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Activity of fosfomycin and comparison of several susceptibility testing methods against contemporary urine isolates☆

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ABSTRACT

Fosfomycin is recommended as first-line treatment for acute uncomplicated cystitis in women. It has demonstrated in vitro activity against a variety of pathogens; however, a paucity of data are available from the USA. We determined the susceptibility of a collection of urine isolates to fosfomycin and compared multiple methods of susceptibility testing. Consecutive non-duplicate Enterobacteriaceae, enterococci and *Pseudomonas aeruginosa* isolates were collected from the clinical microbiology laboratory between August 2013 and January 2014. Isolates represented hospitalised or emergency department patients with monomicrobial bacteriuria. Fosfomycin MICs were determined in duplicate, on separate days, by Etest and disk diffusion and results were compared with agar dilution. Nitrofurantoin and ciprofloxacin were used as comparators. MIC results were categorised using Clinical and Laboratory Standards Institute interpretive criteria for *Escherichia coli* and *Enterococcus faecalis*. Correlation between the three testing methods was evaluated. Overall susceptibility to fosfomycin was 94.4%, 93.5% and 87.9% by agar dilution, disk diffusion and Etest, respectively. Five fosfomycin-resistant isolates were identified, including two *Morganella morganii*, one *P. aeruginosa*, one *Proteus mirabilis* and one *Enterobacter aerogenes*. Across all organisms, rates of essential agreement, categorical agreement, minor errors, major errors and very major errors for Etest/disk diffusion compared with agar dilution were 77.3%/NA, 89.5/93.8%, 7.1/5.0%, 3.6/1.3% and 0/0%, respectively. Fosfomycin displayed fairly consistent activity against a majority of isolates collected when using the susceptibility breakpoint of 64 μg/mL. MICs for *E. coli* were particularly low (≤2 μg/mL). These data lend support to current guidelines that recommend fosfomycin as empirical first-line therapy for uncomplicated UTI.

1. Introduction

In 2010, guidelines published by the Infectious Diseases Society of America (IDSA) and the European Society for Microbiology and Infectious Diseases endorsed oral fosfomycin tromethamine (fosfomycin) as a first-line agent for the empirical treatment of acute uncomplicated cystitis in women [1]. The rationale for this recommendation included minimal documented resistance among target pathogens and a low propensity for collateral damage in comparison with other first-line agents. Despite these recommendations, experience with fosfomycin in the USA is limited, which may lead to clinicians' reluctance regarding its use in comparison with alternative agents.

Fosfomycin, a phosphonic acid derivative, has a broad spectrum of activity against a wide variety of Gram-positive and Gram-negative pathogens, including those commonly implicated in cystitis such as *Escherichia coli*, *Klebsiella pneumoniae* and enterococci [2]. This agent has been used extensively in Europe and Asia both in intravenous and oral formulations; however only the oral formulation is available in the USA. In the face of increasing antimicrobial resistance, renewed interest in fosfomycin likely derives from favourable in vitro susceptibilities reported against multidrug-resistant pathogens such as extended-spectrum β-lactamase-producing and *K. pneumoniae* carbapenemase

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(KPC)-producing isolates [3–7]. The majority of fosfomycin susceptibility data, however, originate from Europe and Asia, while its activity in the USA has not been well described [8–10].

Further compounding the complexity of fosfomycin's use in practice is the difficulty in performing susceptibility testing. Currently, the only two methods for testing approved by the Clinical and Laboratory Standards Institute (CLSI) are agar dilution and disk diffusion, whilst the use of broth dilution is specifically recommended against, therefore automated susceptibility testing cannot be used [11]. Fosfomycin requires glucose-6-phosphate (G6P) to exert its antimicrobial activity; therefore, in the absence of physiological G6P in in vitro testing, it must be added to more closely simulate in vivo conditions [2]. Agar dilution and disk diffusion may be time consuming for laboratory personnel as they require manual processing. In addition, agar dilution is usually only performed in research settings because of the need to make custom media and because of the specialised equipment required. Therefore, the time it takes to relay minimum inhibitory concentration (MIC) data may be increased by using either of these methods. In addition, CLSI guidance only provides interpretive criteria for *E. coli* and *Enterococcus faecalis* species. Etests, on the other hand, are relatively easy to perform and provide quantitative MIC results, making them a potentially attractive method over agar dilution or disk diffusion.

In an effort to better understand the activity of fosfomycin against a representative sample of contemporary urine isolates in the USA, the susceptibility of a clinically relevant collection of isolates to fosfomycin was determined. Moreover, agar dilution, disk diffusion and Etest methods for susceptibility testing were compared.

2. Materials and methods

2.1. Isolate collection

Consecutive non-duplicate monomicrobial urine isolates of Enterobacteriaceae, enterococci and *Pseudomonas aeruginosa* from hospitalised or emergency department (ED) patients were collected from the clinical microbiology laboratory of Beth Israel Deaconess Medical Center (BIDMC, Boston, MA) between August 2013 and January 2014. All isolates were collected from unique patients. Isolates from non-ED outpatient locations or those with more than one species isolated in the urine were excluded. BIDMC is a 649-bed, Level 1 Trauma Center and teaching hospital affiliated to Harvard Medical School. The study was approved by the Institutional Review Boards at BIDMC and Northeastern University (Boston, MA).

2.2. Susceptibility testing

Fosfomycin MICs were determined in duplicate, on separate days, by agar dilution, disk diffusion and Etest. Comparator agents tested by agar dilution included ciprofloxacin (*P. aeruginosa* only) and nitrofurantoin [all other organisms except those with intrinsic resistance (*Morganella*, *Proteus* and *Providencia* spp.)]. CLSI M100-S24 [11] and the manufacturer's package insert were followed for agar dilution/disk diffusion and Etest (bioMérieux, Durham, NC), respectively. Briefly, all test isolates and ATCC reference strains were inoculated onto blood agar plates and were allowed to grow overnight at 35 °C. Single isolated colonies were used to inoculate Mueller–Hinton II broth (BBL, Becton Dickinson, Franklin Lakes, NJ) to a density of ca. 1×10^8 CFU/mL. Agar dilution was performed using Mueller–Hinton agar (Difco, Becton Dickinson, Franklin Lakes, NJ) containing 25 μg/mL G6P. Cell suspensions were further diluted and were delivered onto plates using a Steers replicator, which delivered ca. 10^4 CFU for each isolate. Concentration ranges tested were 0.125–2048 μg/mL for fosfomycin, 1–256 μg/mL for nitrofurantoin and 0.125–256 μg/mL for ciprofloxacin. For disk diffusion, commercially available disks (BBL, Becton Dickinson) containing 200 μg of fosfomycin and 50 μg of G6P were used. For disk diffusion and Etests, the original cell suspensions (ca. $1–5 \times 10^8$ CFU/mL) were used within 30 min of preparation. *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 29212 were used as control strains and were run in parallel with every experiment. Results were read by two independent observers.

2.3. Interpretive criteria used

In the absence of interpretive criteria for all test organisms, CLSI M100-S24 *E. coli* and *E. faecalis* breakpoints (≤64 μg/mL, susceptible; 128 μg/mL, intermediate; and ≥256 μg/mL, resistant) were used to interpret fosfomycin susceptibility for all species, similar to previous investigations [3,5,8,11,12]. Nitrofurantoin interpretive criteria for enterococci and Enterobacteriaceae were used as follows: ≤32 μg/mL, susceptible; 64 μg/mL, intermediate; and ≥128 μg/mL, resistant. Ciprofloxacin interpretive criteria for *P. aeruginosa* were as follows: ≤1 μg/mL, susceptible; 2 μg/mL, intermediate; and ≥4 μg/mL, resistant.

2.4. Evaluation of minimum inhibitory concentration correlations

An evaluation of the correlation between agar dilution, disk diffusion and Etest methods was performed using CLSI M23-A3 guidance with agar dilution as the reference method [13]. Essential agreement was defined as an Etest MIC equal to or within ±1 dilution of the agar dilution MIC. Categorical agreement was met when Etest or disk diffusion interpretive criteria agreed (susceptible/intermediate/resistant) with agar dilution results. A minor error was defined as Etest or disk diffusion with a susceptible or resistant result when agar dilution result was intermediate, or when Etest or disk diffusion results were intermediate and agar dilution was susceptible or resistant. A major error occurred when Etest or disk diffusion results were resistant and agar dilution was susceptible and was calculated only for susceptible isolates. Very major errors occurred when Etest or disk diffusion results were susceptible and agar dilution was resistant and was calculated only for resistant isolates [13].

2.5. Investigation of fosfomycin resistance mechanisms

Isolates resistant to fosfomycin by agar dilution were screened for two of the more frequently reported mechanisms mediating resistance to fosfomycin: mutation of the glycerol-3-phosphate transporter (GlpT), a nutrient transport system; and acquisition of FosA, a fosfomycin-modifying enzyme found in *P. aeruginosa* [14–16]. Bacterial DNA was extracted using an UltraClean® Microbial DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad CA) and was analysed by PCR followed by sequencing for the presence of *glpT* mutations and/or the presence of *fosA* (see Supplementary Table S1).

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2015.08.012.

To assess functionality of the GlpT transporter system, the ability of fosfomycin-resistant isolates to grow on M9 minimal agar supplemented with $MgSO_4$ was tested in the presence of each of the following carbon sources supplemented at 2% (w/v): glycerol; glycerol-3-phosphate; and G6P. All chemicals, except M9 minimal salts, 5× (Difco, Becton Dickinson), were purchased from Sigma-Aldrich (St Louis, MO). Briefly, for each strain, an individual colony was suspended in 500 μL of saline and then 3 μL of the suspension was spotted on the plates and streaked for colonies. Plates were

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