

Susceptibility Pattern of Fosfomycin Against Drug Resistant Bacteria Obtained from Non - Urinary Clinical Samples in a Tertiary Care Hospital, South India

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ABSTRACT

Background: Multi drug resistance (MDR) in bacterial infections has been an ever growing problem worldwide. To combat this some of the old drugs like fosfomycin used in the past are revived. The aim of our study was to determine the susceptibility of fosfomycin against Methicillin sensitive and Resistant Staphylococcus aureus, ESBL producing Escherichia coli, Klebsiella species and Metallo Beta Lactamase producing Pseudomonas aeruginosa isolated from the specimens other than urine and to evaluate the agreement between the two methods, disk diffusion and agar dilution methods performed as per CLSI guidelines.

Methods: First isolate of each species per patient (n=250) were tested for susceptibility to fosfomycin concomitantly by the disk diffusion and agar dilution methods described by CLSI guidelines and comparison of the two methods were studied.

Result: Staphylococcus aureus and ESBL E. coli were showing 100% susceptibility, Whereas ESBL producing Klebsiella species showed 88% susceptibility to fosfomycin 200 µg/disc and 80% and 72% by agar dilution method as per CLSI and EUCAST criteria. For MBL producing Pseudomonas aeruginosa, 90% isolates were susceptible to fosfomycin 200 µg/disc and in agar dilution (60%) ($\leq 32\mu\text{g/ml}$) were susceptible as per EUCAST criteria. Disk diffusion method showed good agreement for S.aureus and E.coli whereas moderate agreement for Klebsiella species and very poor for Pseudomonas aeruginosa.

Conclusion: Fosfomycin can be considered as an alternate drug to treat infections with multi drug resistant bacteria, not only for the UTI but for systemic infections also. This is achievable with establishment of breakpoint values and zone diameter for all common isolates.

Keywords: Fosfomycin, MRSA, ESBL E.coli, ESBL Klebsiella, MBL Pseudomonas Aeruginosa, Disc - Diffusion, Agar Dilution.

Introduction

Multi drug resistance (MDR) in bacterial infections has been an ever growing problem worldwide and emergence of resistance to antimicrobial drugs have been increasing in the organisms like Staphylococcus aureus, Enterococcus, Enterobacteriaceae group of organisms, Pseudomonas aeruginosa and Acinetobacter baumannii.^[1] The isolates of MDR organisms reduce the number of active drugs used to treat these infections.^{[2],[3]} Infections by gram negative bacteria is becoming an immense challenge due to incipient of drug resistance among these pathogens, render even the broad spectrum and newer antibiotics resistant.^[1] Extended spectrum of Beta lactamases (ESBL), Amp C beta lactamases, carbapenemases producing gram negative bacteria and MRSA, VRE among gram positive bacteria have emerged as significant therapeutic challenge. The organisms called as “ESKAPE” ie) Enterococcus faecium, S. aureus, Klebsiella pneumoniae, A. baumannii, P. aeruginosa and Enterobacter species are the greatest threat

in the present scenario, as they easily evade from the action of antibiotics.^[4]

Nonetheless, drugs with considerable antimicrobial activity are dearth of in clinical practice since one decade and escalating progression of drug resistance coupled with a diminished antibiotic pipeline has led some to claim that a post-antibiotic era is eminent.^[5] To battle this problem some of the old drugs used in the past like colistin, polymyxin, tenocillin for gram negative bacteria and Fosfomycin for both gram positive and gram negative bacteria is revived. Out of these fosfomycin is phosphonic acid compound discovered in 1969 in Spain.^[6] It inhibits cell wall synthesis by inactivating phosphophenol pyruvate transferase enzyme. It is a broad spectrum and bactericidal antibiotic showing 90% or greater susceptibility to ESBL producing Enterobacteriaceae, CPE and also to MDR P.aeruginosa, MRSA and VRE.^{[7],[8],[9]} A.baumannii is intrinsically resistant to fosfomycin.^[8] There are few studies showing the action of fosfomycin against these commonly

encountered bacteria isolated from the specimens other than urine.^[10] Hence the aim of our study was to determine the susceptibility of fosfomycin against S.aureus (both MSSA & MRSA), ESBL producing Escherichia coli, Klebsiella species and MBL producing Pseudomonas aeruginosa isolated from the specimens other than urine and to evaluate the agreement between the two methods, disk diffusion and agar dilution method performed as per CLSI guidelines.^[11]

Mateiral & Methods

Sample Collection and Identification: First isolates of each species from a patient were included and total isolates tested were (n=250), of which fifty isolates of each species included were Methicillin sensitive S.aureus (MSSA), Methicillin Resistant S.aureus (MRSA), ESBL producing E.coli & Klebsiella species and Metallo beta lactamase producing (MBL) P.aeruginosa were tested. These bacterial species were isolated from various clinical specimens from 2015 to 2016 as follows. MSSA (n= 50 from Pus -40, Blood -8 and Peritoneal fluid -2), MRSA (n=50 from Pus -43, Blood-6, Synovial fluid-1), ESBL E.coli (n=50 from Pus-28, Blood -18, CSF-1, Synovial fluid -1), ESBL Klebsiella species (n=50 from Pus-14, Blood -15,CSF-2, Ascitic fluid - 1, Sputum -18) and MBL Pseudomonas aeruginosa (n=50 from Pus - 48 and Sputum - 2). Urinary isolates were not tested in our study. All the isolates were identified by conventional methods. MRSA was detected by using Cefoxitin 30mg disc, ESBL by double disc synergy test and MBL by Imipenem - EDTA disk method were carried out.^{[12][13]}

Antimicrobial Testing: The antimicrobial susceptibility testing to fosfomycin was determined concomitantly by the disk diffusion and agar dilution methods described by CLSI guidelines.^[11] For disk diffusion method, Mueller hinton agar with fosfomycin disk 200mg containing 50mg of G_6PO_4 was used. For agar dilution method, Mueller Hinton agar (Himedia) supplemented with 25 mg/ml of Glucose - 6 PO_4 was used. The MIC is defined as the lowest concentration of drug that inhibits visible growth of the organism. Control strains were included viz; S.aureus ATCC 29213, E. coli ATCC 25922 and P.aeruginosa ATCC 27853. (Himedia) Interpretative criteria for disk diffusion was based on CLSI breakpoints given for urinary isolates of E. coli and Enterococcus faecalis, whereas for agar dilution method both CLSI and EUCAST criteria were followed. EUCAST criteria are given for Enterobacteriaceae, Pseudomonas and Staphylococcus species. (Table 1)^[14]

Data Analysis: All the findings were recorded and a susceptibility pattern of the disk diffusion method was

compared with agar dilution method as the reference method. The results were analysed statistically using SPSS version 20. Agreement and discrepancies between the evaluated and reference methods are classified as Very major errors (VME), Major errors (ME), and minor errors (Mi). Value for the kappa coefficient, which gives measure of the percentage of agreement between the categorical results of susceptibility testing methods, were interpreted according to the classification by Landis and Koch. (Table 2)^[15]

Results

The susceptibility pattern of fosfomycin to the different isolates Viz: Staphylococcus aureus (both MSSA & MRSA), ESBL producing Escherichia coli, Klebsiella species and MBL producing Pseudomonas aeruginosa are given in the (Table 3)

Susceptibility Pattern of Fosfomycin Against S.aureus:

Both MSSA 50/50 (100%) and MRSA 50/50 (100%) were showing susceptibility to fosfomycin 200µg/disc and by agar dilution method. As zone diameter breakpoint (mm) is not available for S.aureus, the results were interpreted as per CLSI criteria given for E.coli and Enterococcus faecalis. The MIC value was interpreted according to the EUCAST criteria. All the isolates were having MIC value $\leq 32\mu\text{g/ml}$. (Table 3)

Susceptibility Pattern for Gram Negative Bacteria

ESBL producing E. coli: All the isolates 50/50 (100%) were susceptible to both the fosfomycin 200µg/disc and agar dilution method as per CLSI criteria (Table 3)

ESBL Producing Klebsiella Species: The isolates 44/50 (88%) were susceptible to fosfomycin 200 µg/disc and in agar dilution, 40/50 (80%) were sensitive with MIC value $\leq 64 \mu\text{g/ml}$ and 10/50 (20%) were resistant ($>64\mu\text{g/ml}$) as per CLSI guidelines and following EUCAST criteria 36/50 (72%) isolates were susceptible and 14/50 (28%) were resistant ($\geq 32\mu\text{g/ml}$). (Table 3)

MBL Producing Pseudomonas Aeruginosa: The isolates 45/50 (90%) were susceptible and 5/50 (10%) resistant to fosfomycin 200 µg/disc and in agar dilution 30/50 (60%) ($\leq 32\mu\text{g/ml}$) were susceptible and 20/50 (40%) resistant as per EUCAST criteria. But according to CLSI guidelines given for E. coli, the susceptibility of 70%, ($\leq 64\mu\text{g/ml}$), 10% Intermediate ($128\mu\text{g/ml}$) and resistant 20% (≥ 256) was observed.

Comparison of Susceptibility Tests: The comparison of disk diffusion with gold standard agar dilution method was carried out. The categorical agreement was 100% for Staphylococcus aureus (MSSA & MRSA) and ESBL

producing E. coli. No errors were found. Whereas for ESBL producing Klebsiella, the VME of 40% and 39.2% according to CLSI and EUCAST criteria respectively was perceived. No major and minor errors were observed. The kappa value was 0.70 and 0.51 as per CLSI and EUCAST guidelines which indicate good and moderate agreement respectively. (Table 4 & 5)

In case of MBL producing Pseudomonas aeruginosa, the very major error of 50% and 75% was observed following CLSI and EUCAST criteria correspondingly. No major error was found. Minor error (10%) was observed only by CLSI criteria. The kappa value was 0.412 and 0.286 according to CLSI and EUCAST which indicates moderate and fair agreement respectively. (Table 4 & 5)

Table 1: Interpretive criterion of fosfomycin recommended by CLSI [11] & EUCAST [14]

Standard and Organism	MIC (µg/ml)			Zone diameter breakpoint (mm) for the following interpretive criteria		
	S	I	R	S	I	R
CLSI - (Urinary tract isolate only)	≤64	128	≥ 256	≥ 16	13 - 15	≤ 12
Escherichia coli	≤ 64	128	≥ 256	≥ 16	13 - 15	≤ 12
Enterococcus faecalis	≤ 64	128	≥ 256	≥ 16	13 - 15	≤ 12
EUCAST						
Enterobacteriaceae i.v ^a	≤ 32	-	> 32	NA ^b	NA	NA
Enterobacteriaceae (Fosfomycin trometamol, uncomplicated UTI only)	≤ 32	-	> 32	NA	NA	NA
Pseudomonas species i.v ^d	≤ 32	-	> 32	NA	NA	NA
Staphylococcus species	≤ 32	-	> 32	NA	NA	NA

^a i.v., intravenous, ^bNA, not available, ^cUTI, urinary tract infection; ^d Intravenous fosfomycin may be used in combination with other antibiotics to treat P. aeruginosa infections. S – Sensitive, I – Intermediate & R - Resistant

Table 2: Kappa coefficient - by Landis & Koch^[15].

Kappa value	Strength of agreement
< 0.2	Poor
> 0.2 - < 0.4	Fair
> 0.4 - < 0.6	Moderate
> 0.6 - < 0.8	Good
> 0.8 - < 1	Very good

Table 3: Interpretation of Fosfomycin MIC by CLSI and EUCAST criteria

Organism Tested	Disk Diffusion			Agar dilution-CLSI			Agar dilution - EUCAST	
	S 16	I (13- 15)	R 12	64	128	256	32	> 32
MRSA (n=50)	50/50 (100%)	0	0	50/50 (100%)	0	0	50/50 (100%)	0
MSSA (n= 50)	50/50 (100%)	0	0	50/50 (100%)	0	0	50/50 (100%)	0
ESBL E.coli (n=50)	50/50 (100%)	0	0	50/50 (100%)	0	0	50/50 (100%)	0
ESBL Klebsiella (n=50)	44/50 (88%)	0	6/50 (12%)	40/50 (80%)	0	10/50 (20%)	36/50 (72%)	14/50 (28%)
MBL P. aeruginosa (n=50)	45/50 (90%)	0	5/50 (10%)	35/50 (70%)	5/50 (10%)	10/50 (20%)	30/50 (60%)	20/50 (40%)

Table 4: Correlation of Disk Diffusion method with reference Agar dilution method.

	CLSI (%)			EUCAST (%)		
	VM ^a	M ^b	Mi ^c	VM	M	Mi
ESBL- klebsiella spp	4/10 (40%)	0	0	8/14 (39.2%)	0	0
MBL-Pseudomonas aeruginosa	5/10 50%	0	5/50 10%	15/20 75%	0	0

a - very major error, b-Major error, c- Minor error

Table 5: Kappa value - Comparison of Disk diffusion with agar dilution method.

	Kappa value	Asymp Std error ^a	Approx T ^b	Approx sig
Measure of Agreement (kappa) ESBL Klebsiella species CLSI criteria	0.706	0.135	5.222	0.000
EUCAST criteria	0.519	0.137	4.187	0.000
MBL – P.aeruginosa CLSI criteria	0.412	0.135	4.187	0.000
EUCAST criteria	0.286	0.108	2.887	0.004
No of valid cases	50	50	50	50

^a not assuming the null hypothesis; ^b using the asymptotic standard error assuming the null hypothesis.

Discussion

One form of fosfomycin is Fosfomycin trometamol which is used in the treatment of uncomplicated urinary tract infections (UTI) as an oral single dose regimen. The other is an intravenous (IV) form of fosfomycin - Fosfomycin di sodium salt, has been used in some European countries and in Japan. Successful outcomes were reported in some studies by using fosfomycin intravenously for infections other than UTI, although fosfomycin has not been approved for conditions other than UTI. [16], [17], [18] Our study of susceptibility pattern of fosfomycin against drug resistant gram positive cocci like MRSA and gram negative bacilli like ESBL producing Escherichia coli and Klebsiella species, MBL Producers Pseudomonas aeruginosa correlates well with many studies.

All our isolates tested were from the specimens other than urine, mainly the pus, blood, body fluids, and sputum. MRSA is known for its tenacious problem in both hospital and community acquired infections.[19] Fosfomycin was found to be effective in the treatment of experimental MRSA osteomyelitis in rats.[20] Our study showed that all the isolates of MRSA tested were 100% susceptibility to fosfomycin both by disc diffusion and agar dilution methods as per CLSI and EUCAST criteria. This is in consistent with the study conducted by Falgas etal, where 129 of 130 (99.2%) of MRSA isolates were susceptible to fosfomycin. [21] Even study by Lu CL etal observed that MSSA with 100% susceptibility like our observation and MRSA 89% of susceptibility both by CLSI and EUCAST criteria. [22] But it differs from the study by Oksuz etal reported high fosfomycin resistance (58%) in isolates of a ST 239 - MRSA - III clone. [23]

The effect of fosfomycin against ESBL producing Enterobacteriaceae particularly E. coli and Klebsiella species were studied. In our study E. coli isolates were susceptible to fosfomycin 200µg/disc (100%) and also by agar dilution methods as per CLSI and EUCAST criteria. Studies conducted by Adil karadag etal,[18] Pullukcu H etal, [24] Endimiani etal,[25]Tharavichitkul etal [26] showed

97.5%, 96.5%, 99.4% and 97.3% respectively. All these studies support our findings and also fosfomycin has high susceptibility against carbapenem resistant E. coli (95.1%). Hence fosfomycin can be considered as an alternate drug for ESBL and Carbapenemase producing E. coli. [27], [28]

In our study susceptibility of klebsiella species - ESBL producer to fosfomycin is 88% by DD method and 80% & 70% by AD method following CLSI and EUCAST criteria respectively. This is in par with the study observed by Tharavichitkul etal [26] but the study carried out by Endimiani etal [25] was showing only 63.2% of susceptibility to carbapenemase (KPC) producing Klebsiella pneumoniae, which was not tested in our study.

Moreover, study by Chitra etal [27] in which Klebsiella species were showing 64.2% susceptibility by applying CLSI breakpoints and only 36% by EUCAST break point criteria. Also they stated that klebsiella species isolated from blood and sterile body fluids showed increased resistance compared to urine isolates, while E. coli were unvaryingly susceptible in all isolates. This comparison could not observe in our study as we have done in isolates other than urine. Perdigao etal [29] in his studies reported in the same way that similar isolates tested by E test showed 85% and 48% susceptibility by CLSI & EUCAST respectively.

The susceptibility pattern of fosfomycin against Pseudomonas aeruginosa were 70% and 60% by AD method according to CLSI and EUCAST criteria respectively, whereas 90% by DD method by applying criteria given for E. coli of urinary isolates in CLSI. Most of the studies showed less susceptibility of fosfomycin to Pseudomonas aeruginosa.

The comparison of disc diffusion method with agar dilution was carried out. The categorical agreement between two methods was 100% for S.aureus (both MSSA & MRSA) and ESBL producing E.coli but errors were observed in between these two methods for Klebsiella species and Pseudomonas aeruginosa. In Klebsiella species, the very

major error was 40% and 39.2% by CLSI & EUCAST guidelines respectively. The kappa value was 0.706 and 0.519 in accordance with CLSI and EUCAST guidelines which showed good to moderate agreement. Our findings are different from the study conducted by M de cueto et al^[10] compared the DD method with AD method in which VME 0.7%, Major error 10.9% and Minor error 3.6% was observed and study by Perdigao – Neto et al^[29] perceived very major Error of 4% only, which shows less error rate when compared to our study. To consider a susceptibility test adequate, CLSI recommends that if it obtains < 10% Minor error, < 3% major error and 1.5% VME.^[11] Also in our study disk diffusion method reporting greater susceptibility to ESBL - Klebsiella species than agar dilution method whereas study by M de cueto et al^[10] reported greater resistance. As our study showed VME of 40% for Klebsiella species which concludes that disc diffusion is not satisfactory to study the susceptibility pattern. No breakpoints were given for klebsiella species in CLSI and EUCAST guidelines. The study was done by using the criteria given for E. coli from urinary isolates in CLSI and Enterobacteriaceae in EUCAST. In our study ESBL E. coli and MRSA had significantly lower fosfomycin MICs than klebsiella species which is in accord to previous studies.^{[28][30]}

For *Pseudomonas aeruginosa* the VME was 50% and 75% as per CLSI and EUCAST criteria respectively and kappa coefficient was 0.412 and 0.286 as per CLSI & EUCAST guidelines. Our study is in accordance with the study by Perdigao – Neto et al^[29] where VME was 80% and 100% following CLSI and EUCAST respectively. Hence disc diffusion is not adequate to test the susceptibility pattern.

Conclusion

The existing evidences and our study concluded that fosfomycin has a high level of antimicrobial activity against isolates with high level of resistance to antimicrobial drugs such as MRSA, ESBL producing E. coli, Klebsiella species and MBL producing *Pseudomonas aeruginosa*. Our study has limitations as we tested only for fifty isolates each and clinical outcome following treatment is also not known. The feasible method to know the susceptibility is Disk diffusion method which shows good agreement for S.aureus and E.coli whereas only moderate agreement for Klebsiella species and very poor for *Pseudomonas aeruginosa*. The error rates were also high for these two isolates. To consider a susceptibility test adequate CLSI recommends that if it obtains < 10% Minor error, < 3% major error and 1.5% VME. Therefore, need further studies to establish the breakpoint criteria and to know the clinical outcomes. Hence fosfomycin can be considered as an

alternate drug for treatment of infections with multi drug resistant bacteria, not only for the UTI but for systemic infections also. This is achievable with the establishment of breakpoint values and zone diameter for all the common isolates.

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