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Susceptibility of Urinary Tract Bacteria to Fosfomycin▽

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We evaluated the in vitro activity of fosfomycin against urinary isolates in a region in Greece that exhibits considerable antimicrobial resistance by evaluating retrospectively relevant susceptibility data retrieved from the microbiological library of the University Hospital of Heraklion, Crete, Greece. We examined 578 urinary isolates. In total, 516 (89.2%) were susceptible to fosfomycin; 415 isolates were gram negative, and 101 isolates were gram positive. Fosfomycin appears to exhibit good levels of in vitro activity against the examined urinary isolates.

Urinary tract infections (UTIs) are among the commonest types of bacterial infections (13). The antibiotic treatment for UTIs is associated with important medical and economic implications (12, 17).

Antibiotic agents such as beta-lactams, trimethoprim, and cotrimoxazole have been used for the treatment of UTIs (1, 22). However, the emergence of resistant uropathogens led to a shift to fluoroquinolones (18, 21, 26, 27), shorter antibiotic regimens (24, 39), and early switch practices (38). Yet, resistance to fluoroquinolones has been reported (14, 23). Additionally, the emergence of uropathogens, mainly *Escherichia coli*, exhibiting high rates of resistance due to the production of extended-spectrum beta-lactamases (ESBLs) is worrisome (30, 40).

Fosfomycin is an old broad-spectrum bactericidal antibiotic agent that inhibits the synthesis of the bacterial cell. Its pharmacokinetic profile encourages its use for UTIs; the mean peak urinary concentration of an oral single dose of 3 g fosfomycin tromethamine occurs within 4 h, while concentrations sufficient to inhibit the majority of the urinary pathogens are maintained for 1 to 2 days (28). Thus, fosfomycin tromethamine has been approved as an oral single-dose treatment for acute uncomplicated cystitis (34, 39). Data from studies evaluating the role of fosfomycin in infections other than UTIs are also encouraging (8–11). We aimed to evaluate the in vitro activity of fosfomycin against urinary isolates isolated from patients in a 700-bed university hospital in Heraklion, Crete, Greece.

The data included in our study were retrieved from the database of the microbiological laboratory of the University Hospital of Heraklion, Crete, Greece. We retrospectively tested fosfomycin susceptibility in all clinical urinary isolates that were collected over a 12-month period (January to December 2008). No specific criteria for the selection of isolates to be subjected to fosfomycin susceptibility testing had been set. Only the first isolate of each species per patient could be included in our study.

Quantitative urine cultures were performed by standard techniques using Columbia blood and MacConkey agar plates (bioMérieux, Marcy l'Étoile, France). Plates were incubated for 18 to 24 h at 36°C. The bacterial species identification was performed by using standard biochemical methods, the API system, or the Vitek 2 automated system (bioMérieux, Marcy l'Étoile, France) (36). Antimicrobial susceptibility testing was performed using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) (5). Due to the lack of acknowledged fosfomycin breakpoints for bacteria other than *Escherichia coli* and *Enterococcus faecalis* (2, 5, 33), we used the fosfomycin breakpoints for *E. coli* proposed by the CLSI for the remaining evaluated gram-negative isolates, a practice that was also followed by authors of similar studies (6). Similarly, we used the CLSI breakpoint regarding *E. faecalis* for the other evaluated gram-positive bacteria. Identification of ESBL-producing strains was performed by phenotypic testing based on the synergy between clavulanic acid and extended-spectrum cephalosporins (5). Carbapenemase production was detected by the modified Hodge test (29).

A total of 578 clinical urinary isolates were included. Two-hundred seven (35.8%) of these 578 isolates originated from adult outpatients, 167 (28.8%) from patients hospitalized in medical wards, 74 (12.8%) from adult patients hospitalized in surgical wards, 17 (2.9%) from intensive care unit adult patients, 9 (1.5%) from adult patients in renal replacement therapy clinics, and 14 (2.4%) from patients from areas other than the above-mentioned hospital units. Ninety (15.5%) of the 578 isolates originated from pediatric patients. Eighty-six (95.5%) of these 90 isolates originated from pediatric inpatients.

The 578 tested urinary bacterial isolates represented 456 (78.8%) gram-negative isolates and 122 (21.1%) gram-positive isolates. The 456 gram-negative isolates represented 404 (88.5%) members of the *Enterobacteriaceae*, 266 (65.8%) of

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TABLE 1. Susceptibility to fosfomycin of gram-negative and gram-positive urinary isolates tested

Isolate	Total no. of isolates	No. of isolates (%) with:	
		Susceptibility	Intermediate susceptibility
Gram-negative bacteria[a]			
Members of the *Enterobacteriaceae*			
Escherichia coli	266	266 (100.0)	0 (0)
Enterobacter species	16	12 (75.0)	2 (12.5)
Klebsiella pneumoniae	68	56 (82.3)	5 (7.3)
Proteus mirabilis	31	30 (96.7)	0 (0)
Other members of the *Enterobacteriaceae*	21	14 (66.6)	2 (10.0)
Nonfermentative gram-negative bacilli			
Acinetobacter baumannii	11	1 (9.0)	0 (0)
Pseudomonas aeruginosa	40	34 (85.0)	2 (5.0)
Gram-positive bacteria[b]			
Staphylococcus aureus	13	13 (100.0)	0 (0)
MRSA[c]	4	4 (100.0)	0 (0)
S. saprophyticus	16	16 (100.0)	0 (0)
Enterococcus faecalis	74	68 (91.8)	1 (1.3)
Enterococcus faecium	12	0 (0)	0 (0)
Streptococcus agalactiae	6	3 (50.0)	0 (0)

[a] Three single urinary isolates, namely, a *Salmonella* sp., *Serratia marcescens*, and *Stenotrophomonas maltophilia*, were also included in the total of 456 tested gram-negative isolates. Both the *Salmonella* and the *Serratia* isolates were susceptible to fosfomycin, whereas the *Stenotrophomonas maltophilia* isolate was resistant to fosfomycin.

[b] A single isolate of *Staphylococcus epidermidis* was also included in the total of the 122 tested gram-positive urinary isolates. This isolate was susceptible to fosfomycin.

[c] Resistant to cefoxitin or oxacillin.

TABLE 2. Susceptibility to fosfomycin in specific categories of resistant isolates tested

Isolate	Total no. of isolates	No. of isolates (%) susceptible to fosfomycin
Gram-negative bacteria		
K. pneumoniae nonsusceptible to colistin[a]	12	7 (58.3)
K. pneumoniae nonsusceptible to carbapenem[a]	25	15 (60.0)
Carbapenemase-producing *K. pneumoniae*	25	15 (60.0)
P. aeruginosa nonsusceptible to carbapenem[a]	9	8 (88.8)
Members of the *Enterobacteriaceae* nonsusceptible to cefotaxime, ceftriaxone, cefepime, ceftazidime, and aztreonam[a]		
E. coli	14	14 (100.0)
K. pneumoniae	40	30 (75.0)
Enterobacter species	5	5 (100.0)
P. aeruginosa	17	16 (94.1)
ESBL-producing bacteria		
E. coli	14	14 (100.0)
K. pneumoniae	15	15 (100.0)
Klebsiella oxytoca	1	0 (0)
Gram-positive bacteria		
MRSA	4	4 (100.0)
Vancomycin-resistant *E. faecium*	4	0 (0)

[a] "Nonsusceptible" includes resistant isolates and isolates with intermediate susceptibility.

which were *Escherichia coli*, and 52 (11.5%) of which were gram-negative, nonfermentative bacilli. The 122 tested gram-positive isolates consisted of 74 (60.6%) *Enterococcus faecalis* isolates, 16 (13.1%) *Staphylococcus saprophyticus* isolates, 13 (10.6%) *Staphylococcus aureus* isolates, 12 (9.8%) *Enterococcus faecium* isolates, 6 (4.9%) *Streptococcus agalactiae* isolates, and a single (0.8%) *Staphylococcus epidermidis* isolate. In total, 516 (89.2%) of the 578 tested urinary isolates were found to be susceptible to fosfomycin; 415 were gram negative, and 101 were gram positive. Specific data are presented in Table 1. The above-mentioned group of 516 fosfomycin-susceptible urinary isolates included specific categories of resistant isolates. Specific data are presented in Table 2.

The main finding of our study is that fosfomycin exhibits considerably high antimicrobial activity against urinary clinical isolates with relatively high levels of antimicrobial resistance that were collected recently in a university hospital in Crete, Greece. Specifically, fosfomycin was active against all tested *E. coli*, *S. aureus* (including methicillin [meticillin]-resistant *S. aureus* [MRSA]), and *S. saprophyticus* isolates. However, the number of the above-mentioned gram-positive isolates was rather limited. Considerable rates of susceptibility to fosfomycin were found for *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (including the respective carbapenem-resistant isolates), and *Enterobacter* spp., as well as *Enterococcus faecalis* and *E. faecium*.

In our study, fosfomycin susceptibility rates followed only those of colistin, imipenem, and meropenem for the majority of the tested gram-negative urinary isolates. Regarding *E. coli*, rates of susceptibility to fosfomycin were maximal and equaled those to colistin and to carbapenems. Increasing resistance of nosocomial or community-acquired *E. coli* strains to ampicillin, cotrimoxazole, or fluoroquinolones has been reported previously in various clinical settings (4, 18, 27, 35). In contrast, the reported rates of *E. coli* resistance to fosfomycin were lower (3, 16, 25). In our study, all the *E. coli* tested isolates were susceptible to fosfomycin. The emergence of ESBL-producing *E. coli* strains is also an evolving problem (40). There is some evidence that fosfomycin might be a promising solution for the treatment of such infections (19, 20, 30). It is also suggested that the antimicrobial activity of fosfomycin against ESBL-producing *E. coli* may be accompanied by an immunomodulating activity (37). Notably, in our study, fosfomycin was active against all the ESBL-producing *E. coli* and *K. pneumoniae* isolates.

Since fosfomycin has been used extensively in many countries for the treatment of UTIs (7, 15, 32), the extrapolation of our findings should be dealt with cautiously. However, the alarming resistance rates observed in Greece necessitate the evaluation of alternative antibiotic agents (31). Moreover, genetic identification techniques were not implemented in our study. Thus, one may consider that a proportion of the tested urinary isolates might have been of the same clonal origin.

In conclusion, our study indicates that fosfomycin is active in vitro against a considerable percentage of urinary isolates, which simultaneously exhibit high rates of antimicrobial drug resistance to the conventionally used antimicrobial agents for the treatment of UTIs. Consequently, fosfomycin may be considered a useful antibiotic agent in our armamentarium for the treatment of UTIs.

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