To the editor in chief,

South Asian Journal of Research in Microbiology,

Dear SIR,

Please find attached research paper for publication in your journal. The Title of manuscript "Fosfomycin an alternative for the treatment of *METHICILLIN RESISTANT STAPHYLOCOCCCUS AUREUS (MRSA)".*

Thankyou

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Tittle

Fosfomycin an Alternative for the Treatment of Methicillin Resistant Staphylococcus aureus (MRSA)

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, prostate gland, 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*(MRSA) 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 MRSA 143(97.3%) were sensitive to fosfomycin and only 4 (2.7%) were found to be resistant.

Conclusion

Fosfomycin proved to be a very good alternative for treating MRSAbecause 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

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-acetylmuramic 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 MRSAposed concern for clinical microbiologist(1). MRSA infections have been increasing over theyears. In Pakistan, the prevalence of MRSA increased from 5% in 1989 to up to 51% in recent years(2). MRSAhas shown resistance to multiple antibiotics such as gentamicin, fusidic acid, norfloxacin, clindamycin, and erythromycin(3).

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 remainedthe 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 MRSA infections. Furthermore, increasingMRSAinfections 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).

Fosfomycin combination regime has shown effective response in treating various MRSA infections includingbiofilm-associatedMRSA, 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 MRSA were sensitive to fosfomycin having MICs ranging from MIC ≥0.38>1024ug/ml(10).

Therefore, the purpose of this study was to evaluate fosfomycin as an alternative treatment against MRSA 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 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 thegenus level by routine biochemical tests. *Staphylococcus aureus* bacteria were 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% HClandmannitol 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(11). Resistance and sensitive cases were differentiated by measuring zone diameter around antibiotics.MRSA was identified by resistant to cefoxitin 30ug 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<16mm were categorized as being sensitive (S) or resistant (R) accordingly(6).

Statistical Analysis

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.

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 MRSA 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)

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)

Source of Specimen	Fosfomycin		Total	P-Value
	Sensitive	Resistant		
Blood	109(74.1%)	4(2.7%)	113(76.9%)	0.744
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)

Drug Resistance	Sensitive	Resistant
	f(%)	f(%)
Fusidic acid (FD)	130(88.4%)	17(11.6%)
Clindamycin (DA)	101(68.7%)	46(31.3%)
Ciprofloxacin (CIP)	50(34%)	97(66%)
Erythromycin (E)	88(59.9%)	59(40.1%)
Vancomycin (VA)	146(99.3%)	1(0.7%)
Trimethoprim sulfamethoxazole (SXT)	125(85%)	22(15%)
Tetracycline (TET)	71(48.3%)	76(51.7%)

Discussion

MRSAhas become the leading cause of hospital-acquired infections all around the world. The emergence of MRSA has also been seen in developing countries like Pakistan. Previous data showed avariable prevalence of MRSAobtained from different cities like 61% in Lahore, 57% in Karachi, 54% in Peshawar, and 46% in Rawalpindi(3). The increased emergence of MRSA isolates with the passage of time may be due to thetransfer of resistant genes between bacterial cells due to the persistence of bacteria in hospital environment resulting in antibiotic resistance (2). In our study, the susceptibility of fosfomycin was found to be 97.3% and 2.7% were resistant to fosfomycin against MRSA. One of the study conducted in university of Virginia showed broader range of fosfomycin activity against MRSA and only 12% strains are found to be

resistant in addition to it other anti-staphylococcal agents like penicilins, 1st generation cephalosporins, clinadmycin, erythromycin and aminoglycosides showed in-vitro resistance against MRSA.(12). Similar study was conducted in Taiwan in the same year 2011 showed 89 % of fosfomycin was sensitive against MRSA(13).

Beside fosfomycin other antibiotics likevancomycin 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 MRSAinfection but its role is limited because of its nephrotoxic effect. One of the studies was conducted in Spain in 2012 that showedan effective synergistic effect of fosfomycin plus daptomycin in treating MRSA. Furthermore, the synergistic effect of fosfomycin with daptomycin was proved to be effective in treating glycopeptide intermediate resistance(14).

Our study showed 85% of isolates were sensitive to trimethoprim-sulfamethoxazole against MRSA and 15% resistant. In other countries like sub-Saharan Africa, 19% of resistance to trimethoprim-sulfamethoxazole against MRSA has been reported(15). 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(15).Community-acquired MRSA usually show susceptible results to clindamycin [16]. The results of our study showed 68.7% clindamycinsensitivity and 31.3% resistant against MRSA. One of the side effects of clindamycin is its association with *Clostridium difficle* induced diarrhea(16). Moreover, the treatment failure has been seen with clindamycin because of inducible resistance and if the local rate of clindamycin resistance exceeds10% to 15% clindamycin cannot be used as an empirical antibiotic for treating skin and soft tissue infections caused by community-acquired MRSA(16).

One of the studiesconducted in California in 2006, showed higher resistance rate of erythromycin 93% against community-acquired MRSA whereas other studies have reported 69% of resistance to erythromycin in Alaska and 61% of resistance to erythromycin in San Francisco against community-acquired MRSA(17). Our study showed 40.1% resistant to erythromycin

against MRSA. This high resistance rate among non-beta lactam antibiotics may complicate efforts to manage infections within the community(18).

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 MRSA(19).

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 MRSA(20)whereas the results of our study showed 66.0% resistant for MRSA. 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(20).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(21).

Conclusion

MRSA are a global threat and we need to limit the use of Vancomycin and similar antimicrobial agents which are expensive and some are inhibitory and expensive while Fosfomycin is less expensive and has an unique property of binding the mast cellswhich would help in reducing the nephrotoxicity. In this study fosfomycin and vancomycinhas shown good results for MRSA(22).Therefore the fosfomycin was considered the drug of choice with good penetration to the various body sites and no such renal complications andhas shown results of 97.3% sensitivity and only 2.7% resistant.

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