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Anti-Microbial Susceptibility Pattern of Fosfomycin in Various Clinical Isolates

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Abstract

To identify the susceptibility pattern of Fosfomycin in various clinical isolates by estimating the frequency in terms of percentages. A convenient sampling technique was adopted for study proceedings. Total 748(n) specimens for culture and sensitivity were received in the microbiology section of pathology department**.** Out of these 748(n) specimens, positive cultures were seen in 144(n). For culture and sensitivity proceedings, the recommended CLSI – 2014 (clinical and laboratory standard institute) guidelines were followed. The bacterial isolation was done by biochemical tests. The zone diameter of ≥16 mm for 50µgm fosfomycin disc was considered as sensitive zone. While ≤15-12mm was considered as intermittent one and <12mm was the resistant zone. Data was recorded and analyzed by using SPSS version 20 for statistical inference. For numerical variables frequencies were calculated in terms of percentages. Seventy six 76.06% (n=143) gram negative and 68.42 % (n=13) gram positive organisms were sensitive to Fosfomycin. The efficacy of fosfomycin is more for gram negative (76%) as compared to gram positive organisms (68%).

1 Introduction

Fosfomycin belongs to the group of phosphonic acid derivatives. It is available in tablets and sachets preparation. The sachets have been given a license by food & drug administration of United States for the management of complicated and uncomplicated UTIs[1]. The drug harbors a wide spectrum against many gram positive and gram negative bacteria[2]. Moreover, various multi drug resistant pathogens especially the carbapenam resistant *Pseudomonas aeruginosa*, *Klebsiella pneumonia,* extended-spectrum β-lactamase (ESBL) producing bacteria and the vancomycin resistant enterococci (VRE)[3, 4].

Many researches are available regarding the importance of fosfomycin usage in urinary tract infection[5]. However, the studies on its efficacy for the isolates of other specimens like pus, stool, high vaginal swabs and blood are deficient.

In view of all this, current study was designed to identify the sensitivity pattern of fosfomycin for both gram positive and gram negative isolates in various specimens.

2 Materials and Methods

The study was done at Microbiology (Pathology) Department of Al Nafees Medical College & Hospital, Islamabad, Pakistan. The study was carried out over the period of two years i.e 01st Oct 2015 to 20th Jan 2017. For ethical considerations informed consent was taken from all the enrolled participants.

A Convenient sampling technique was adopted for this study. All the indoor and outdoor specimens received for culture and sensitivity were included in the study. While the samples sent in wrong containers, dry swabs, or delay in specimen sending to laboratory (without preservatives) were excluded from the study.

The CLSI-2014 guidelines were followed for microbiological sample processing. Three days proceedings were done for the processing of urine, high vaginal swabs (HVS), pus, stool, and

sputum specimens. While Blood cultures were followed for 07 days.

CLED (cysteine lysine electrolyte deficient media) was used for urine cultures. While for rest of all specimens Macconkey's agar and Blood agar were used. Nutrient agar was used for obtaining the drug susceptibility of fosfomycin.

The first day inoculation for pus, blood, sputum, HVS, and stool specimens were done on Macconkey's and Blood agar. CLED agar was used for first day inoculation of urine specimens. Bacteriuric strips were used for the purpose and to count the number of bacteria per ml. The inoculated plates were incubated at 37^0C for 24 hours. The remaining urine specimens were then transferred to the disposable test tubes for centrifugation at 3000rpm/05minutes. The number of pus cells were identified by direct microscopy of deposits and correlated with bacteriuria afterwards.

On second day the morphology of colonies were assessed. Further identification of bacteria was done by gram staining and biochemical tests. The fosfomycin with a disc potency of 50µgm was used to detect the susceptibility pattern on Nutrient agar on same day.

On third day, interpretation of biochemical tests and drug susceptibility were done as per recommended CLSI guidelines. According to which, fosfomycin disc with a clearing zone of $\geq$16mm was considered sensitive. A zone diameter of $\geq$12-15mm was considered as intermittently sensitive and <12 mm was considered resistant.

All the blood cultures were processed accordingly (recommended protocols) uptil 07 days.

Frequencies and percentages were the numerical variables extracted by using the SPSS version 20. Mean zone diameters along with standard deviation were assessed as well.

3 Results

Total 748 (n) specimens received in microbiology section of pathology department from 01st Oct 2015 to 20th Jan 2017. Out of which 207(n) yielded significant growth. The positive urine cultures were seen in 70.04% (n=145) cases, followed by pus i.e 12.5% (n=26), high vaginal swabs (HVS) i.e 7.72% (n=16), sputum i.e 4.83% (n=10), blood i.e 2.8% (n=6), and lastly the stool i.e 1.9% (n=04). This is shown in table 1.

In case of positive urine cultures 70.04 (n=145), *Escherichia coli* was seen in 57.9% (n=84), followed by *Klebsiella pneumoniae* i.e 14.4 % (n=21), *Pseudomonas aeruginosa* i.e 11% (n=16), *Satphylococcus saprophyticus* i.e 5.5 (n=08), *Proteus vulgaris* i.e 04% (n=06), *Morganella marganii* i.e 3.44% (n=05), *Serratia marcecens* i..e 2.75%(n=04), and Citrobacter freundii i.e 0.68% (n=01). This is shown in table - 2.

Table 1: Distribution of positive cultures (n=207)

Specimens	Total Number of Positive Cultures (N=207)	
	(n)	(%)
Urine	145	70.04
Pus	26	12.5
High vaginal swabs (HVS)	16	7.72
Sputum	10	4.83
Blood	06	2.89
Stool	04	1.93
Total	207	99.91

In nineteen 12.5% (n=26) positive pus specimens, *Staphylococcus aureus* was seen in 38.46% (n=10), followed by *Pseudomonas aeruginosa* i.e 19.2% (n=05), *Klebsiella pneumonia* i.e 15.3%(n=04), *Proteus vulgaris* & *Escherichia coli* i.e 11.53% (n=03), each. While Morganella morganii was seen in only 3.84% (n=01).

In sixteen 7.7% (n=16) positive HVS specimens, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* were seen in 25% (n=04) each. Next in sequence were *Proteus vulgaris* 18.7% (n=03) and *Streptococcus agalactiae* in 6.2% (n=01).

Amongst nine positive sputum specimens 4.83% (n=10), *Klebsiella pneumoniae* was present in 50% % (n=05). It was followed by *Morganella morganii* i.e 30% (n=03), and *Moraxella catarrhalis* in 20% (n=02).

Amongst six positive blood cultures 2.83% (n=06), *Salmonella typhi* was seen in 100% (n=06) cases.

In case of four positive stool cultures 1.9% (n=04), *Escherichia coli* was present in 100% (n=04) specimens. This is shown in table 2.

The total distribution revealed that 90.8% (n=188) isolates were gram negative, while 17% (n=19) were gram positive. The mean zone diameter in mm (SD) for gram negative organisms was 17.34$\pm$1.03. However for gram positive organisms it was 17.6$\pm$1.01. This is shown in table 3.

Amongst the gram negative organisms, 76.06% (n=143) were sensitive to fosfomycin. While 68.42% (n=13) gram positive organisms were sensitive to fosfomycin..

For gram negative organisms highest sensitivityof 100% was seen for *Citrobacter freundii* and *Pseudomonas aeruginosa.* Next in sequence was *Salmonella typhi* (83.3%), *Escherichia*

coli (67.5%), *Klebsiella pneumonia* (55.8%), *Morganella morganii* (55.5%), *Moraxella catarrhalis* (50%), *Proteus vulgaris* (41.6%), and *Serratia marcescens* (25%) respectively.

Table 2: Frequencies of bacteria in various isolates (N= 207)

Organisms	Urine		Pus		HVS		Sputum		Blood		Stool	
	n = 145	%	n=26	%	n=16	%	n=10	%	n= 06	%	n=04	%
E. coli	84	57.93	03	11.53	04	25	-	-	-	-	04	100
K. pneumoniae	21	14.48	04	15.38	04	25	05	50	-	-	-	-
P. aeruginosa	16	11.03	05	19.23	04	25	-	-	-	-	-	-
S. saprophyticus	08	5.51	-	-	-	-	-	-	-	-	-	-
P. vulgaris	06	4.13	03	11.53	03	18.75	-	-	-	-	-	-
S. marcecens	04	2.75	-	-	-	-	-	-	-		-	-
M. morganii	05	3.44	01	3.84	-	-	03	30	-	-	-	-
S. aureus	-	-	10	38.46	-	-	-	-	-	-	-	-
S. typhi	-	-	-	-	-	-	-	-	06	100	-	-
M. catarrhalis	-	-	-	-	-	-	02	20	-	-	-	-
C. freundii	01	0.68	-	-	-	-	-	-	-	-	-	-
S. agalactiae	-	-	-	-	01	6.25	-	-	-	-	-	-

For gram positive isolates highest sensitivity was seen i.e 100% for each, *Staphylococcus saprophyticus* and *Streptococcus agalactiae*. This was followed by *Staphylococcus aureus* (40%) including the methicillin resistant *Staphylococcus aureus* (MRSA). This is shown in table 3.

4 Discussions

Literature review highlights the facts that there is increased incidence of drug resistance cases like the methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus* (VRSA) or extended spectrum beta lactamases (ESBL). The reported incidence of such cases in Japan, Korea and China is about 60%. In Europe it is 35%[6, 7]. While in Pakistan it is 36.1%. Hence, the management of simple infections is becoming challenging for the health care professionals globally[8]. *Falagas ME* etal in 2010 described that besides treatment for urinary tract infection, this drug can be used for the management of systemic infections as well[9].

As mentioned in the results of current study, a wide spectrum of susceptibility was observed for fosfomycin for various isolates in different specimens. It was assessed from the current study results that, 76.06% (n=143) gram negative organisms, and 68.42% (n=13) gram positive organisms. This is in favor of two studies conducted by *Falagas ME* etal in 2008 & 2010. He narrated that fosfomycin harbors good efficacy against various gram positive cocci like the *Staphylococcus aureus* and *Enterococcus faecalis.* While for gram negative organisms like *Enterobacteriaceae* family members and *Pseudomonas aeruginosa*, it has yielded good results[10, 11].

Amongst the gram negative isolates highest sensitivity was seen for *Pseudomonas aeruginosa* (100%), *Citrobacter freundii* (100%), *Salmonella typhi* (83.3%), *Escherichia coli* (67.5%), *Klebsiella pneumonia* (55.8%), and *Morganella morganii* (55.5%). This is different from the study results by Samonis etal (2010). He narrated in his published research that almost all *Escherichia coli* are susceptible to fosfomycin. However, all *Salmonella typi* are found susceptible, just like the results of our study. Considerable susceptibility was observed for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter.* This supports the findings of current study[12].

Regarding the susceptibility pattern of gram positive isolates, highest sensitivity was seen i.e 100% for each, *Staphylococcus saprophyticus* and *Streptococcus agalactiae*. It was followed by *Staphylococcus aureus* (40%). His is different from the study results by Falagas etal (2010). He concluded that highest efficacy amongst gram positive organisms is for *Staphylococcus aureus* (MSSA), followed by penicillin-resistant *Streptococcus pneumonia, MRSA, Enterococci and* lastly for *vancomycin resistant enterococci* (VRE)[13]. Maviglia R etal (2009) also supports the finding of current study that fosfomycin is amongst promising management options for *Staphylococcus saprophyticus* and *enterococci*[14]. Samonis etal (2010) described that the efficacy of drug for *Streptococcus agalactiae* is there but to lesser extent as compared to other gram positive

organisms. This is a different finding form current study results[12].

The mean zone diameter for gram negative isolates is 17.34±1.03. While for gram positive isolates, it was 17.6±1.01. The literature review is deficient for justification of specific finding of current study.

Table 3: Susceptbility pattern of fosfomycin (N=207)

Organisms	Total		Sensitive		Mean zone diameters	
	N= 207	%	n	%	Mean (mm)	SD
	Gram negative organisms		**90.8% (n=188)**			
Escherichia coli	120	57.97	81	67.5	17.34	1.0.3
Klebsiella pneumonia	34	18.08	19	55.88		
Pseudomonas aeruginosa	25	13.29	25	100		
Proteus vulgaris	12	6.38	05	41.66		
Morganella morganii	09	4.78	05	55.5		
Salmonella typhi	06	3.19	05	83.3		
Serratia marcescens	04	2.12	01	25		
Moraxella catarrhalis	02	1.06	01	50		
Citrobacter freundii	01	0.53	01	100		
Total			143	76.06		
	Gram positive organisms		**9. 17% (n=19)**			
Staphylococcus aureus	10	4.83	4	40	17.6	1.01
Staphylococcus saprophyticus	08	3..86	08	100		
Streptococcus agalactiae	01	0.48	1	100		
Total			13	68.42		

The susceptibility pattern of fosfomycin extracted from current study will be a guide for initiating the prophylactic management decisions in various clinical sittings.

5 Conclusion

Besides the urine isolates, wide spectrum of susceptibility is observed for fosfomycin. The efficacy of fosfomycin is more for gram negative 76% (n=143) as compared to gram positive organisms 68.4% (n=13).

6 Recommendations

- Fosfomycin can be used for the management of either gram negative or gram positive severe infections.
- The studies with larger sample size are required to assess the efficacy of fosfomycin.
- Fosfomycin can be considered as a good option for the management infections like methicillin resistant *Staphylococcus aureus* (MRSA) or extended spectrum beta lactamases (**ESBL)**

7 Limitations of study

- Small sample size
- Anaerobic culture and sensitivity not performed
- Study is conducted in one setting only

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9 Conflicts of Interests

There are no conflicts of interests regarding publication of this manuscript in UKJPB

10 Author's contributions

HZ: Provoking the idea of study, corresponding author, Abstract, Methodology & Result writing, along with final formatting of entire manuscript.

NN: Data gathering and analysis

KTB: Introduction writing, Summarizing the tables for Results, Collection of latest references for discussion.

SH: Data gathering and analysis

NKL: Supervising all laboratory technicalities and final proof reading of manuscript.

11 References

1. Falagas ME. Antimicrobial susceptibility of multidrug-resistant Gram negative bacteria to fosfomycin. Eur. J. Clin. Microbiol. Infect. Dis. 2008; 27(1):439–443.
2. Maraki S.. Susceptibility of urinary tract bacteria to fosfomycin. Antimicrob. Agents Chemother. 2009; 53(3):4508–4510.
3. Endimiani A. In vitro activity of fosfomycin against blaKPC-containing Klebsiella pneumoniae isolates, including those nonsusceptible to tigecycline and/or colistin. Antimicrob. Agents Chemother.2010; 54 (3):526–529.
4. Shrestha NK. Antimicrobial susceptibility of vancomycin-resistant Enterococcus faecium: potential utility of fosfomycin. Scand J Infect Dis. 2003; 35(4):12–14.
5. Elizabeth A, Sekeers NJ, Hall GS, Duin DV. Experience with Fosfomycin for Treatment of Urinary Tract Infections Due to Multidrug-Resistant Organisms Antimicrob Agents Chemother. 2012; 56(11): 5744–5748.
6. Kim HB, Park WB, Lee KD, Choi YJ, Park SW. Nationwide surveillance for Staphylococcus aureus with reduced susceptibility to vancomycin in Korea. J Clin Microbiol. 2003; 41(6): 2279–2281.
7. Bell JM, Turnidge JD. High prevalence of oxacillin-resistant Staphylococcus aureus isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY antimicrobial surveillance program, 1998–1999. Antimicrob Agents Chemother.2002; 46(3): 879–881.
8. Ullah A, Qasim M, Rahman H, Khan J, Haroon M, Muhammad N. High frequency of methicillin-resistant Staphylococcus aureus in Peshawar Region of Pakistan. Springerplus. 2016; 5 (11): 600-601.
9. Falagas ME, Kastoris AC, Kapaskelis AM. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum β-lactamase producing, Enterobacteriaceae infections: a systematic review. Lancet Infect Dis 2010;10(2):43-50.
10. Falagas ME, Kanellopoulou MD, Karageorgopoulos DE. Antimicrobial susceptibility of multidrug-resistant Gram negative bacteria to fosfomycin. Eur J Clin Microbiol Infect Dis 2008;27(8):439-43.
11. Falagas ME, Maraki S, Karageorgopoulos DE. Antimicrobial susceptibility of Gram-positive non-urinary isolates to fosfomycin. Int J Antimicrob Agents 2010;35(10):497-499.
12. Samonis G, Maraki S, Rafailidid PI, Kapaskelis A, Kastoris AC, Falagas ME. Antimicrobial susceptibility of Gram-negative nonurinary bacteria to fosfomycin and other antimicrobials. J Antimicrob. 2010; 5(6): 961-970.
13. Falagas ME, Vouloumanou EK, Togias AG, Karadima M, Kapaskelis AM, Rafailidis PI, et al. Fosfomycin versus other antibiotics for the treatment of cystitis: a meta-analysis of randomized controlled trials. J Antimicrob Chemother. 2010;65(4):1862–77.
14. Maviglia R, Nestorini R, Pennisi MA. Role of old antibiotics in multidrug resistant bacterial infections. Curr Drug Targets. 2009;10(1):895–905