Fosfomycin resistance in community-acquired urinary pathogens from Western Cape, South Africa

Oral fosfomycin is commonly used to treat uncomplicated urinary tract infections (UTI) and whilst resistance has been reported in many healthcare facilities in South Africa, the current prevalence remains unknown. This study investigated the prevalence and mechanisms of fosfomycin resistance amongst urinary pathogens in the Western Cape, South Africa. Of the 200 isolates collected during the study period (2019–2020), seven (3.5%) were fosfomycin resistant. Mutations in the glpT and uhpT transporter genes were the most common mechanism of resistance detected. These findings support the ongoing use of fosfomycin as an empiric antibiotic choice for the treatment of community-acquired UTI in this setting.

Keywords: fosfomycin resistance; resistance mechanisms; prevalence; urinary tract infections; empiric therapy; Enterobacterales; Enterococcus spp.; community-acquired UTI.

Introduction

Bacterial urinary tract infections (UTI) are common worldwide, affecting almost 150 million people annually.¹ The most common causes of UTI are members of the Enterobacterales order as well as Enterococcus spp. Owing to increasing antibiotic resistance and side-effect concerns with the use of antibiotics such as ciprofloxacin and trimethoprim/sulfamethoxazole, fosfomycin has become an empiric antibiotic treatment option for uncomplicated UTI.² Fosfomycin is active against both gram-positive and gram-negative pathogens, including Enterococcus spp., Escherichia coli, Klebsiella spp., Enterobacter spp., and Proteus mirabilis. In addition to nitrofurantoin and intramuscular gentamicin, fosfomycin is recommended by the South African Department of Health as a first-line agent for the treatment of uncomplicated UTI in women.

Fosfomycin resistance primarily occurs by modification of the antibiotic target because of mutations in the murA gene, which reduces the affinity between the murA protein and the fosfomycin molecule. Fosfomycin resistance may also be as a result of the inactivation of the hexose phosphate (UhpT) and glycerol-3-phosphate (GlpT) transport systems, thereby decreasing uptake of the antibiotic.³ Another mechanism of fosfomycin resistance involves the production of enzymes such as FosA, FosB, FosC and FosX, encoded by the fos genes, which inactivate fosfomycin by cleaving the oxirane ring.⁴ Of these, FosA enzymes are most frequently reported and are common in Enterobacterales.⁵

Reports of fosfomycin resistant E. coli are increasing worldwide, with resistance rates of approximately 3.2% reported in Europe, Asia and the United States (US).⁶ Similarly, fosfomycin resistance rates of up to 16% have been reported in vancomycin-resistant Enterococcus faecium in North America.⁷ In South Africa, fosfomycin resistance rates of 4.3% – 4.5% have been reported in UTI isolates from Gauteng with resistance in major Enterobacterales pathogens (E. coli, Klebsiella spp., P. mirabilis and Enterobacter spp.) ranging between 2.0% and 8.0% and resistance rates in Enterococcus spp. reaching 2.0%.⁸

At the Tygerberg Hospital National Health Laboratory Service (NHLS) Medical Microbiology diagnostic laboratory (Cape Town, Western Cape, South Africa), fosfomycin susceptibility testing is routinely performed on all Enterobacterales isolates, except E. coli, when resistance to other commonly prescribed oral antibiotics is noted. Despite the occasional detection of fosfomycin resistance in our setting, the current prevalence and underlying mechanisms of fosfomycin resistance remain unknown. This study aimed to determine the prevalence of fosfomycin resistance amongst community-acquired urinary pathogens from the Western Cape and to describe the mechanisms of resistance in these isolates.

Note: Additional supporting information may be found in the online version of this article as Online Appendix 1.
Methods and materials

Study design
This was a laboratory-based descriptive study performed at the Division of Medical Microbiology at Tygerberg Hospital in the Western Cape of South Africa, which serves approximately 2.6 million people. Over a period of four months (October 2019 – January 2020), 200 isolates were cultured from urine samples received from antenatal clinics. Pregnant women visiting antenatal clinics routinely submit urine samples for medical health screening. Any organisms isolated from these samples were considered to be representative of community carriage.

Isolation, identification and antimicrobial susceptibility testing (AST) of urinary pathogens were performed as part of routine diagnostic procedures in the laboratory. This included urine culture on UriSelect™ selective chromogenic agar medium (Diagnostic Media Production, Green Point, South Africa) for isolation and differentiation of urinary pathogens. Identification of organisms and routine AST were performed on the automated VITEK® 2 (bioMérieux, France) platform. The VITEK® 2 provides AST results in the form of estimated minimum inhibitory concentrations (MIC) for multiple organism/antimicrobial combinations including gram-positive and gram-negative bacteria. Organism susceptibility was interpreted according to the 2019 Clinical and Laboratory Standards Institute (CLSI) guidelines.

Fosfomycin susceptibility testing
Fosfomycin susceptibility of all isolates was determined by the Kirby-Bauer disc diffusion method using a fosfomycin disc (200 μg) containing 50 μg glucose-6-phosphate (G-6-P) (Mast Group Ltd, United Kingdom) on Mueller-Hinton (MH-Sens) agar plates (Diagnostic Media Production, Green Point, South Africa). For fosfomycin resistant isolates, the fosfomycin MICs were determined by gradient diffusion with fosfomycin E-test® strips (0.064 μg/mL – 1024 μg/mL) (Liofilchem, Italy). Strips were placed on MH-Sens agar plates inoculated with a 0.5 McFarland standard bacterial suspension, and incubated at 37 °C for 16 h in the presence of 5% carbon dioxide. All zone sizes were measured from the disc to the closest colony growth and E-tests® were read as per the manufacturer’s guidelines. No mutant colonies grew within the E-test® ellipses. The disc diffusion and MIC results were interpreted according to the CLSI 2019 guidelines and reported as either susceptible (zone of inhibition [ZOI] ≥ 16 mm, MIC ≤ 64 μg/ml), intermediate (ZOI 13–15 mm, MIC 128 μg/μl), or resistant (ZOI ≤ 12 mm, MIC ≥ 256 μg/ml). The CLSI guidelines only provide breakpoint definitions for E. coli amongst Enterobacterales and E. faecalis amongst Enterococcus spp.; therefore, these breakpoints were inferred for all Enterobacterales and Enterococcus spp., respectively.

Molecular detection of fosfomycin resistance genes
DNA was extracted from fosfomycin resistant isolates using a crude heat-freeze DNA extraction method. All isolates were screened for fosA1-7 by polymerase chain reaction (PCR) amplification using previously described primers.7 Isolates positive for fosA3/4 and fosA5/6 were obtained from an in-house isolate collection and used as positive controls following Sanger sequencing to confirm target specificity. In the absence of a control for fosA7, the fosA7 PCR product was confirmed by Sanger sequencing and used as a positive control for subsequent PCR reactions. There were no controls available for fosA1/2. Fosfomycin resistant E. coli and K. pneumoniae were also subjected to PCR and Sanger sequencing to characterise mutations in the chromosomal genes murA, glpT and uhpT, using previously described primers.11,12 All PCR reactions were performed using KAPA Taq ReadyMix (KAPA Biosystems, US). Primer sequences and PCR reaction conditions are described in Online Appendix 1, Table 1. The PCR products were visualised by agarose gel electrophoresis and Sanger sequencing was performed at Inqaba Biotech14 (South Africa). E. coli and K. pneumoniae chromosomal gene sequences were aligned to E. coli strain K-12 substr. MG1655 (ref: NC_000913.3) or K. pneumoniae subsp. pneumoniae HS11286 (ref: NC_016845.1), respectively, using the BioEdit Sequence Alignment Editor 20 to identify potential mutations in murA, glpT and uhpT.

Ethical considerations
Because of the inclusion of secondary non-human data in this study, ethical approval for waiver of consent was obtained from the Stellenbosch University Health Research Ethics Committee (reference number: S19/08/168).

Results

Study samples and species distribution
Of the 200 isolates cultured from urine samples received from antenatal clinics, E. coli was the most predominant species (n = 138; 69%), followed by E. faecalis (n = 24; 12%) (see Figure 1).

Fosfomycin susceptibility
Seven (3.5%) of the 200 isolates were resistant to fosfomycin:
3/138 (2.2%) E. coli, 2/5 (40%) E. cloacae, 1/16 (6.3%) K. pneumoniae and 1/10 (10%) P. mirabilis. One isolate (E. cloacae) had an MIC of 512 μg/ml and the rest of the isolates had MICs of >1024 μg/ml, which were all interpreted as fosfomycin resistant according to the CLSI 2019 criteria. All fosfomycin resistant isolates were susceptible to ciprofloxacin and trimethoprim/sulfamethoxazole and most were intermediate (3/7, 43%) or susceptible (3/7, 43%) to nitrofurantoin. Fosfomycin resistance was not detected amongst the Enterococcus spp. isolates.

Fosfomycin resistance mechanisms
All fosfomycin resistant isolates were screened for the fosA1-7 genes, however, only fosA7 was detected in a single E. coli isolate. Fosfomycin resistant E. coli (n = 3) and K. pneumoniae (n = 1) isolates were screened for chromosomal
In this study, fosfomycin resistance was detected in 2.2% of isolates, which is similar to the recently reported 2% resistance in hospital-acquired UTI $E. coli$ isolates in Johannesburg. $E. coli$ is not routinely tested for fosfomycin resistance at the Tygerberg Hospital NHLS Medical Microbiology diagnostic laboratory because of the presumed low prevalence of resistance and these findings support this practice.

$fosA7$ was only detected in one fosfomycin resistant isolate, which suggests that $fosA$ activity is not a common cause of resistance amongst community-acquired urinary pathogens in this population. The deletions observed in $uhpT$ in two $E. coli$ and one $K. pneumoniae$ isolate are likely to confer resistance as $uhpT$ deletions have been reported to be the most common mutations involved in gene inactivation in both clinical and in vitro generated fosfomycin resistant isolates. This should be further investigated in follow-up functionality studies.

There was growth of single colonies within the ZOI, making disc diffusion interpretation difficult and operator dependant. Elliot et al. suggested that the growth of single colonies within the ZOI may be caused by the presence of chromosomal $fosA$ genes rather than chromosomal mutations. Whole genome sequencing of scattered colonies’ genomes may indicate the common genes that are harboured by these colonies and could improve the interpretation of diffusion susceptibility testing methods in the laboratory.

None of the $E. coli$ isolates in this study harboured mutations in the $murA$ gene, but all three had mutations identified in the $glpT$ gene and two had additional mutations identified in the $uhpT$ transporter genes (Table 1). Mutations in the $murA$ gene are common in most fosfomycin resistant organisms except $E. coli$, where they have been associated with a high biological cost. The role of the previously reported Thr348Asn mutation, detected in the $glpT$ gene of one of the $E. coli$ isolates, in fosfomycin resistance has not been established. The Glu374Ala, Gly415Asp and Asn450Thr mutations detected in $glpT$ in $E. coli$ have not been described before, therefore their role in fosfomycin resistance remains unknown. Other mutations such as Leu297Phe, Glu434Gln and Gln444Glu, that have been previously described, were detected in the $glpT$ gene of three $E. coli$ isolates, but they have previously been proven not to confer resistance. There were no positive controls for $fosA1/2$ genes, making it possible that these genes were missed during PCR detection. The small fosfomycin resistant sample set and the general lack of correlation between genetic mechanisms of resistance and phenotypic expression, complicated the interpretation of our results. Furthermore, we could only base our findings on the selected resistance genes and mutations investigated in this study.

Future studies on this sample set could use whole genome sequencing to describe other potential fosfomycin resistance mechanisms, including the detection of other $fos$ genes and mutations in genes such as $ptsl$, $uhpA$ and $cyaA$, that have also been previously reported to contribute to fosfomycin resistance. Functional characterisation of previously uncharacterised
mutations should also be performed to confirm their role in fosfomycin resistance.

Conclusion
The prevalence of fosfomycin resistance in community acquired UTI in the Western Cape of South Africa remains low (3.5%). The most common mechanism of fosfomycin resistance was deletions in the transporter gene *uhpT*. This study served as a reminder of the challenges related to fosfomycin susceptibility testing and highlighted the need to improve these testing methods. Our findings support the ongoing use of fosfomycin as an empiric choice for the treatment of community acquired UTI. Close clinical follow-up of patients is however essential when treating UTIs caused by pathogens other than *E. coli* and *E. faecalis*.

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

Author’s contributions
L.B.M., M.N-F. and P.N. designed the study, L.B.M. performed all the experiments and data analysis. L.B.M., M.N-F. and P.N. collectively interpreted the results. L.B.M. wrote the manuscript, with the support and supervision of P.N. and M.N-F., and all authors reviewed and approved the final manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding author, P.N., upon reasonable request.

Disclaimer
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