Original Research Article

Fosfomycin an Alternative for the Treatment of Methicillin Resistant Staphylococcus aureus

Abstract

Introduction

Fosfomycin an antibiotic having unique chemical structure possess broad-spectrum activity against numerous pathogenic organisms including both gram negative and gram positive bacteria including multi-resistant strains. In early 1970 this antibiotic was accepted in clinical practice, but for several years the use of fosfomycin was limited for treating uncomplicated lower urinary tract infections. However, fosfomycin achieves clinically relative concentrations in serum, cerebrospinal fluid, other body fluids, lung, kidney, bladder wall, prostrate, heart valve tissues, other inflamed tissues, abscess, and bone as well and has shown the good activity in treating severe infections caused by multi-resistant pathogens at various body sites. The objective of the study was to evaluate fosfomycin as an alternative treatment against methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital.

Materials and methods

The Prospective Descriptive cross-sectional study was conducted at the Department of Clinical Microbiology Laboratory at the Sindh Institute of Urology and Transplantation (SIUT) Hospital, Karachi, June 2017 till January 2018. A total of 147 specimens were collected from various body sites include blood, fluids like pleural fluid, synovial fluids, broncho-alveolar lavage, urine, pus, and tissues were identified to genus level by a routine biochemical test. Antimicrobial sensitivity was determined by Kirby-Bauer disk diffusion method. All fosfomycin susceptible isolates *i.e.* Zone size of ≥16 mm and resistant isolates as zone size <16 mm were evaluated.

Results

Out of 147 isolates 113 (76.9%) isolates were from blood, 12 (8.2%) were from other body fluids, 14((5%) were from pus and 8 (4%) from Urine. All 147 isolates were resistant to methicillin. Out of 147 isolates of methicillin-resistant *Staphylococcus aureus* 143 (97.3%) were sensitive to fosfomycin and only 4 (2.7%) were found to be resistant.

Conclusion

Comment [DM1]: Write abbreviation as MRSA, once the full-form is provided

Fosfomycin proved to be a very good alternative for treating methicillin-resistant *Staphylococcus aureus* because of good activity against this pathogen as well as good penetration of fosfomycin in serum, tissues, cerebrospinal fluid, and other body fluids make this drug effective in treating infections at various body sites.

Keywords

Fosfomycin, methicillin-resistant *Staphylococcus aureus* MRSA, minimum inhibitory concentration MICs, vancomycin resistant enterococci VRE

Introduction

Fosfomycin is a bactericidal drug having broad-spectrum activity against both gram-negative and gram-positive bacteria. Fosfomycin act by inhibiting synthesis of peptidoglycan by blocking formation of N-acetyl muramic acid and therefore also effective in treating multi resistant strain of organisms like methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptides intermediate *Staphylococcus aureus* (GISA) and vancomycin-resistant Enterococci (VRE). Years back, gram-positive bacteria particularly methicillin-resistant *Staphylococcus aureus* (MRSA) posed concern for clinical microbiologist [1]. Methicillin-resistant *Staphylococcus aureus* infections have been increasing over the years. In Pakistan, the prevalence of MRSA increased from 5% in 1989 to up to 51% in recent years [2]. Methicillin-resistant *Staphylococcus aureus* has shown resistance to multiple antibiotics such as gentamicin, fusidic acid, norfloxacin, clindamycin, and erythromycin [3].

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates causes both nosocomial as well as community-acquired infections leading to bacteremia, septic arthritis, prosthetic joint infections, artificial graft infections and infective endocarditis causing significant morbidity and mortality [4]. Vancomycin has been remained the drug of choice of treatment but has shown poor efficacy in recent years leading to increased MIC ranged from 0.25 mg/L to 2.0 mg/L thus making difficult to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Furthermore, increasing methicillin-resistant *Staphylococcus aureus* (MRSA) infections and limited treatment options available including glycopeptides, oxazolidinones, lipopepdides and fifth-generation cephalosporin's such as ceftaroline and ceftobiprole [5]. Therefore, to overcome this problem several combination regimes have been proposed [4]. Besides this, fosfomycin is indicated as a single dose in women for treating the uncomplicated urinary tract infections due to other pathogens like *Escherichia coli* and *Enterococcus fecalis* [6].

Comment [DM2]: Drug name is always written in small alphabets

Fosfomycin combination regime has shown effective response in treating various methicillin-resistant *Staphylococcus aureus* MRSA infections including biofilm-associated methicillin-resistant *Staphylococcus aureus* MRSA, in venous catheter-related infections [5-7], in bone and soft tissues infections as it achieves good penetration [1] and therefore effective in treating acute and chronic osteomyelitis [8]. Fosfomycin when co-administered with other antibiotics has

shown synergistic effects and has shown reduce nephrotoxic effects associated with aminoglycoside [9]. There is no cross-resistance reported between fosfomycin and other antibacterial agents because of its unique mode of action. Surveillance data have shown a low frequency of resistance to fosfomycin from clinical isolates [5]. It has also shown a useful component for topical preparation for otology purposes [9]. In a study from Thailand, 70% of isolates of methicillin-resistant *Staphylococcus aureus* were sensitive to fosfomycin having MICs ranging from MIC ≥0.38>1024 ug/ml [10].

Therefore, the purpose of this study was to evaluate fosfomycin as an alternative treatment against methicillin-resistant *Staphylococcus aureus* isolates because of cost-effectiveness, is cheaper, available in oral as well injectable form and has shown no nephrotoxic effect compared with other antibiotics like vancomycin for treating methicillin-resistant *Staphylococcus aureus* (MRSA) infection.

Materials and Methods

The Prospective descriptive cross-sectional study was conducted in the Department of Diagnostic Microbiology Laboratory, Sind Institute of Urology and Transplantation (SIUT) from June 2017 till Jan 2018. One hundred and forty-seven consecutive clinical isolates of *Staphylococcus aureus* resistant to methicillin were selected from clinical samples including blood, fluids, urine, sputum, bronchoalveolar lavage, middle ear fluid, nasopharyngeal swab/aspirate, sinus aspirate, pus, and tissue received were included in the study. Age range between 16 to 75 years of either gender was selected. To avoid duplication caution was taken to exclude repeat specimen from the same patient. Non Probability consecutive sampling techniques were used in this study.

Isolates were identified to the genus level by routine biochemical tests. Staphylococcus aureus bacteria was identified by colony morphology, Gram stain appearance, catalase positive and biochemical characteristics. The organisms fulfilling the inclusion criteria isolated from clinical samples were confirmed as *Staphylococcus aureus* by conventional identification methods such as positive catalase which were seen as appearance of bubbles as result of conversion of hydrogen peroxide into water and oxygen, positive tube coagulase which were seen as formation of fibrin clot in a tube containing plasma, positive DNASE test was seen as clear zone on DNA test medium after addition of 1% HCl and mannitol fermentation was seen as change of color from red to yellow on Mannitol Salt agar. Confirmed organisms were saved in 1 ml aliquots containing glycerol-phosphate buffer at -80°C. The organisms were revived on the respective media. A single colony was emulsified in 1 ml of normal saline and was adjusted equivalent to 0.5 McFarland standards and was spread with a sterile cotton swab on the Muller Hinton agar. Staphylococcus aureus was tested for sensitivity to antibiotics like cefoxitin, vancomycin, erythromycin, tetracycline, clindamycin, trimethoprim-sulfamethoxazole, and fosfomycin. The antibiotics were placed on the inoculated plate and were incubated aerobically for 24 hours at 35°C. ATCC *Staphylococcus aureus* 25923 was included as a control strain. Sensitivity/Resistance of antibiotics was evaluated by Kirby Bauer's disk diffusion method according to CLSI (Clinical Laboratory and Standards Institute) guidelines. Resistance and sensitive cases were differentiated by measuring zone diameter around antibiotics. Methicillin-resistant *Staphylococcus aureus* was identified by resistant to cefoxitin 30 ug disc that is zone

size <21mm and the interpretative criteria were established according to Clinical Laboratory and Standards Institute (CLSI) on Muller Hinton agar by Kirby-Bauer disk diffusion method. All fosfomycin susceptible isolates *i.e.* zone size of ≥16mm and resistant isolates as zone size <16 mm were categorized as being sensitive (S) or resistant (R) accordingly [6]. Data was entered and analyzed using SPSS version 19. The fosfomycin was the unit of analysis and each unit like (susceptible & resistant) was considered as an individual statistical analysis. Descriptive statistics was assessed. Shapiro Wilk's test was applied to check the normality of quantitative variable age. Mean ± SD or median (IQR) was computed for age as appropriate. Frequency and percentage was computed for qualitative variables like source of specimen (urine, sputum, wounds swabs, & sterile body fluids). Fosfomycin (susceptible & resistant) antibiotic was calculated. Stratification was done with regards source of specimen to see the impact association on Fosfomycin susceptibility. Chi-square test or Fisher exact test was applied and p-value <0.05 was taken as significance.

Comment [DM3]: A separate section with heading statistical treatment would be better for understanding

Results

Out of 147 isolates received in the Department of Diagnostic Laboratory SIUT from June 2017 till Jan 2018 from different body sites included blood specimens (76.9%), fluids specimens other than blood (8.2%), pus specimens (9.5%) and urine specimens were (5.4%) as shown in (Table 1). Out of 147 specimens, 143 specimens showed 97.3% susceptibility to fosfomycin and only 2.7% were resistant as shown in (Table 2). Fosfomycin was found to be sensitive 74.1% from blood specimens, 8.2% from fluid, 9.5% from pus and 5.4% from urine (p value 0.744) as shown in (Table 3). Besides fosfomycin other antibiotics like vancomycin, fusidic acid, trimethoprim sulfamethoxazole, clindamycin, erythromycin, tetracycline, and ciprofloxacin were also tested against methicillin-resistant *Staphylococcus aureus* which constitute 99.3% susceptibility and 0.7% resistance to vancomycin, fusidic acid showed 88.4% of sensitivity and 11.6% resistant, Trimethoprim sulfamethoxazole 85% sensitive and 15% resistant, clindamycin 68.7% of sensitivity and 31.3 % resistant, erythromycin 59.9% sensitive and 40.1% resistant, tetracycline 48.3% sensitivity and 51.7% resistant, ciprofloxacin 34.0% sensitivity and 66.0% resistant as shown in (Table 4).

Table 1: CLASSIFICATION OF SOURCE OF SPECIMEN (n=147)

Comment [DM4]: Standardization is required

Source of Specimen	Frequency (f)	Percentages (%)
Blood	113	76.9%
Fluid	12	8.2%
Pus	14	9.5%
Urine	8	5.4%
Total	147	100%

Table 2: CLASSIFICATION OF FOSFOMYCIN (FOT) (n=147)

Fosfomycin	Frequency (f)	Percentages (%)
Sensitive	143	97.3%
Resistant	04	2.7%

Total	147	100%

Table 3: COMPARISON ASSOCIATION OF FOSFOMYCIN WITH SOURCE OF SPECIMEN DISTRIBUTION (n=147)

P-VALUE=0.744

Source of Specimen	Fosfomycin		P-Value
	Sensitive	Resistant	
Blood	109(74.1%)	4(2.7%)	113(76.9%)
Fluid	12(8.2%)	0(0%)	12(8.2%)
Pus	14(9.5%)	0(0%)	14(9.5%)
Urine	8(5.4%)	0(0%)	8(5.4%)
Total	143(97.3%)	4(2.7%)	147(100%)

Table 4: DIFFERENT CLASSIFICATION OF DRUGS RESISTANCE (n=147)

DRUGS RESISTANCE	FREQUENCY	PERCENTAGES
Fusidic acid (FD)	Sensitive 130	88.4%
	Resistant 17	11.6%
Clindamycin (DA)	Sensitive 101	68.7%
	Resistant 46	31.3%
Ciprofloxacin (CIP)	Sensitive 50	34%
	Resistant 97	66%
Erythromycin (E)	Sensitive 88	59.9%
	Resistant 59	40.1%
Vancomycin (VA)	Sensitive 146	99.3%
	Resistant 01	0.7%
Trimethoprim sulfamethoxazole (SXT)	Sensitive 125	85%
	Resistant 22	15%
Tetracycline (TET)	Sensitive 71	48.3%
	Resistant 76	51.7%

Discussion

Methicillin-resistant *Staphylococcus aureus* has become the leading cause of hospital-acquired infections all around the world. The emergence of methicillin-resistant *Staphylococcus aureus* has also been seen in developing countries like Pakistan. Previous data showed a variable prevalence of methicillin-resistant *Staphylococcus aureus* obtained from different cities like 61% in Lahore, 57% in Karachi, 54% in Peshawar, and 46% in Rawalpindi [3]. The increased emergence of methicillin-resistant *Staphylococcus aureus* isolates with the passage of time may be due to the transfer of resistant genes between bacterial cells due to the persistence of bacteria in hospital environment resulting in antibiotic resistance [3]. In our study, the susceptibility of fosfomycin was found to be 97.3% and 2.7% were resistant to fosfomycin against methicillin-resistant *Staphylococcus aureus*. One of the study conducted in Thailand in 1999 showed 96% of fosfomycin sensitivity against methicillin-resistant *Staphylococcus aureus*. Similar studies were conducted in Thailand by routine disc susceptibility test in the different time period from 1994 to 1998 showed 98%, 97%, 98%, 96% and 95% of fosfomycin sensitivity against methicillin-resistant *Staphylococcus aureus* in the years. The persistently high rates of fosfomycin sensitivity in their study were due to the limited use of fosfomycin and also the combined drug regime of Fosfomycin with other antibiotics [11]. Similar study was conducted in Taiwan in the same year 2011 showed 89 % of fosfomycin was sensitive against methicillin-resistant *Staphylococcus aureus* [12].

Beside fosfomycin other antibiotics like vancomycin showed 99.3% sensitivity and 0.7% resistant. Similar results of this study coincide with the results of the study that was conducted in Pakistan in 2013 that showed 99.5% sensitivity to vancomycin and only one isolate was found to be resistant [3]. As vancomycin is the choice of antibiotic for treating methicillin-resistant *Staphylococcus aureus* infection but its role is limited because of its nephrotoxic effect. One of the studies was conducted in Spain in 2012 that showed an effective synergistic effect of fosfomycin plus daptomycin in treating methicillin-resistant *Staphylococcus aureus*. Furthermore, the synergistic effect of fosfomycin with daptomycin was proved to be effective in treating glycopeptide intermediate resistance [13].

Our study showed 85% of isolates were sensitive to trimethoprim-sulfamethoxazole against methicillin-resistant *Staphylococcus aureus* and 15% resistant. In other countries like sub-Saharan Africa, 19% of resistance to trimethoprim-sulfamethoxazole against methicillin-resistant *Staphylococcus aureus* has been reported. As trimethoprim is recommended for treating uncomplicated skin and soft tissue infections and cannot be used for treating severe infections

like bacteremia or pneumonia and therefore vancomycin remains the primary drug of choice for such severe infections [14]. Community-acquired methicillin-resistant *Staphylococcus aureus* usually show susceptible results to clindamycin. Our study showed clindamycin 68.7% sensitivity and 31.3% resistant against methicillin-resistant *Staphylococcus aureus*. One of the side effects of clindamycin is its association with *Clostridium difficle* induced diarrhea. Moreover, the treatment failure has been seen with clindamycin because of inducible resistance and if the local rate of clindamycin resistance exceeds 10% to 15% clindamycin cannot be used as an empirical antibiotic for treating skin and soft tissue infections caused by community-acquired methicillin-resistant *Staphylococcus aureus* [15].

One of the studies conducted in California in 2006, showed higher resistance rate of erythromycin 93% against community-acquired methicillin-resistant *Staphylococcus aureus* whereas other studies have reported 69% of resistance to erythromycin in Alaska and 61% of resistance to erythromycin in San Francisco against community-acquired methicillin-resistant *Staphylococcus aureus* [16]. Our study showed 40.1% resistant to erythromycin against methicillin-resistant *Staphylococcus aureus*. This high resistance rate among non-beta lactam antibiotics may complicate efforts to manage infections within the community [17].

Tetracycline one of the alternative treatment for less serious infections which can be given orally, showed good absorption by the gastrointestinal tract and have shown excellent tissue penetration. Our study showed 48.3% sensitivity and 51.7% resistant to tetracycline, because of bacteriostatic effect its role is limited in treating severe infections caused by methicillin-resistant *Staphylococcus aureus* [18].

Ciprofloxacin resistance has been increasing since years and according to the data of the United State from 2010, the rate of resistance to ciprofloxacin was found to be 70% against methicillin-resistant *Staphylococcus aureus* whereas our study showed 66.0% resistant for methicillin-resistant *Staphylococcus aureus*. This high resistance has mainly been resulted due to mutations occurring in quinolone resistance determining region (QRDR) of parC, encoding topoisomerase IV, and gyrA, encoding DNA gyrase. Moreover, the fluoroquinolone resistance can also be chromosomally mediated encoding multidrug resistance efflux pumps NorA, NorB, and NorC and are present widely in different strain [19]. Due to limited usage of fosfomycin, good penetration into various body fluids as well as in tissues and low resistance rate reported in other studies making this drug as an effective antibiotic in treating severe infections [20].

Conclusion

Our study showed 97.3% sensitivity and 2.7% resistant to fosfomycin against methicillin-resistant *Staphylococcus aureus*. Fosfomycin and vancomycin showed good results in this study for methicillin-resistant *Staphylococcus aureus* but the use of vancomycin is limited because of its nephrotoxic effect in contrast to fosfomycin that has no such complications of renal or liver function disturbances.

Comment [DM5]: A better conclusion is needed

References

Comment [DM6]: References are not uniform

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