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Fosfomycin susceptibility of carbapenem-resistant Enterobacterales and methicillin-resistant *Staphylococcus aureus* bloodstream isolates in a tertiary care hospital, South India

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Abstract

Background: The emergence of multidrug-resistant organisms has limited the choice of therapeutic options to treat infections. The lack of development of new antimicrobials paved the way for considering the reassessment of older antibiotics like fosfomycin. In this context, we assessed the in-vitro effect of fosfomycin against carbapenem-resistant Enterobacterales and methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream isolates by agar dilution, disk diffusion, and screen agar.

Methods: In this study, 141 consecutive blood isolates resistant to carbapenem and 62 MRSA blood culture isolates were collected over a period of 8 months. The methods used were fosfomycin agar dilution (0.25 µg/ml to 512 µg/ml), Kirby-Bauer disk diffusion (150 µg of fosfomycin + 50 µg of glucose-6-phosphate), and fosfomycin screen agar (32 µg/ml, 48 µg/ml, and 64 µg/ml). All three methods were interpreted using the European Committee on Antimicrobial Susceptibility Testing guidelines. The agreement between the new method and the reference method was calculated.

Results: Among the tested isolates, 100% of MRSA, followed by *Escherichia coli* (*E. coli*) (86.4%), *Klebsiella pneumonia* (*K. pneumonia*) (65.2%), and *E. cloacae* (50%) were susceptible to fosfomycin. The MIC50 and MIC90 of fosfomycin were 0.5 µg/ml and 2 µg/ml for MRSA, 16 µg/ml and 32 µg/ml for *K. pneumoniae*, 4 µg/ml and 16 µg/ml for *E. coli*, and 8 µg/ml and 32 µg/ml for *E. cloacae*, respectively.

Conclusion: Fosfomycin demonstrated a good *in-vitro* effect on most of the carbapenem-resistant Enterobacterales and MRSA isolates tested.

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Introduction

Antimicrobial resistance is an increasing concern for public health. The emergence of drug-resistant pathogens such as multidrug-resistant, extensively drug-resistant, and pan-drug-resistant gram-negative organisms remains a major threat worldwide, responsible for increasing mortality and morbidity (1-3). The emergence of drug-resistant organisms has limited the choice of therapeutic options to treat infections. Of particular concern is the spread of carbapenemases, because these beta-lactamases are resistant to almost all beta-lactam antibiotics (4,5). Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) (MRSA), which is resistant to almost all available beta-lactam antimicrobial drugs (Except 5th-generation cephalosporins), has been increasing over the last decades. In India, the MRSA rate is around 30-40%, although it varies between years and locations (6). The increasing resistance rates among both gram-positive and gram-negative pathogens necessitate the implementation of alternative treatment options. The lack of development of new antimicrobials paved the way for considering the reassessment of older antibiotics like fosfomycin.

One such promising agent is fosfomycin, a bactericidal antibiotic active against both gram-positive and gram-negative pathogens. It inhibits the initial step of cell wall synthesis involving phosphoenolpyruvate synthetase. The World Health Organization classified fosfomycin in the category of a “critically important” antimicrobial for investigation of efficacy against gram-negative infections (7). Fosfomycin was previously used as an oral treatment for uncomplicated urinary tract infections (8,9). It has a low level of existing resistance and also has activity in biofilms (8). Considering the potential utility of fosfomycin against multidrug-resistant bacteria, we undertook this study to determine the fosfomycin susceptibility of carbapenem-resistant Enterobacterales (CRE) and MRSA.

Very few studies on fosfomycin susceptibility are available for bloodstream isolates. According to the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, only agar dilution is a valid method for fosfomycin to determine the minimum inhibitory concentration (MIC) (10-12). This study aimed to detect fosfomycin susceptibility patterns in isolates of CRE and MRSA from bloodstream infections to determine its therapeutic utility in our healthcare facility (4,13).

Methods

The study was conducted at Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), a tertiary care referral center and an institution of national importance under the Ministry of Health and Family Welfare, Government of India. This study was approved by the JIPMER Institutional Ethics Committee (IEC) with the approval number JIP/IEC/2021/070. A certificate for waiver of informed consent was also obtained from the IEC, as this study included bacterial isolates routinely obtained in our hospital laboratory and did not involve human subjects directly. Therefore, informed consent was not taken from the patients as per our institute's policy. The samples were collected from April 2021 to November 2021. During this study period, all consecutive 141 CRE isolates, which included *Escherichia coli* (*E. coli*), *Klebsiella pneumonia* (*K. pneumonia*), *Enterobacter* spp., *Morganella* spp., and *Providencia* spp. showing carbapenem resistance (Meropenem, imipenem, ertapenem, doripenem), along with MRSA isolates, were collected over a period of 8 months. Carbapenem and methicillin resistance were confirmed by either disc diffusion or VITEK 2™. Subculture was performed from stocked isolates. The identification of the isolated colonies was confirmed with VITEK MS™ - bioMérieux

(Matrix-assisted laser desorption ionization-time of flight mass spectrometry technique).

The only approved MIC method for fosfomycin testing is the agar dilution method (11). Fosfomycin (Sigma-Aldrich) (P5396) was obtained as powder and disk (200 µg). The agar dilution method was performed according to CLSI M100 guidelines. The MIC of fosfomycin was determined by the agar dilution method. An MIC >32 µg/ml is considered resistant to fosfomycin according to EUCAST 2022 guidelines. A series of Mueller-Hinton agar plates containing 25 µg/ml of glucose-6-phosphate and fosfomycin in concentrations ranging from 0.25 µg/ml to 512 µg/ml were prepared, as this range covers all current clinical breakpoints of fosfomycin for various organisms, plus two dilutions on either side, with the lowest susceptible and highest resistant breakpoints being 8 µg/ml and 256 µg/ml, respectively. The bacterial inoculum was prepared and adjusted to 0.5 McFarland (1-2 × 10^8 CFU/ml). The final inoculum on the agar needed was 10^4 CFU/ml, so 0.1 µl of this inoculum was pipetted onto the agar surface. The plates were incubated overnight at 37°C and interpreted according to EUCAST guidelines (10). As per the literature, the recommended concentration of the drug in a fosfomycin screen agar plate is 32 µg/ml. Because of variability in the susceptible breakpoint, it was difficult to decide on a single concentration of the drug plate to be tested as a screen that could cover all organisms (10,11). In the present study, we evaluated the utility of using three different concentrations of fosfomycin as screen agar, i.e., 32 µg/ml (to detect the susceptible breakpoint of 32 µg/ml), 64 µg/ml (To detect the resistant breakpoint of 128 µg/ml), and 48 µg/ml (Intermediate between both breakpoints). The interpretation of fosfomycin screen agar was done according to EUCAST guidelines (10). The Kirby-Bauer disk diffusion method was performed only on CRE isolates. The fosfomycin disk (150 µg of fosfomycin + 50 µg of glucose-6-phosphate) was placed onto a Mueller-Hinton agar plate and incubated at 37°C overnight and interpreted according to EUCAST guidelines. According to EUCAST guidelines, disk diffusion breakpoints are available only for *E. coli.* There are no disk diffusion breakpoints for other species of the family Enterobacterales. The results were interpreted using the *E. coli* disk diffusion breakpoints.

Statistical analysis was performed using Stata version 14 software, and the p-value was calculated. The comparison of agar dilution and disk diffusion was analyzed using McNemar's chi-square test.

Results

In this study, a total of 203 isolates were included, consisting of 141 CRE isolates and 62 MRSA isolates. Among the 141 CRE isolates, *K. pneumoniae* was the most common (67.3%; 95/141), followed by *E. coli* (26.2%), *E. cloacae* (4.2%), *P. stuartii* (1.4%), and *M. morganii* (0.7%). All three methods were evaluated according to EUCAST guidelines. The MICs of quality control strains were within the limits: 0.5-2 µg/ml for ATCC *E. coli* 25922 and 0.5-4 µg/ml for ATCC *S. aureus* 25923 on all occasions. Out of the 203 isolates, MRSA (62/62), E. coli (32/37), K. *pneumoniae* (64/95), *E. cloacae* (3/6), and *P. stuartii* (2/2) were shown to be susceptible to fosfomycin (Figure 1). The MICs of the test isolates obtained by fosfomycin screen agar were the same as those of the reference method. The test method essentially agreed with the reference method. The essential agreement between fosfomycin screen agar and the reference method was 100%.

The categorical agreement of 141 CRE isolates for fosfomycin disk diffusion compared with the reference method was 96%. The categorical disagreement of 141 CRE isolates for fosfomycin disk diffusion compared with the reference method was found to be 4.2%, the majority of which were highly major errors (VME, 5.0%) followed by major errors (ME, 4.0%) (Table 1). There was high concordance between agar dilution MICs and disk diffusion breakpoints for *E. coli.* The p-value between the agar dilution and disk diffusion for *E. coli* was found to be <0.05 (Significant), while others were not significant. According to EUCAST guidelines, the MIC of fosfomycin for Enterobacterales and *S. aureus* is (S ≤32, R ≥64). The MIC_{50} and MIC_{90} of fosfomycin for MRSA were 0.5 µg/ml and 2 µg/ml, respectively. The MIC_{50} and MIC_{90} of fosfomycin for *K. pneumoniae* were 16 µg/ml and 32 µg/ml. For *E. coli*, the MIC_{50} and MIC_{90} were 4 µg/ml and 16 µg/ml, respectively. For *E. cloacae*, the MIC_{50} and MIC_{90} were 8 µg/ml and 32 µg/ml, respectively.

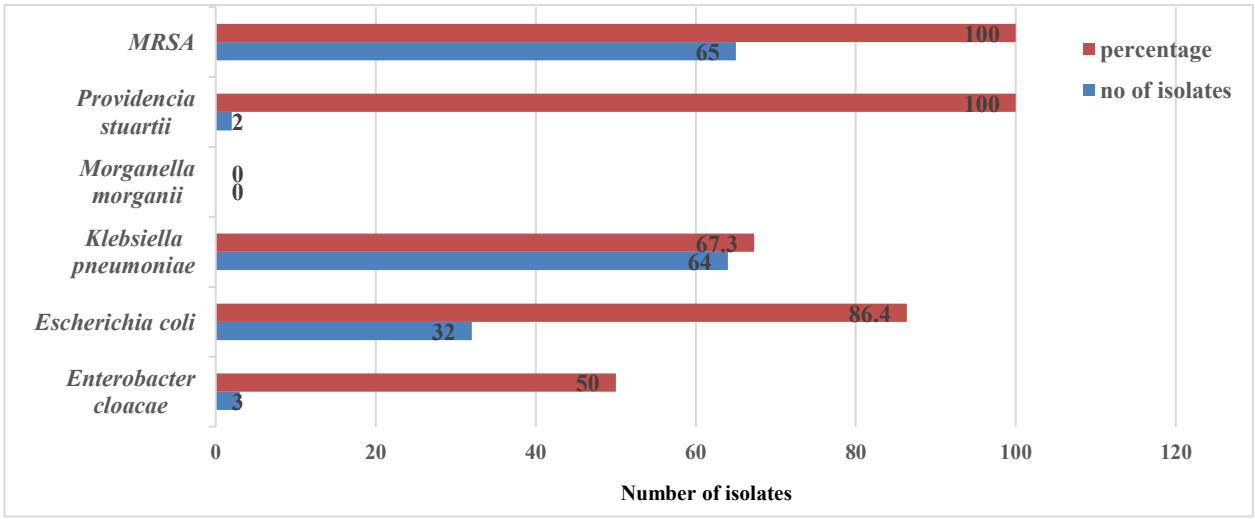

Figure 1. Column chart depicting isolates susceptible to fosfomycin by agar dilution.
Abbreviation: MRSA- Methicillin-Resistant *Staphylococcus aureus*

Table 1. Agreement between disk diffusion and agar dilution

Organism	Categorical agreement		Categorical disagreement		Major error		Highly major error		Statistical analysis (P-value)
	No. of isolates	Percentage	No. of isolates	Percentage	No. of isolates	Rate (%)	No. of isolates	Rate (%)	
Carbapenem-resistant Enterobacterales	135/141	96	6/141	4.2	4/101	4.0	2/40	5	0.41
Enterobacter cloacae	6/6	100	0	0	0	0	0	0	0.08
Escherichia coli	37/37	100	0	0	0	0	0	0	0.03
Klebsiella pneumoniae	89/95	93.6	6/95	6.31	4/64	6.25	2/31	6.4	0.41
Morganella morganii	1/1	100	0	0	0	0	0	0	-
Providencia stuartii	2/2	100	0	0	0	0	0	0	-

Discussion

In the era of increasing drug resistance with the available antibiotics, gram-positive and gram-negative pathogens often cause difficult-to-treat infections. The outcomes of patients infected with multidrug-resistant bacteria are worse than those infected with susceptible strains. The dramatic increase in drug resistance and the limited availability of novel antibiotics necessitate the implementation of alternative treatment strategies. Fosfomycin has a significant role in carbapenem-sparing treatment strategies in multidrug-resistant sepsis. However, the susceptibility testing method for fosfomycin is challenging due to issues involved in preparation and interpretation. In this study, we tested isolates against fosfomycin by agar dilution, screen agar, and disk diffusion methods.

A total of 203 non-repetitive CRE and MRSA isolates consecutively obtained from bloodstream infections were collected and subjected to fosfomycin susceptibility testing by various methods. *K. pneumoniae* was the most common isolate among all CRE isolates, followed by E. *coli, E. cloacae, P. stuartii,* and *M. morganii*. The EUCAST breakpoints were used in this study. In the current CLSI M100, the zone diameter and MIC breakpoints are restricted only to urinary isolates of *E. faecalis* and *E. coli*, while the MIC values from the current EUCAST breakpoints apply to all isolates from Enterobacterales. Zone diameter breakpoints are available only for *E. coli* from all samples. In this study, disk diffusion breakpoints for Enterobacterales were interpreted using *E. coli* disk diffusion breakpoints.

We observed that most of the isolates obtained from bloodstream infections included in this study were sensitive to fosfomycin. Our study results demonstrate the potential activity of fosfomycin against MRSA isolates, and this finding is similar to previous studies (13). Among all isolates, MRSA seemed to be the most susceptible (100%, 62/62) to fosfomycin, followed by *E. coli* (86.4%, 32/37), *K. pneumoniae* (67.3%, 64/95), and *E. cloacae* (50%, 3/6). In this study, *K. pneumoniae* exhibited the highest non-susceptibility (33%, 31/95), followed by *E. cloacae* (50%, 3/6) and *E. coli* (13.5%, 5/37).

In our study, MRSA (0.5 µg/ml) strains had significantly lower fosfomycin MICs, followed by *E. coli* (4 µg/ml), *E. cloacae* (8 µg/ml), and *K. pneumoniae* (16 µg/ml), which is in accordance with previous reports (14-17). Williams PC et al. (18) studied 247 multidrug-resistant gram-negative isolates from sepsis and analyzed the *in-vitro* activity of these isolates against fosfomycin. The reference method used in their study was agar dilution. They reported that 90% (202/224) of Enterobacterales were highly susceptible to fosfomycin as per EUCAST (≤32 µg/ml) criteria. Among these Enterobacterales, *K. pneumoniae* was found to be highly non-susceptible, followed by *E. coli* and *E. cloacae*, which matched our study results. They also found high concordance between agar dilution and disk diffusion for *E. coli*, which is similar to our study.

In our study, we found 72% (101/141) of the CRE isolates to be susceptible to fosfomycin. These data are similar to those reported by Livermore et al. (17), where 66.7% of strains were sensitive to fosfomycin among Enterobacterales-producing carbapenemases using the agar dilution method. In a study by Falagas et al. (16), 84.8% of isolates of Enterobacterales were susceptible to fosfomycin by the E-strip test method, although carbapenemase types were not characterized. Endimiani et al. (19) found that 75% (MIC ≤32 µg/ml) of KPC-producing *K. pneumoniae* strains were susceptible to fosfomycin using the agar dilution method. Pasteran et al. (20) found 86.7% of strains susceptible to fosfomycin, most of which were KPC-producing Enterobacterales.

In the work of Behara et al. (21) on 137 non-urinary isolates (Pus, tracheal aspirate, and blood), 81 (59.1%) isolates were resistant to fosfomycin according to EUCAST breakpoints. By using CLSI breakpoints, among 142 urinary isolates, 129 were sensitive to fosfomycin. They found a slightly higher resistance rate in non-urinary isolates (57%) compared to urinary isolates (9.2%). Maximum susceptibility was observed in *E. coli* (62%, 18/29), followed by *K. pneumoniae* (44.4%, 24/54).

Gopichand et al. evaluated the fosfomycin effect on multidrug-resistant gram-negative bacteria from urinary tract infections (22). In their study, AmpC β-lactamases, β-lactamases, and carbapenemase-producing strains of *E. coli*, *K. pneumoniae*, *Enterobacter* spp., and *Pseudomonas aeruginosa* were included. They also found a good inhibitory effect of fosfomycin on *K. pneumoniae* and *E. coli.* Sayantan Banerjee et al. included extended-spectrum β-lactamases, multidrug-resistant, and β-lactamase–producing uropathogens and found that 98.14% of *E. coli* and 95.52% of *K. pneumoniae* were susceptible to fosfomycin.

Mittal et al. (23) found that fosfomycin was 100% effective against uropathogenic *E. coli.* In a study by Rajenderan et al. (24), fosfomycin effectively inhibited 90% of *Klebsiella* and *E. coli* strains. Sahni et al. (25) found that fosfomycin susceptibility was 83% for *E. coli*, similar to our study. *M. morganii* was resistant to fosfomycin in this study. In the work of Floriana Campanile et al. (26), 99 isolates of Enterobacterales and 80 isolates of *S. aureus* were tested using agar dilution. According to EUCAST guidelines, 61% of *S. aureus* and 76% of Enterobacterales were inhibited by fosfomycin. These results are similar to those of our present study.

Inclusion of a greater number of isolates is necessary to further validate the results. This study was purely laboratory-based, and the test results did not clinically correlate with patient outcomes. Lack of molecular analysis is another limitation of this study in determining the mechanisms of fosfomycin resistance.

Abbreviations

CLSI: Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Testing; MIC: Minimum Inhibitory Concentration; MRSA: Methicillin-Resistant *Staphylococcus Aureus*; CRE: Carbapenem-Resistant Enterobacteriaceae; DD: Disk Diffusion; UTI: Urinary Tract Infection; G-6-P: Glucose-6-Phosphate; MALDI: Matrix-Assisted Laser Desorption Ionisation-Time of flight

Conclusion

In this study, we observed that fosfomycin has a positive in vitro effect on most of the carbapenem-resistant Enterobacterales and MRSA isolates tested. Therefore, we propose that fosfomycin could be considered as a therapeutic option for the treatment of extensively drug-resistant Enterobacterales where no alternative therapeutic options are available. For MRSA isolates, fosfomycin can be considered as an alternative to vancomycin in scenarios such as raised renal parameters or when vancomycin MIC is >1 µg/ml, where a vancomycin-sparing regimen is preferred.

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Ethical statement

The study was approved by the Institutional Ethics Committee of JIPMER (JIP/IEC/2021/070). Informed consent was not taken from patients as per institute policy.

Conflicts of interest

None

Author contributions

The authors contributed equally to the conception, design, analysis, and writing of this manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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