Brief Communication

In vitro Activity of Fosfomycin against Multidrug-Resistant Urinary and Nonurinary Gram-Negative Isolates

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Abstract

Background: The era of multidrug-resistant (MDR) Gram-negative bacilli (GNB) has renewed interest in fosfomycin. **Aim:** The present study evaluated the *in vitro* activity of fosfomycin against MDR urinary and nonurinary GNB isolates. **Materials and Methods:** Fosfomycin susceptibility was carried out by agar dilution for a total of 279 (142 from urine and 137 from other samples) MDR-GNB. Disk diffusion was done for urinary isolates only. **Results:** Urinary tract isolates had a high degree of susceptibility to fosfomycin (overall susceptibility, 90.8%), whereas only 42.9% of nonurinary isolates retained susceptibility to the drug. Percentage susceptibility rates for urinary and nonurinary isolates of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter* spp. were 99%, 91.3%, 66%, 0% and 62%, 44.4%, 32%, 11%, respectively. **Conclusion:** Fosfomycin showed excellent *in vitro* activity for uropathogens. Large-scale evaluation of fosfomycin against MDR systemic isolates is required to evaluate its therapeutic efficacy.

Keywords: Fosfomycin, multidrug-resistant Gram-negative bacilli, urinary tract infection

Introduction

In the era of increasing drug resistance to Gram-negative bacilli (GNB) and lack of new antibiotics, old forgotten antibiotics such as polymyxins and fosfomycin have made an excellent comeback, with polymyxins now enjoying a cult status which it did not in its first innings. The usage of fosfomycin has not been so widespread compared to the polymyxins.

Fosfomycin, originally named phosphonomycin, is a broad-spectrum, bactericidal antibiotic, first identified and reported from various strains of *Streptomyces* in 1969 in Spain.[1] It is the only member of the epoxide group of antibiotics and is structurally unrelated to any other agent currently approved for clinical use.[1] Fosfomycin interferes cell wall synthesis by inhibiting the initial step of peptidoglycan synthesis involving phosphoenolpyruvate synthetase.[1] It is available as fosfomycin tromethamine and fosfomycin calcium for oral use and as fosfomycin disodium for intravenous (IV) use.[1] Fosfomycin tromethamine (oral form) is recommended as one of the first-line therapies for uncomplicated cystitis and pyelonephritis.[2] The IV form is available only in some selected countries.[1]

The renewed interest in fosfomycin in recent years is mainly to address the treatment of urinary tract infections (UTIs) as an oral agent as well as systemic therapy of severe infections due to multidrug-resistant (MDR)-GNB in hospitalized patients.[1] There are a few technical limitations in the *in vitro* susceptibility testing as well as in the interpretative criteria of fosfomycin.[3] The Clinical and Laboratory Standards Institute (CLSI) guidelines are available only for the urinary isolates of *Escherichia coli* and *Enterococcus faecalis*.[4] Susceptibility breakpoints for these organisms, performed by disk diffusion (DD) and agar dilution (AD) methods by the CLSI, are representative of only the oral formulations.[4] The CLSI has not published IV fosfomycin breakpoints till date.[4] On the other hand, the European Committee of Antimicrobial Susceptibility Testing (EUCAST) recommends AD or broth microdilution methods for minimum inhibitory concentration (MIC) determination of fosfomycin for both oral and IV

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formulations.[5] In addition, it has recently published zone diameter breakpoints for IV fosfomycin since January 2017.[5] Both the EUCAST and CLSI MIC breakpoints differ [Table 1].[4,5] There is no consensus regarding the breakpoints for non-*Enterobacteriaceae* isolates such as *Pseudomonas aeruginosa* and *Acinetobacter* spp., which are important nosocomial pathogens. Considering the potential utility of fosfomycin against MDR-GNB and the relative paucity of data from India, we undertook the present study to determine the *in vitro* activity of fosfomycin against MDR-GNB isolates from urinary and nonurinary samples.

Materials and Methods

The study was performed over a period of 8 months (August 2016–March 2017) at the microbiology laboratory of a tertiary care teaching and referral hospital in the eastern part of India. Consecutive, nonduplicate MDR-GNB isolated from urine and various other samples (pus, blood, and endotracheal secretion/sputum) of the admitted patients were included. MDR was defined as nonsusceptibility to at least one agent in three or more antimicrobial categories.[6]

Fosfomycin susceptibility of all the isolates was determined by both DD and AD. For MIC determination by AD, fosfomycin disodium salt (Sigma Aldrich Corporation, St. Louis, MO. USA, Catalog no: P5396) in solution was added to Mueller–Hinton agar (HiMedia, lab Pvt Ltd, Mumbai,India) supplemented with 25 mg/L glucose-6-phosphate (HiMedia) to provide serial two-fold dilutions ranging in concentrations from 0.25 to 512 mg/l.[7] For DD, commercially available fosfomycin disks (HiMedia) containing 200 μg of fosfomycin and 50 μg of G6P were used.

CLSI MIC and zone diameter breakpoints for *E. coli* were used to interpret fosfomycin MIC and DD results for urinary isolates of GNB. For nonurinary isolates, EUCAST IV breakpoints were used.[7] The zone diameters of urinary isolates were also interpreted using the current EUCAST DD breakpoints in addition to the CLSI breakpoints. Isolated colonies within the inhibition zone were ignored. The interpretive criteria used are tabulated in Table 1. Categorical agreement (CA) between AD and DD was defined as results within the same susceptibility category.

Results

A total of 279 consecutive nonduplicate MDR-GNB were included in the present study. The sample-wise distribution of isolates is summarized in Table 2.

Using the CLSI MIC breakpoints, 129 out of 142 (90.8%) urinary isolates of GNB were susceptible to fosfomycin, whereas 7 (4.9%) and 6 (4.2%) were classified as intermediate and resistant, respectively [Table 3]. There was complete CA between AD and DD results among the susceptible and resistant isolates using CLSI breakpoints. However, all the seven intermediate isolates by AD were observed to be sensitive by DD. Applying EUCAST MIC breakpoints to the urinary isolates, the total number of resistant isolates was 21 (21/142, 14.7%), which included all the 13 isolates classified as intermediate and resistant using the CLSI criteria plus 6 isolates of *Klebsiella pneumoniae* and 2 isolates of *P. aeruginosa* with MICs of 64 μg/ml by CLSI.

A total of 137 nonurinary MDR-GNB isolates were tested for fosfomycin susceptibility applying the EUCAST IV MIC breakpoints for *Enterobacteriaceae* to nonurinary isolates. Maximum susceptibility was observed for *E. coli* (18/29, 62%), followed by *K. pneumoniae* (24/54, 44.4%), *P. aeruginosa* (8/25, 32%), and *Acinetobacter* spp. (3/26, 11%) [Table 4].

When the fosfomycin activity was analyzed as per the site of infection, the highest *in vitro* susceptibility was observed for urine (121/142, 85.3%), followed by pus (25/58; 43.1%), sputum/tracheal aspirate (17/60, 28.3%), and blood (5/19; 26.3%) [Figure 1]. Thus, a higher resistance rate was detected among isolates recovered from samples other than urine compared to the urinary isolates (57% vs. 9.2%; $P < 0.0001$).

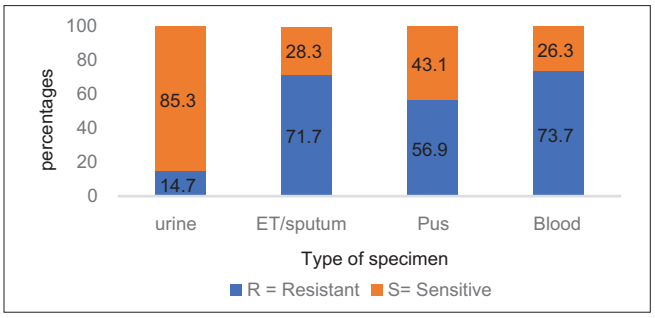

Figure 1: Analysis of fosfomycin activity as per the site of infection

Table 1: Fosfomycin minimum inhibitory concentrations and zone diameter breakpoints for Gram-negative bacilli according to the European Committee of Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute criteria 2017

Criteria	Organism(s) and delivery route	MIC (mg/l)			Zone diameter (mm)		
		S	I	R	S	I	R
EUCAST	*Enterobacteriaceae*						
	Intravenous (for systemic isolates)	≤32		>32	≥24		<24
	Oral (for uncomplicated UTI)	≤32		>32	≥24		<24
CLSI (for urinary isolates only)	*Escherichia coli*	≤64	128	≥256	≥16	13-15	≤12

EUCAST: European Committee of Antimicrobial Susceptibility Testing; CLSI: Clinical and Laboratory Standards Institute; UTI: Urinary tract infection; MIC: Minimum inhibitory concentration; S: Sensitive; R: Resistant; I: Intermediate

Discussion

The present study aimed to assess the *in vitro* activity of fosfomycin as a suitable oral agent for the treatment of UTI as well as a last resort therapeutic option in severe infections due to MDR-GNB in hospitalized patients.

Isolates recovered from the urinary tract (n = 142) had a high degree of susceptibility to fosfomycin (overall susceptibility, 90.8%) including a high percentage of *E. coli* and *K. pneumoniae,* the predominant urinary pathogens, which demonstrated a susceptibility of 99% and 91.3%, respectively. For urinary *P. aeruginosa* isolates, fosfomycin may be useful as 66.6% isolates were sensitive. We analyzed *P. aeruginosa* according to the CLSI *E. coli* breakpoints, which may not be appropriate. Some studies recommend that the ecological cutoff value of 128 mg/L can be used as a reference in interpreting the results in the absence of clinical breakpoints for *Pseudomonas*.[7] If the cutoff value of 128 mg/L is used, the percentage of susceptible isolates will be 75%. However, the total number of *P. aeruginosa* urinary isolates is very less (n = 12) to draw any conclusion. Similarly, in the absence of interpretative criteria and very less number of *Acinetobacter* isolates (n = 5), it is difficult to discuss the findings.

Table 2: Sample-wise distribution of various isolates

Bacterial isolates	Urine	Pus	ET/Sputum	Blood	Total
Escherichia coli	74	13	10	6	103
Klebsiella pneumoniae	46	17	26	11	100
Pseudomonas aeruginosa	12	15	7	2	36
Acinetobacter spp.	4	11	15	0	30
Other *Enterobacteriaceae*	6	4	0	0	10
Total	142	60	58	19	279

ET: Endotracheal secretion

Our findings of fosfomycin susceptibility against uropathogens are similar to that of other Indian studies which have reported susceptibilities in the ranges of 90%–100%.[8] In our study, 99% of urinary *E. coli* retained susceptibility to fosfomycin. In case of urinary isolates of *Klebsiella*, in our study, 91.3% retained susceptibility to fosfomycin, whereas other studies from India have reported 95.5%, 90%, and 88.2%.[8] The overall CA between AD and DD was 98.6%, 95.6%, 91.6%, and 25% for *E. coli, Klebsiella, P. aeruginosa, and Acinetobacter* spp., respectively. The CA between the study by Perdigão-Neto *et al*. and our study is comparable for *Klebsiella* spp. (96% vs. 95.6%), whereas wide discrepancies were observed for *P. aeruginosa* (7% vs. 91.6%) and *Acinetobacter* spp. (86% vs. 25%).[9]

The second objective of our study was to study the *in vitro* susceptibility of fosfomycin against systemic infections. Among the 137 isolates tested, 81 (59.12%) were resistant to fosfomycin using the EUCAST breakpoints. Among nonurinary isolates, the rates of resistance for *E. coli, Klebsiella, P. aeruginosa, and Acinetobacter* spp. were 38%, 55.6%, 68%, and 89%, respectively. Our study found a little high rate of resistance in nonurinary isolates (59.12%) compared to a recent Indian study by Chitra *et al*. which found 48.8% resistance to fosfomycin among nonurinary isolates of *E. coli and Klebsiella* spp.[3] In the study by Chitra *et al*., *Klebsiella* isolates from blood and sterile body fluid showed increased resistance (24.5%) compared to urinary isolates (5.8%), while *E. coli* isolates were uniformly susceptible to both blood/body fluids (97%) and urinary isolates (100%).[3]

A notable finding of our study was 99% of MDR *E. coli* isolates from UTI retained susceptibility to fosfomycin, whereas among nonurinary tract isolates, the fosfomycin sensitivity was 62%. Similarly, 91.3% of MDR *K. pneumoniae* isolates

Table 3: Minimum inhibitory concentration (μg/ml) distribution of various urinary tract isolates to fosfomycin (Clinical and Laboratory Standards Institute, 2017) (n=142)

	>512 (μg/mL)	256	128	64	32	16	8	4	2	1	0.5	0.25	Total
Escherichia coli	0	0	1	0	3	7	5	9	9	24	11	5	74
Klebsiella pneumoniae	1	1	2	6	9	6	5	9	5	2	0	0	46
Pseudomonas aeruginosa	1	2	1	2	2	2	2	0	0	0	0	0	12
Acinetobacter spp.	0	1	3	0	0	0	0	0	0	0	0	0	4
Other *Enterobacteriaceae*	0	0	0	0	0	0	1	2	1	2	1	1	6
Total	2	4	7	8	14	15	12	18	14	26	11	5	142

Table 4: Minimum inhibitory concentration distribution of various nonurinary tract isolates (n=137) (interpreted by European Committee of Antimicrobial Susceptibility Testing minimum inhibitory concentration, 2017)

	>512 (μg/mL)	256	128	64	32	16	8	4	2	1	0.5	0.25	Total
Escherichia coli	2	1	4	4	3	4	8	1	0	1	1	0	29
Klebsiella pneumoniae	5	5	9	11	7	7	8	0	1	1	0	0	54
Pseudomonas aeruginosa	2	3	5	7	6	1	1	0	0	0	0	0	25
Acinetobacter spp.	5	4	10	4	1	1	1	0	0	0	0	0	26
Other *Enterobacteriaceae*	0	0	0	0	0	0	0	0	1	2	0	0	3
Total	14	13	28	26	17	13	18	1	1	2	1	0	137

from UTI retained susceptibility to fosfomycin, whereas among nonurinary tract isolates, the sensitivity was 44.5%. The discrepancy in the rates of resistance between urinary and nonurinary isolates is difficult to explain and more work is needed to understand the differences in susceptibility profile between isolates responsible for UTI and other systemic infections.

Conclusion

Although fosfomycin appears promising as a suitable agent for UTI based on *in vitro* data, high rates of resistance in nonurinary isolates of *Enterobacteriaceae* is a cause of concern. There is a need for harmonization of CLSI and EUCAST breakpoints and optimization of DD, particularly for nonurinary isolates. Furthermore, breakpoints for *Pseudomonas* spp. and *Acinetobacter* spp. need to be defined.

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Conflicts of interest

There are no conflicts of interest.

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