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ORIGINAL ARTICLE

Evaluation of fosfomycin susceptibility using CLSI vs EUCAST criteria among multi drug resistant uropathogens in a tertiary care Hospital

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ABSTRACT

Background and Objectives: Urinary Tract Infections (UTIs) are most frequently caused by uropathogenic *Escherichia coli*, which accounts for approximately 80% of the cases. Other causative agents include *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Enterococcus* spp., and *Staphylococcus saprophyticus*. The main objectives of the study were to estimate the in vitro antimicrobial activity of fosfomycin against multidrug-resistant uropathogens (MDR) isolated from patients with suspected UTI using CLSI and EUCAST criteria and to describe the antimicrobial susceptibility pattern of uropathogens isolated during the study.

Materials and Methods: This was a descriptive study in which a total of 900 urine samples were collected from patients presenting with physician-assessed signs and symptoms suggestive of a UTI. Only samples exhibiting significant bacteriuria that were also multidrug-resistant (MDR) were included. Although fosfomycin disk diffusion criteria, according to CLSI and EUCAST, are only validated for *E. coli*, susceptibility among other Gram-negative bacteria was also interpreted using the same criteria. This represents a major limitation of the study.

Results: In the study, 251 samples grew multi drug resistant organisms. Only 57% of the Gram-negative isolates were sensitive according to EUCAST guidelines, while 87.6% of all isolates were sensitive by CLSI criteria. Among the 161 carbapenem-resistant isolates, 135 (83.9%) were fosfomycin-susceptible and 18 (11.2%) were resistant according to CLSI. In contrast, by EUCAST criteria, only 40 (24.9%) isolates were fosfomycin-susceptible, and the remaining 121 (75.1%) were resistant.

Conclusion: Our study showed that using fosfomycin disc diffusion criteria of *E. coli* for other organisms is not ideal; therefore, performing an alternative form of susceptibility testing for non-*E. coli* isolates is recommended. Continuous monitoring of fosfomycin susceptibility is warranted to detect any emerging resistance and to guide its clinical application.

Keywords: Breakpoints; Discrepancy; Uropathogens; Infection; Susceptibility

INTRODUCTION

Urinary tract infections (UTIs) are among the most commonly encountered diseases in clinical practice, with an annual global incidence of at least 250 million (1). UTIs can be either community-acquired or nosocomial (2). Urinary tract infections are most frequently caused by uropathogenic *Escherichia coli*. Other causative agents include *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Enterococcus* spp., and *Staphylococcus saprophyticus* (3).

Antibiotic misuse has caused widespread resistance, which in turn has made outpatient oral therapy extremely difficult. Various studies have reported

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high resistance rates to ciprofloxacin, norfloxacin, and nalidixic acid among uropathogenic organisms (4-7). The emergence of extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, and carbapenemases has drastically reduced treatment options, making the choice of empiric antimicrobials for UTIs more challenging (8, 9).

Fosfomycin has the unique property of lacking cross-resistance with other antimicrobial agents. It can be used as a single-dose oral treatment for uncomplicated UTIs (10). The intravenous formulation can be considered in combination with other agents, such as polymyxins, for the treatment of severe infections, including bacteremia and pneumoniae (11). Fosfomycin prevents bacterial cell wall synthesis by irreversibly inhibiting the enzyme UDP-N-acetylglucosamine enolpyruvyl transferase (MurA), thereby blocking the formation of the cell wall precursor N-acetylmuramic acid (12). Studies have demonstrated in vitro fosfomycin activity against carbapenem-resistant *Klebsiella pneumoniae* (CR-Kp), *Pseudomonas aeruginosa*, ESBL-producing bacteria, and vancomycin-resistant *Enterococcus* (VRE) (13-16). The Infectious Diseases Society of America (IDSA) 2010 guidelines for uncomplicated UTIs recommend a single 3 g oral dose of fosfomycin as a first-line treatment option (17).

Fosfomycin susceptibility testing requires the incorporation of glucose-6-phosphate (G6P) in either the media or the disk/Etest strip to ensure adequate bacterial uptake of the antibiotic (18). The interpretative criteria for fosfomycin from the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are shown in Table 1. CLSI endorses only disk diffusion and minimum inhibitory concentration (MIC) testing by agar dilution, and exclusively for urinary isolates of *E. coli* and *Enterococcus faecalis* (19). EUCAST initially suggested only MIC testing by agar dilution and broth dilution for fosfomycin (20). EUCAST introduced disk diffusion criteria for fosfomycin in 2017. These criteria are applicable only to *E. coli* isolates.

Table 1. CLSI and EUCAST interpretation criteria for fosfomycin

Methods	Disc	CLSI			EUCAST	
	Content	S	I	R	S	R
Disc Diffusion (Zone diameter in mm)	200 µg	≥16	13-15	≤ 12	≥24	<24
MIC (µg/ml)	-	≤64	128	≥ 256	≤8	≥8

CLSI- Clinical Laboratory Standards Institute (CLSI)
EUCAST- European Committee on Antimicrobial Susceptibility Testing (EUCAST)
MIC- Minimum inhibitory concentration
S- Susceptible, I- Intermediate, R- Resistant

The main objectives of the study were:

To estimate the in vitro antimicrobial activity of fosfomycin against multidrug-resistant uropathogens isolated from patients with suspected UTI using CLSI and EUCAST criteria.

To describe the antimicrobial susceptibility pattern of uropathogens isolated during the study.

MATERIALS AND METHODS

This was a prospective study conducted over a period of seven months from June to December 2021 in a tertiary care hospital in South India after getting clearance from ethical committee (Ref No-IEC/2021/05/207). Informed consent was obtained from patients and all patient identifiers were removed. A total of 900 urine samples were collected from inpatients and outpatients based on physician assessment of signs and symptoms suggestive of a UTI. Patients were advised to collect mid-stream urine samples into a sterile wide mouth container with all aseptic measures. Samples from catheterised patients were taken with all aseptic measures. Collection from patients with comorbidities was performed based on physician recommendation. Samples were transported to the laboratory and processed immediately. Relevant demographic details and clinical information were also collected.

Urine samples were plated using a semi-quantitative method on CLED agar and MacConkey agar (Hi-Media Laboratories, Mumbai, India) and incubated at 37°C for 24 hours. Identification was performed using standard biochemical tests and the VITEK 2 Compact System (BioMérieux, France). Antimicrobial susceptibility testing for these isolates was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates and interpreted according to CLSI guidelines. Fosfomycin susceptibility (Hi-Media Laboratories, Mumbai, India) was interpreted using both CLSI and EUCAST criteria. For quality control, *E. coli* ATCC 25922 and *Staphylococcus au-*

reus ATCC 29213 were used. The results consistently fell within the acceptable ranges specified by EUCAST and CLSI. Only samples exhibiting significant bacteriuria that were also multidrug-resistant (MDR) were included in the study. In this study, the fosfomycin disk diffusion criteria recommended by CLSI and EUCAST for *E. coli* were applied to all Gram-negative isolates (including *E. coli*, *Citrobacter*, and *Klebsiella*). This was done because MDR *Klebsiella* is another major uropathogen in our institute, along with *E. coli*, and a quicker, cheaper method for determining fosfomycin susceptibility in these isolates was required. This was one of the limitations of the study.

Inclusion criteria: Only samples exhibiting significant bacteriuria that were also multidrug-resistant were included in the study.

Exclusion criteria: Samples yielding bacterial growth of < 10^4 CFU/mL in asymptomatic individuals or that were not multidrug-resistant (MDR) were excluded.

Criteria for significant bacteriuria and MDR. Significant bacteriuria (21):

- Urinary catheter: ≥10^4 CFU/mL.
- Midstream urine: ≥10^5 CFU/mL. A lower threshold of 10,000–50,000 CFU/mL was included for cases with high clinical suspicion (e.g., fever with pyuria/bacteriuria or in patients with renal disease).

Multidrug resistance (MDR) (22):

Acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

Statistical methods. Rate of susceptibility was estimated using proportions.

RESULTS

In this study, a total of 251 isolates were determined to be MDR. Among them, 132 (52.6%) isolates were from male patients and 119 (47.4%) were from female patients. Additionally, 100 (39.8%) isolates were from the General Medicine Department. The majority of isolates (77.7%) were recovered from patients admitted to the ward, while 21.1% were from patients in the intensive care unit. Only 3 (1.2%) samples were from patients attending outpatient facilities. Among the 251 MDR isolates, the most common were *Klebsiella pneumoniae* (66.1%) and *Escherichia coli* (28.3%). There were seven (2.8%) isolates each of *Enterococcus faecalis* and *Citrobacter koseri.*

A comparison of fosfomycin susceptibility according to CLSI and EUCAST criteria is shown in Table 2. Only 57% of Gram-negative isolates were susceptible by EUCAST guidelines, while 87.6% of all isolates were susceptible by CLSI criteria. Only 9.2% of the isolates were resistant according to CLSI criteria. The antimicrobial resistance pattern of the isolates is shown in Table 3. For *E. coli*, resistance to nitrofurantoin was relatively low (14.1%), as was resistance to meropenem (25.4%). In contrast, meropenem resistance was very high in *K. pneumoniae* (84.9%). Among the 244 Gram-negative isolates in our study, 161 (66%) were resistant to meropenem.

Table 2. Comparison of fosfomycin susceptibility using CLSI and EUCAST

Fosfomycin	CLSI	EUCAST
Susceptible	220	139
Intermediate	8	Nil
Resistant	23	105

CLSI- Clinical Laboratory Standards Institute (CLSI)
EUCAST- European Committee on Antimicrobial Susceptibility Testing (EUCAST)

Among the 161 isolates, *K. pneumoniae*, *E. coli*, and *C. koseri* accounted for 141, 18, and 2 isolates, respectively. Fosfomycin susceptibility according to CLSI and EUCAST criteria among carbapenem-resistant organisms is shown in Tables 4 and 5. For *E. coli*, fosfomycin resistance was more common among non-carbapenem-resistant organisms than among carbapenem-resistant organisms. Among the carbapenem-resistant isolates in the study, 135 (83.9%) were fosfomycin-susceptible and 18 (11.2%) were resistant to fosfomycin according to CLSI criteria. The remaining eight isolates were in the intermediate category. In contrast, by EUCAST criteria, only 40 (24.9%) isolates were fosfomycin-susceptible and 121 (75.1%) were resistant.

DISCUSSION

In this study, fosfomycin demonstrated excellent in vitro activity against uropathogens according to CLSI criteria (87.6% susceptibility). Susceptibility

Table 3. Antimicrobial resistance pattern of the MDR isolates

Antibiotics	*Escherichia coli*	*Klebsiella pneumoniae*	*Citrobacter koseri*	*Enterococcus faecalis*
	n (%)	n (%)	n (%)	n (%)
Amikacin	23 (32.4)	127 (76.5)	2 (28.6)	NA
Gentamicin	33 (46.5)	145 (87.3)	3 (42.9)	NA
Cefazolin	69 (97.2)	166 (100)	7 (100)	NA
Cefuroxime	69 (97.2)	166 (100)	7 (100)	NA
Cefotaxime	68 (95.8)	165 (99.4)	7 (100)	NA
Ceftriaxone	68 (95.8)	165 (99.4)	7 (100)	NA
Cefepime	59 (83.1)	163 (98.2)	5 (71.4)	NA
Ciprofloxacin	66 (93)	165 (99.4)	7 (100)	7 (100)
Norfloxacin	66 (93)	165 (99.4)	7 (100)	7 (100)
Nitrofurantoin	10 (14.1)	149 (89.8)	3 (42.9)	7 (100)
Cotrimoxazole	56 (78.9)	141 (84.9)	6 (85.7)	NA
Amoxiclav	19 (26.8)	115 (69.3)	2 (28.6)	NA
Cefoperazone sulbactam	25 (35.2)	152 (91.6)	3 (42.9)	NA
Piperacillin-tazobactam	24 (33.8)	149 (89.8)	2 (28.6)	NA
Meropenem	18 (25.4)	141 (84.9)	2 (28.6)	NA
Ampicillin	71 (100)	NA	NA	7 (100)
Vancomycin	NA	NA	NA	1 (14.3)
Teicoplanin	NA	NA	NA	1 (14.3)
Linezolid	NA	NA	NA	0 (0)

N- Number, NA- Not Applicable, MDR- Multi drug resistant

Table 4. Fosfomycin susceptibility among carbapenem resistant and non-carbapenem resistant organisms using CLSI

Fosfomycin	*E. coli*		*K. pneumoniae*		*C. koseri*		Total
	CR	Non CR	CR	Non CR	CR	Non CR	
Susceptible	18	50	116	23	1	5	213
Intermediate	0	0	8	0	0	0	8
Resistant	0	3	17	2	1	0	233
Total	18	53	141	25	2	5	244

CR- Carbapenem (Meropenem) resistant

Table 5. Fosfomycin susceptibility among carbapenem resistant and non-carbapenem resistant organisms using EUCAST

Fosfomycin	*E. coli*		*K. pneumoniae*		*C. koseri*		Total
	CR	Non CR	CR	Non CR	CR	Non CR	
Susceptible	16	45	23	15	1	5	105
Resistant	2	8	118	10	1	0	139
Total	18	53	141	25	2	5	244

CR- Carbapenem (Meropenem) resistant

was reduced to 57% by EUCAST criteria, which employ higher breakpoints. A similarly high fosfomycin susceptibility rate (89.2%) against uropathogens was reported by Maraki et al. (23). A study by Sabharwal et al. showed that 94.4% of the isolates were susceptible to fosfomycin (24). Increased resistance to fosfomycin according to EUCAST criteria was also reported by Patel et al. (25). The large discrepancy in susceptibility rates between CLSI and EUCAST criteria is concerning. Since most microbiology laboratories in India use CLSI breakpoints, microbiologists and clinicians should be aware of the risk of treatment failure when applying these criteria for interpretation. Among the Gram-negative isolates in the present study, the carbapenem resistance rate was 66%. Of these, 83.9% and 24.9% were fosfomycin-susceptible according to CLSI and EUCAST criteria, respectively. High fosfomycin susceptibility rates among carbapenem-resistant organisms have been described by others (Table 6) (25-29). This study found 100% fosfomycin susceptibility among carbapenem-resistant *E. coli*. This finding is concordant with studies conducted by Banerjee et al., Amladi et al., and Pogue et al. (30-32). A surprising finding in our study was that fosfomycin resistance was present among carbapenem-susceptible *E. coli* by CLSI criteria, while no resistance was found among carbapenem-resistant (CR) *E. coli*. The high fosfomycin susceptibility observed among carbapenem-resistant (CR) organisms in this study provides hope for using this drug as an alternative to other nephrotoxic agents like colistin.

In this study, fosfomycin susceptibility among *K. pneumoniae* isolates was 83.7% by CLSI criteria, compared to only 22.9% by EUCAST criteria. This finding is discordant with the study by Kaase et al., which showed 68% fosfomycin susceptibility among *Klebsiella* isolates using EUCAST criteria (26). The high susceptibility reported by Kaase et al. is likely due to their use of the agar dilution method for fosfomycin testing. The study by Endimiani et al. suggested that, since agar dilution is a time-intensive method, disk diffusion may be the most practical system for evaluating fosfomycin susceptibility in *Klebsiella* spp. (29). Both CLSI and EUCAST guidelines state that the disk diffusion criteria for fosfomycin are applicable only to urinary isolates of *E. coli*. In this study, we applied this criterion to other Gram-negative isolates, such as *Klebsiella* spp. Our findings indicate that while using the CLSI disk diffusion criteria for fosfomycin susceptibility in *K. pneumoniae* yields relatively high susceptibility rates, the same is not true for the EUCAST criteria.

In the outpatient department, where oral antibiotics are preferred, minimal options are available for treatment of UTI. The antimicrobial resistance pattern of the isolates in this study showed that for *E. coli*, nitrofurantoin and amoxicillin-clavulanate (amoxiclav) remain good options for oral therapy, with resistance rates of only 14.1% and 26.8%, respectively. For patients requiring intravenous therapy, meropenem, with a resistance rate of 25.4%, remains a good option against *E. coli*. For *K. pneumoniae* isolates, resistance rates were very high for all tested drugs. Therefore, fosfomycin represents a good therapeutic choice for UTIs caused by *K. pneumoniae* in our hospital setting.

CONCLUSION

In conclusion, our findings indicate that fosfomycin is a highly effective option against UTIs caused by MDR Gram-negative organisms. While 87.6% of isolates were susceptible according to CLSI criteria, susceptibility was only 57% by EUCAST criteria. Only 22.9% of *K. pneumoniae* isolates were fosfomycin-susceptible by EUCAST criteria. In a country

Table 6. Fosfomycin susceptibility among carbapenem resistant organisms in other studies

Author	No of isolates	Susceptibility	Testing method	Reference number
Patel et al.	47	72.34%	E test	25
Kaase et al.	107	78%	Agar dilution	26
Falagas et al.	79	94.9%	E test	27
Livermore et al.	81	60.5%	Agar dilution	28
Endimiani et al.	68	63.2%	Disc diffusion	29

E test- Epsilometer test

like India, where there is a high prevalence of MDR pathogens, an oral drug like fosfomycin is very useful. Hence, it is essential to determine the susceptibility of this drug against common organisms such as *E. coli* and *K. pneumoniae*. Our study demonstrates that applying the fosfomycin disk diffusion criteria established for *E. coli* to other organisms is not ideal; therefore, performing an alternative form of susceptibility testing for non-*E. coli* isolates is recommended. The antimicrobial resistance patterns showed that for *E. coli*, fosfomycin, nitrofurantoin, and amoxicillin-clavulanate (amoxi-clav) remain good oral options. In contrast, for *K. pneumoniae*, fosfomycin was the only effective option. The rising trend of MDR uropathogens poses a significant challenge to the current therapeutic armamentarium for UTIs. Fosfomycin shows excellent efficacy for treating UTIs due to its unique mechanism of action, low incidence of resistance, oral availability with single-dose administration, and low propensity for cross-resistance with other antibiotics.

Continuous monitoring of fosfomycin susceptibility is warranted to keep a check on any increase in resistance pattern and further to aid in its clinical application.

One limitation of this study was the inability to perform other methods of fosfomycin susceptibility testing, such as Etest or agar dilution.

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