]

It is important to note that the CLSI recommend the addition of 25 µg/ml of glucose-6-phosphate to the media when doing agar dilution, as it reduces the rate of false resistance. [13] With regard to the disk diffusion method and the E test method, there is no need for glucose-6-phosphate in the media as they are both supplemented with glucose-6-phosphate. [7]

Agar dilution method:

Fosfomycin trometamol powder (Sigma Aldrich, SA) was dissolved in distilled water and supplemented with 25 µg/ml of glucose-6-phosphate (Sigma Aldrich, SA). Mueller Hinton agar plates were then prepared using the fosfomycin solution that has been serially double diluted with distilled water to achieve dilutions of 16,32,64,128 and 256 µg/ml. Each plate had a different concentration and was divided to accommodate 35 isolates plus controls. An antibiotic free plate was also prepared, to ensure viability and purity of isolates. For each isolate, a 0.5 McFarland standard suspension was made which was then further diluted (1:10). 1 µl of this suspension was then inoculated onto each plate. *Enterococcus faecalis* ATCC 29212 was used as control strains in each batch which had a minimum inhibition concentration (MIC) range of 32 – 128 µg/ml. The inoculated plates were then incubated for 16-20hrs at 35^{o}C +/- 2^{o}C. The minimum inhibition concentration (MIC) for each antimicrobial agent was defined as the lowest concentration that inhibited visible growth of the organism. [16]

Disk Diffusion method:

Mueller Hinton agar plates (Diagnostic Media Products, SA) were seeded with a 0.5 McFarland standard suspension of the isolate and a disk containing 200 µg fosfomycin and 50 µg of glucose-6-phosphate (MastDiscs ®AST, Davies Diagnostic, SA) was placed on the plate. *E. coli* ATCC 25922 was used as control strain in each batch. The inoculated plates were incubated for 18-24hrs at 35^{o}C +/- 2^{o}C. After incubation, zone diameters were measured and interpreted using CLSI guidelines. [13]

E test method:

A subset of 27 *E. coli* isolates were randomly selected and further tested for fosfomycin susceptibility using an E test strip (Biomerieux SA, France). Mueller Hinton agar plates (Diagnostic Media Products, SA) were seeded with a 0.5 McFarland standard suspension of the isolate. A fosfomycin E test strip containing glucose-6-phosphate (Biomerieux SA, France), was placed onto the agar surface, ensuring that the whole length of the antibiotic gradient was in complete contact with the agar surface. *E. coli* ATCC 25922 which has a MIC range of 0.5 – 2 µg/ml, was used as a control strain in each batch. The inoculated plates were incubated for 16-20hrs at 35^{o}C +/- 2^{o}C. After incubation, the area where the ellipse intersects the strip was read as the MIC. [13]

Data Analysis

Data was entered onto an Excel spread sheet, where the rate of susceptibility of fosfomycin to commonly isolated MDR urinary pathogens was calculated. The percent categorical agreement, and percentage error rates were also calculated using agar dilution as the reference method. Categorical agreement was defined as the percentage of isolates that fell within the same CLSI zone size interpretive category for fosfomycin [13,17,18] The acceptable performance rate for categorical agreement is ≥90%. [17,18] A very major error (VME) was defined as false susceptibility to fosfomycin and was calculated as the number of VMEs/ total number of fosfomycin resistant isolates. VME rates should be ≤3%. [17,18] A major error (ME) indicates false resistance to fosfomycin and was calculated as the number of MEs/ total number of fosfomycin susceptible isolates. ME rates should be ≤3%. [17,18] A minor error (mE) was defined as an isolate that tested as intermediate to fosfomycin by one method and as either resistant or susceptible by another method. Minor error rate was calculated as the number of mEs/ total number of isolates tested. [17,18]

Results

A total of 200 MDR urinary isolates were collected from September 2019 to July 2020, of which 178 isolates remained viable for testing.

In Table 1, of the 178 viable isolates, 165 isolates were Gram-negative bacilli, followed by 13 isolates that were Gram-positive cocci. *E. coli* was the most frequent Gram-negative followed by *Klebsiella* spp. *Enterococcus* spp. was the most common Gram-positive cocci.

The susceptibility of fosfomycin in Gram-negative bacilli and the Gram-positive cocci was 85.5% and 7.7% respectively, with an overall susceptibility rate of approximately 80%. Of the Gram-negative bacilli, *E. coli* had the highest rate of susceptibility to fosfomycin at >90%, while *Klebsiella* spp. had the second highest rate of susceptibility at >70%. All 12 *Enterococcus* spp. were resistant to fosfomycin.

Table1: Antimicrobial susceptibility of commonly isolated MDR urinary pathogens to fosfomycin using agar dilution.

Organism	***Total Number of isolates* (N=178)**		***Susceptibility to fosfomycin* (N=142)**	
	N	**%**	**N**	**%**
Gram negative bacilli	**165**	**92.7**	**141**	**85.5**
Escherichia coli	97	54.5	91	93.8
Klebsiella spp.	55	30.9	43	78.2
Enterobacter spp.	12	6.7	6	50
Citrobacter spp.	1	0.6	1	100
Gram positive cocci	**13**	**7.3**	**1**	**7.7**
Enterococcus spp.	12	6.7	0	0
Staphylococcus aureus	1	0.6	1	100

(N = number, % = percentage of susceptibility)

When comparing disk diffusion to agar dilution (reference method), 91% categorical agreement was achieved. The rate of VMEs were high at 42.9%, where of the 35 isolates that tested resistant on agar dilution, 15 isolates tested susceptible to disk diffusion. Although there were no major errors, the rate of mE's were 11%.

There was no correlation found between zone diameter and MICs as the zone diameters fell in a wide spectrum, ranging from 13 mm to 40 mm.

Table 2: Categorical agreement and error rates.

Method	%CA (N)	%VME (N)	%ME (N)	%mE (N)
Agar Dilution Vs Disk Diffusion (All isolates)	91% (162/178)	42.9% (15/35)	0% (0/142)	11.0% (15/178)
Agar Dilution Vs Disk Diffusion *E. coli*	93.8% (91/97)	50% (3/6)	0% (0/91)	3.1% (3/97)

(N = number, % = percentage of susceptibility)

Of the 27 isolates tested using E tests, 3 isolates were resistant on agar dilution and 24 were susceptible, while with disk diffusion 1 isolate was intermediate and 26 isolates were susceptible. When comparing these results to the E test we found that all 27 isolates tested as susceptible, this translates to an 88.9% categorical agreement between E tests and agar dilution.

Table 3. Discrepant fosfomycin MIC results between Agar dilution and E Tests

Organism	AD	AD Int	DD	DD Int	E Test	E Test Int
E. coli	>256 µg/ml	R	31 mm	S	12 µg/ml	S
E. coli	>256 µg/ml	R	31 mm	S	16 µg/ml	S
E. coli	>256 µg/ml	R	15 mm	I	48 µg/ml	S

AD = Agar Dilution; AD Int = Agar dilution Interpretation; DD = Disk Diffusion; DD Int = Disk Diffusion Interpretation; E Test Int = E Test interpretation.

Discussion

The purpose of this study was to determine the susceptibility of commonly isolated MDR urinary pathogens to fosfomycin. Secondly, a comparison was made between the reference method (agar dilution) and disk diffusion as well as E Tests. We established that fosfomycin was fairly effective to frequently isolated MDR uropathogens. We also showed that while there was 91% categorical agreement between agar dilution and disk diffusion, disk diffusion had a tendency towards false susceptibility.

In this study, as with many others, *E. coli* was the most frequently isolated urinary pathogen, next was *Klebsiella* spp., followed by *Enterobacter* spp. and *Enterococcus* spp. (Table 1). [19] This is an important observation, as a key finding of our study revealed that Gram negative bacilli had a high level of susceptibility to fosfomycin. In fact, MDR *E. coli* in particular had a susceptibility rate of >90% making fosfomycin ideal for the use as an oral treatment option in a UTI where a MDR pathogen is the suspected cause.

These findings are comparable to local studies as well as those done internationally. [1,14,20,21] In a local study conducted by Mothibi *et al*., 95.7% of all *Enterobacterales,* and 98.1% of *E. coli* were susceptible to fosfomycin. In the same study, fosfomycin demonstrated activity against 94.4% of ESBL producers and 90.7% carbapenemase producers. [12] The higher susceptibility rate found in the Mothibi study could possibly be attributed to disk diffusion being the method of choice. It is also important to note that our study was based on MDR isolates, which would also affect the susceptibility rate.

A Mexican study conducted in 2020, which used disk diffusion and broth dilution, reported a resistance rate of only 6.6% in ESBL producing *E. coli*. [22] Similarly, a study conducted in the United Kingdom, in 2019, revealed that ESBL producing *E. coli* had a 98% susceptibility rate to fosfomycin, likewise an Indian study conducted in 2019, which used agar dilution, also demonstrated 100% susceptibility of ESBL producing *E. coli* to fosfomycin. [2,23]

In contrast, our study as well as many others, revealed a higher rate of resistant for *Klebsiella* spp. which falls above the 10% resistance rate allowed in which a drug is recommended for empiric therapy use. [3,21,23]

Similarly, we discovered a very high rate of resistance of *Enterococcus* spp. to fosfomycin, with all isolates having an MIC ≥ 256 µg/ml, though it is important to note that there were only twelve

isolates available for testing. On further investigation, we found that of the twelve strains isolated, eleven were *Enterococcus faecium.*

As current CLSI guidelines are only available for *E. coli* and *Enterococcus faecalis*, high resistance rates should be interpreted with caution. [13]

In the second part of our study, using CLSI guidelines, we determined that there was significant agreement categorically between agar dilution and disk diffusion, particularly in *E. coli,* but the percentage of VMEs and mEs where higher than the 3% recommended by the CLSI guidelines. [17,18] We also tested a small subset of randomly selected *E. coli* isolates for fosfomycin susceptibility using E test strips, which revealed 88.8% categorical agreement. We were unable to detect the number of VMEs, MEs and mEs as the number of isolates were too low. [17] Although a high categorical agreement rate was noted for both methods, the high rate of false susceptibility in disk diffusion, as well as a categorical agreement rate that was below the recommended level for E tests, render both methods as unreliable for antimicrobial susceptibility testing of fosfomycin. Ultimately, between disk diffusion and E tests, we find that disk diffusion would be more reliable than E tests, as categorical agreements was achieved. Even though E tests had a high categorical agreement at 88%, it was below the CLSI recommended rate of >90%. [17] It is important to note that whilst reading the disk diffusion as well as the E tests the phenomenon of colonies with the inhibition zone was not experienced in this study.

Findings similar to this study, were demonstrated in a study conducted in the Netherlands in 2018, where using EUCAST guidelines a high categorical agreement rate was recorded for both disk diffusion and E Tests, but the detection of resistance was low. [24] Similarly, a Canadian study in 2020, also demonstrated high categorical agreement for both disk diffusion and E tests using both CLSI and EUCAST guidelines, but the rate of VMEs differed depending on the guidelines used. [25] EUCAST has a lower breakpoint for fosfomycin susceptibility (<32 μg/ml) compared to the CLSI guidelines (<64 μg/ml).[26]

Limitations

A limitation of our study was the small number of isolates tested, having a larger number of isolates would give us a clearer picture of the *in vitro* activity of fosfomycin over a wider spectrum of organisms.

Secondly, as this study was based solely on resistant organisms, the propensity for a higher rate of resistance is increased, therefore studies conducted on a wider sample base would possibly change the antimicrobial picture.

Lastly, the activity of fosfomycin against the other causes of UTI was not as satisfactory as with *E. coli*, this could be attributed to clinical breakpoints being extrapolated from those available in the current CLSI guidelines.

Conclusion

The takeaway message from our study is that *E. coli* is very susceptible to fosfomycin regardless of whether it is a MDR strain or not. This is an important finding, as *E. coli* is the leading cause of a UTI.It is also our finding that due to the high rate of false susceptibility in the disk diffusion and E test methods, agar dilution remains the most reliable method in testing fosfomycin susceptibility.

It is our recommendation that more research is needed on the *in vivo* activity and resistance mechanisms of fosfomycin which are important factors to consider when looking at its suitability as a treatment option for UTIs.

Acknowledgements

We would like to acknowledge the management and staff of both RK Khan Laboratory (NHLS) and Inkosi Albert Luthuli Central Hospital, Microbiology Department (NHLS) for all their assistance while completing this project. We would also like to acknowledge the National Health Laboratory Services Academic Affairs Department, for all their financial support.

Competing interests

The authors declare that they have no financial or personal relationship that may have inappropriately influenced them in writing this article.

Author Contributions

AN: conceptualisation and implementation of the research project, data collection, data analysis, literature review, writing of the first draft, writing, and editing of all subsequent drafts and writing of final draft; NRM: Conceptualisation of research project, input on all drafts, editing and analysis; KSS-H: input on implementation of the project and editing the first and final draft

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Data Availability

All required data is available within this article.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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CHAPTER 4

Discussion and Conclusion

Urinary tract infections are quite a burden on the health care system with multi drug resistance compounding the problem. Globally, multiple studies have been conducted on the antimicrobial resistance of advised therapy as well as on finding a way forward with regards to treatment options for multi drug resistant infections. Unfortunately, South African based studies on urinary infections are a rarity, with interest from the research community only recently taking off.

Due to this paucity of studies, this research was aimed at bringing to light the current antimicrobial susceptibility rates of commonly isolated urinary pathogens to the recommended treatment plan, whilst also looking at fosfomycin as a possible alternative to the current treatment regime.

While investigating the susceptibility patterns of common uropathogens we discovered that in South Africa, much like the rest of the world, the prevalence of antimicrobial resistance to frequently prescribed antibiotics was extremely high. This was regardless of clinical setting, as with both inpatients and outpatients the causative organisms showed high levels of resistance to what is generally considered oral drugs of choice. Ciprofloxacin and amoxicillin clavulanic acid

had resistance rates that were almost three times the recommended rates for empiric therapy. Trimethoprim sulfamethaxole had a resistance rate that was more than six times what is recommended, contributing to it being removed from the South African Treatment Guidelines as empiric treatment for a UTI. [25] While this research clearly illustrates that antimicrobial resistance in UTIs pose a challenge to clinicians, it also raises the issue of what antibiotics are available that will effectively treat a MDR UTI.

It was that question that fuelled the thinking behind exploring fosfomycin as a possible oral treatment option for MDR UTIs. We again found very limited local information on fosfomycin, which was surprising considering it does form part of the recommended empiric treatment plan for UTIs in South Africa. This possibly could be attributed to the fact that the CLSI recommended antimicrobial susceptibility testing (AST) method, agar dilution, is very time consuming and not cost effective. Bearing that in mind, we also considered alternative AST methods, namely disk diffusion and E tests, which are far more user friendly. Our method comparison revealed that while there was a correlation between agar dilution and disk diffusion, the rate of false susceptibility was too high. It was also determined that there was no categorical agreement between E tests and agar dilution. Therefore, agar dilution remains the best method in which to determine fosfomycin susceptibility.

With regards to fosfomycin susceptibility, our results revealed that *E. coli* is quite susceptible to fosfomycin regardless of whether it was a MDR strain or not.

It was also concluded in both studies that *E. coli* was the most predominant urinary pathogen, which makes fosfomycin an attractive option in the treatment of UTIs, more especially MDR UTIs. This would definitely benefit clinicians as MDR in urinary pathogens is a growing problem.

The findings of this research have emphasised the need for local antimicrobial susceptibility surveillance to be carried out on a regular basis as this will help clinicians to keep up to date with the changing antimicrobial patterns and to make the necessary modifications to treatments plans. It is also the recommendation of this study that further research on the resistance mechanisms and *in vivo* activity of fosfomycin be carried out to ensure we keep abreast of the changes in the pharmodynamics and pharmokinetics of fosfomycin.

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APPENDICES

Appendix A- Initial Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

11 October 2020

Mrs Alicia Naidoo (200005216)
School of Lab Med & Medical Sc
Medical School

Dear Mrs Naidoo,

Protocol reference number: BREC/00001578/2020
Project title: Fosfomycin: an oral treatment option for multi drug resistant uropathogens
Degree: MMedSci

EXPEDITED APPLICATION: APPROVAL LETTER

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application.

The conditions have been met and the study is given full ethics approval and may begin as from 11 October 2020. Please ensure that outstanding site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is subject to national and UKZN lockdown regulations dated 26th August 2020, see (http://research.ukzn.ac.za/Libraries/BREC/BREC_Lockdown_Level_2_Guidelines.sflb.ashx). Based on feedback from some sites, we urge PIs to show sensitivity and exercise appropriate consideration at sites where personnel and service users appear stressed or overloaded.

This approval is valid for one year from 11 October 2020. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be noted by a full Committee at its next meeting taking place on 10 November 2020.

Yours sincerely,

[redacted]

Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Email: BREC@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

Appendix B – Amendment to Initial Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

16 September 2021

Mrs Alicia Naidoo (200005216)
School of Laboratory Medicine & Medical Science
Medical School

Dear Mrs Naidoo,

Protocol reference number: **BREC/00001578/2020**
Project title: **Fosfomycin: an oral treatment option for multi drug resistant uropathogens**
Degree: Master of Medicine

We wish to advise you that your application for amendments (to extend the study period from: Jan 2019 to Dec 2019 to: an 2018 to Dec 2020) received on 14 September 2021 for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee.

The committee will be advised of the above at its next meeting to be held on 12 October 2021.

Yours sincerely

[redacted]

Ms A Marimuthu
(for) Prof D Wassenaar
Deputy Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
UKZN Research Ethics Office Westville Campus, Govan Mbeki Building
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Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx
Founding Campuses: Edgewood Howard College Medical School Pietermaritzburg Westville

Appendix C – Renewed Ethics Approval

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

05 October 2021

Mrs Alicia Naidoo (200005216)
School of Laboratory Medicine & Medical Science
Medical School

Dear Mrs Naidoo,

Protocol reference number: BREC/00001578/2020
Project title: Fosfomycin: an oral treatment option for multi drug resistant uropathogens
Degree: MMedSci

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	**11 October 2021**
Expiration of Ethical Approval:	**10 October 2022**

I wish to advise you that your application for recertification received on 30 September 2021 for the above study has been **noted and approved** by a subcommittee of the Biomedical Research Ethics Committee (BREC). The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 09 November 2021.

Yours sincerely

[redacted]

.....

Ms A Marimuthu
(for) Prof D Wassenaar
Chair: Biomedical Research Ethics Committee

Biomedical Research Ethics Committee
Chair: Professor D R Wassenaar
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Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx