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RESEARCH ARTICLE

In vitro efficacy of fosfomycin against clinical strains

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ABSTRACT

Objective: Fosfomycin is an alternative drug for treatment of uncomplicated urinary tract infections. This study aimed to investigate in vitro activity of fosfomycin against methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase negative staphylococci (MRCoNS), vancomycin-resistant *Enterococcus faecium* (VR *E. faecium*), *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter isolates*.

Methods: Clinical isolates of MRSA, MRCoNS, *E. coli, K. pneumoniae* and *Enterobacter spp.* and VRE isolates which were isolated from rectal swaps were identified with Vitek 2 Compact (Biomeriux, France) and BD Phoenix (BD USA) automated systems. The Kirby-Bauer disc diffusion method was used to determine the susceptibility to fosfomycin.

Results: All the MRSA (n=40), MRCoNS (n=40), and VR *E. faecium* (n=62) isolates were susceptible to fosfomycin. The fosfomycin susceptibility rates for *E. coli*, *K. pneumoniae*, and *Enterobacter spp.* were 97.5% (39 of 40), 97.3% (36 of 37), and 86.9% (20 of 23), respectively. One (2.7%) isolate of *K. pneumoniae* and three (13.1%) isolates of *Enterobacter spp.* showed intermediate susceptibility to fosfomycin. Resistance to fosfomycin was detected in only one (2.5%) isolate of *E. coli.*

Conclusion: Based on the results of our study, fosfomycin is highly active against a collection of several gram-positive and gram-negative bacteria, including multidrug resistant isolates, and is an alternative drug in the treatment option. *J Microbiol Infect Dis 2014;4(2): 55-58*

Key words: Fosfomycin, gram-positive bacteria, gram-negative bacteria, antimicrobial agent

Fosfomisinin klinik izolatlara karşı in vitro etkinliği

ÖZET

Amaç: Fosfomisin komplike olmayan üriner sistem enfeksiyonlarının tedavisinde alternative bir ilaçtır. Bu çalışmanın amacı fosfomisinin in vitro etkinliğini, metisiline dirençli *Staphylococcus aureus* (MRSA), metisiline dirençli koagülaz negatif stafilokoklar (MRKNS), vankomisine dirençli *Enterococcus faecium* (VR- *E. faecium*), *Escherichia coli*, *Klebsiella pneumoniae* ve Enterobacter izolatlarında araştırmaktır.

Yöntemler: MRSA, MRKNS, *E. coli*, *K. pneumoniae* ve *Enterobacter spp.* klinik örnekleri ve rektal sürüntüden izole edilen VRE izolatlarının tanımlanması Vitek2 Compact (Biomeriux, Fransa) ve Phoenix (BD, ABD) otomatize sisteminlerinde yapıldı. Fosfomisin duyarlılığı belirlenmesinde Kirby-Bauer disk difüzyon yöntemi kullanıldı.

Bulgular: Tüm MRSA (n=40), MRKNS (n=40) ve VR- *E. faecium* (n=62) suşları fosfomisine duyarlı olarak saptandı. *E. coli*, *K. pneumoniae* ve *Enterobacter spp.* için fosfomisin duyarlılık oranları sırasıyla; % 97,5 (40'da 39), % 97,3 (37'de 36), % 86,9 (23'te 20) olarak bulundu. Bir *K. pneumoniae* izolatı (% 2,7) ve üç *Enterobacter spp.* izolatı (% 13,1) fosfomisine ortaduyarlı olarak saptandı. Fosfomisine karşı direnç sadece bir *E. coli* izolatında (% 2,5) saptandı.

Sonuçlar: Fosfomisin çoklu ilaç direncine sahip izolatlar dahil birçok Gram pozitif ve Gram negatif bakteriye karşı invitro yüksek etkinliğe sahiptir ve alternatif bir tedavi seçeneği olarak kullanılabilir.

Anahtar kelimeler: Fosfomisin, gram pozitif bakteri, gram negatif bakteri, antimikrobiyal ajan

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INTRODUCTION

Fosfomycin is an antibiotic and a derivative of phosphoric acid (cis-1,2-epoxyropylphosphare acid) and has effect on bacterial cell wall synthesis at an earlier stage than beta-lactams and glycopeptides.[1-3] Fosfomycin is recommended for the treatment of uncomplicated urinary system infections caused by *Escherichia coli* and Enterococcus faecalis. It is also effective against various gram-negative and gram-positive bacteria, such as Pseudomonas aeruginosa, extended spectrum beta lactamase (ESBL), and carbapenemase-producing Enterobacteriaceae, as well as methicillin-resistant Staphylococcus aureus.[3-5]

The increasing rate of antibacterial resistance in Enterobactericeae limits the number of agents that may be used in infections caused by these bacteria. ESBL enzymes are important both in community-acquired and hospital-acquired isolates.[6] Increasing antimicrobial resistance leads difficulties in the treatment of enterococcal infections. Glycopeptide resistance has become a significant problem in nosocomial infections.[7]

S. aureus is an important cause of nosocomial bacteremia. Coagulase-negative staphylococci are one of the most common sources of blood- and catheter-associated infections, particularly in hematology and oncology patients.[8] Methicillin-resistant staphylococci bear a staphylococcal cassette chromosome (Scc) in their genome that gives rise to resistance against methicillin, as well as other antimicrobials. As the beta-lactam group antimicrobials cannot be used in the treatment of methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant coagulase negative staphylococci (MRCoNS) infections, glycopeptides are the drug of choice.[9] On the other hand, the detection of vancomycin-resistant and intermediate *S. aureus* strains are also of concern today.[10]

The increasing rate of antimicrobial resistance is an emerging problem. It is an important issue for clinicians to choose appropriate agents in treatment of infections by bacterium which are resistant to several antimicrobials. It is known fact that there is need to discover new treatment options and fosfomycin, may be one of this options.[11]

In this study, we aimed to evaluate the fosfomycin susceptibility of MRSA, MRCoNS, vancomycin-resistant *Enterococcus faecium* (VR *E. faecium*), *E. coli*, *K. pneumoniae*, and *Enterobacter spp.* isolates from clinical specimens.

METHODS

A total of 180 non-duplicate isolates of bacterial species recovered from various clinical specimens received from different clinics were included in the study. The distribution of the isolates, which were collected between January 2011 and September 2011, were as follows: MRSA (n=40), MRCoNS (n=40), *E. coli* (n=40), *K. pneumoniae* (n=37), and *Enterobacter spp.* (n=23). During the study period, the specimens were routinely inoculated onto 5% sheep blood agar and eosin methylene blue (EMB) agar and incubated at 35°C for 20–22 h. Conventional methods and Vitek 2 Compact System (Biomeriux, France) and a BD Phoenix (BD Diagnostic Systems, USA) automated system were used in the identification of the bacteria. The antibiotic susceptibility of the isolates and ESBL production of the gram-negative bacteria were analyzed with the Vitek 2 Compact System and the BD Phoenix automated system.

Sixty-two VR *E. faecium* strains detected in hospitalized patients were included in the study. These isolates were collected by rectal swaps by an infection control committee. The rectal swaps were inoculated onto VRE screening agar containing 6 µg/ml of vancomycin and incubated at 35° C for 24 h. Gram staining, catalase, and pyrolidonly-beta naphilamide tests were performed for enterococci-suspicious bacteria for preidentification. After the preidentification, final identification and antibiotic susceptibility were tested with the Vitek 2 Compact System and the BD Phoenix system.

The Kirby-Bauer disc diffusion method was used for the determination of fosfomycin susceptibility using discs (HIMEDIA, India) containing 200 µg/ml of fosfomycin with 50 µg/ml of D-glucose-6 phosphate as recommended by the Clinical and Laboratory Standards Institute (CLSI).[12] The recommendations of the CLSI for urinary isolates of *E. feacalis* were used to determine the fosfomycin susceptibility of gram-positive bacteria, and the disc diffusion criteria for urinary tract isolates of *E. coli* recommended by the CLSI were used to identify the fosfomycin susceptibility of gram-negative bacteria.[12]

RESULTS

All the enterococcoci isolates from the rectal swap specimens were determined as *E. faecium*, and all (n=62) were susceptible to fosfomycin by the disc diffusion method. All the MRSA (n=40) and MR-

CoNS (n=40) isolates were susceptible to fosfomycin. Of the 100 gram-negative bacteria, 55 (55%) were ESBL positive. Among the *E. coli* isolates 39 (97.5%) of 40 were fosfomycin susceptible. Of 37 *K. pneumoniae* isolates, 36 (97.3%) were susceptible, and one (2.7%) showed intermediate susceptibility to fosfomycin. Of 23 *Enterobacter spp.* isolates, 20 (86.9%) were susceptible to fosfomycin, and three (13.1%) showed intermediate susceptibility to fosfomycin (Table 1).

Table 1. Fosfomycin susceptibility rates of the isolates

Bacteria	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Total n (%)
MRSA	40 (100)	-	-	40 (100)
MRCoNS	40 (100)	-	-	40 (100)
VR *E. faecium*	62 (100)	-	-	62 (100)
E. coli	39 (97.5)	-	1 (2.5)	40 (100)
K. pneumoniae	36 (97.3)	1 (2.7)	-	37 (100)
Enterobacter spp.	20 (86.9)	3 (13.1)	-	23 (100)

DISCUSSION

Fosfomycin trometamol is a form of fosfomycin and used orally in a single dose in the treatment of non-complicated urinary system infections. Fosfomycin disodium salt is an intravenous (i.v.) form of fosfomycin and used in some European countries and in Japan. Although fosfomycin has not been approved for conditions other than urinary system infections, successful outcomes were reported in some studies that used i.v. fosfomycin for several types of infections other than those affecting the urinary tract.[11,13]

Various treatment regimens, including aminoglycosides, linezolid, daptomycin and quinupristin-dalfopristin, have been used in the treatment of VRE. However, resistance to these agents was reported.[7,14] In this study, we determined that all VR *E. faecium* isolates were susceptible to fosfomycin. Allerger et al.[15] reported that 96% of VRE were susceptible to fosfomycin according to the disc diffusion method. In another study, all the VR *E. faecalis* (n=23) and 98.1% of VR *E. faecium* (51/52) isolates were found to be susceptible to fosfomycin.[16] Shrestha et al.[17] reported that only one (1.3%) of 75 VR *E. faecium* isolates isolated from urinary and blood culture specimens was resistant to fosfomycin. In another study, 98.4% of 193 VR *E. faecalis* isolates were fosfomycin susceptible.[7] In a study carried out in Turkey, fosfomycin resistance was not determined in VRE isolates, similar to the results of our study.[18]

MRSA is considered an important problem in both hospital- and community-acquired infections.[19] In a study conducted by Falagas et al.,[20] 129 of 130 (99.2%) MRSA isolates and 745 of 961 (77.5%) MRCoNS isolates were susceptible to fosfomycin. Fosfomycin was found to be effective in the treatment of experimental MRSA osteomyelitis in rats.[5] Synergistic and additive effects were detected following treatment with a combination of fosfomycin with linezolide, rifampicin and antistaphylococcal beta-lactams.[9] Oksuz et al.[21] reported high fosfomycin resistance (58%) in isolates of a ST239-MRSA-III clone, which was the most frequently detected clone in their study, and identified fosB in all the isolates of that clone.

A recent study demonstrated that fosfomycin was effective against ESBL-producing Enterobactericeae, particularly *E. coli*.[6] In our study, 97.5% of *E. coli* isolates were susceptible to fosfomycin. In another study carried out in Turkey, 96.5% (332/344) of ESBL- producing *E. coli* isolates were reported to be fosfomycin susceptible.[22] Endimiani et al.[23] found that 63.2% (43/68) of *K. pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* isolates were susceptible to fosfomycin. In a study of the fosfomycin susceptibility of 157 multidrug-resistant *E. coli* (89 of which were ESBL producers) by the disc diffusion method, 99.4% (98.9% of ESBL producers) were susceptible to fosfomycin.[24] Tharavichitkul et al.[25] reported that 88.4% of ESBL-producing *K. pneumoniae* isolates and 97.3% of ESBL-producing *E. coli* isolates were susceptible to fosfomycin. However, in an investigation of an outbreak in 1994, all the ESBL-producing *K. pneumoniae* isolates (n=12) analyzed with the disc diffusion method were fosfomycin resistant.[26] In another evaluation of an outbreak, of 76 multidrug-resistant E. aerogenes isolates identified, only 3.9% were susceptible to fosfomycin.[27] These reports point to the possibility of the spread of fosfomycin-resistant isolates in hospitals. However, fosfomycin resis-

tance is not common in daily clinical practice. Demir et al.[28] reported the resistance to fosfomycin in clinical isolates of *E. coli*, *K. pneumoniae*, and *Enterobacter spp.* as 2%, 9.4%, and 4.4%, respectively. In our study, 97.3% of *K. pneumoniae* isolates and 86.9% of *Enterobacter spp.* isolates were fosfomycin susceptible.

According to the CLSI, when testing fosfomycin susceptibility, breakpoint values for agar dilution and zone diameters for disc diffusion exist only in *E. coli* and *E. faecalis* isolates isolated from urinary specimens. The agar dilution method is a reference method used to determine the minimum inhibitory concentration values.[12] However, it is time consuming and not easily applicable in daily practice. On the other hand, the disc diffusion method is quick and easy to apply. As zone diameters and breakpoints have not yet been determined for bacteria other than *E. coli* and *E. faecalis* by the CLSI, there is a need for further studies that include numerous isolates for determination of both zone diameters and breakpoints values.

In conclusion, fosfomycin was found to be highly active against MRSA, MRCoNS, VRE, *E. coli*, *K. pneumoniae*, and *Enterobacter spp.* isolates. Fosfomycin can be considered an alternative drug for the treatment of infections.

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