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In Vitro Activity of Fosfomycin and Other Commonly Used Oral Antimicrobial Agents against Urinary Isolates of AmpC-Phenotype versus Extended-Spectrum β-Lactamase (ESBL)-Producing Community Strains of *Escherichia coli*

C-176

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ABSTRACT

Background: Infections due to organisms harboring broad-spectrum β-lactamases, such as ESBLs and/or AmpC-type β-lactamases, have been reported worldwide, with significant morbidity and mortality. The objective of this study was to assess the *in vitro* activity of fosfomycin, an antimicrobial agent with a novel mechanism of action, along with other commonly used oral antimicrobials, against AmpC phenotype versus ESBL-producing *E. coli.*

Methods: During a 14 month study, all urinary isolates of *E. coli* (*n* = 5,532) were screened for ESBL production, followed by confirmatory testing if warranted, in accordance with CLSI guidelines. The *in vitro* antimicrobial susceptibility profiles of ESBL-producing isolates and those expressing the AmpC phenotype were analyzed for eight oral antimicrobials: fosfomycin (FOS), ampicillin (AM), cephalothin (CF), ciprofloxacin (CIP), nitrofurantoin (F/M), norfloxacin (NOR), tetracycline (TE), and trimethoprim/sulfamethoxazole (TMP/SMX). Duplicate isolates from the same patient were excluded from the study.

Results: A total of 77 isolates were confirmed as AmpC phenotype (*n* =35) or ESBL-producing (*n* =42) *E. coli*. All of the isolates were resistant to both AM and CF. Of the AmpC phenotype vs the ESBL producing strains, 5/35 (14%) vs 32/42 (76%) were resistant to both CIP and NOR (p <0.001), respectively; 8/35 (23%) vs 28/42 (67%) were resistant to TMP/SMX (p <0.001); 18/35 (51%) vs 28/42 (67%) were resistant to TE; 1/35 (3%) vs 1/42 (2%) were resistant to FOS; and 0/35 (0%) vs 1/42 (2%) were resistant to F/M.

Conclusions: *E. coli* isolates harboring ESBLs are more likely to be resistant to multiple other antimicrobial agents, such as ciprofloxacin, norfloxacin, and trimethoprim-sulfamethoxazole than those expressing the AmpC phenotype. Resistance to fosfomycin and nitrofurantoin among urinary isolates of *E. coli* with ESBLs or the AmpC phenotype occurred very infrequently. These agents may be useful in the treatment of uncomplicated urinary tract infections caused by *E. coli* with ESBLs or expressing the AmpC phenotype.

INTRODUCTION

Pathogens with multidrug resistance mediated by plasmids encoding broad-spectrum β-lactamases, such as AmpC-type and extended-spectrum β-lactamases (ESBLs) can cause serious infections. These potent enzymes are able to confer resistance *in vivo* against a large number of β-lactams and β-lactam/ β-lactamase inhibitor combinations, which constitute the largest arsenal of antimicrobial agents used in clinical practice throughout the world.

The rapid propagation of resistance genes and the recently reported emergence of carbapenem resistance in ESBL and AmpC producers, including *E. coli* and *Klebsiella* strains, calls for further studies to identify alternative agents that can be used against ESBL- and AmpC-associated infections.

Fosfomycin is an antimicrobial agent with a novel mechanism of action, that has the advantage of providing a single oral dose for the treatment of uncomplicated urinary tract infection (UTI) due to *E. coli* or *Enterococcus faecalis*. It has been recommended as a quinolone-sparing agent for the treatment of UTIs in areas with resistance to trimethoprim-sulfamethoxazole exceeding 20%.

We conducted this study to assess the *in vitro* activity of fosfomycin and seven other oral antimicrobial agents commonly used in the treatment of community-associated UTIs, against ESBL-producing and AmpC phenotype-expressing urinary strains of *E. coli.*

METHODS

Bacterial Strains: Over a 14 month period ending in December 2005, urine specimens submitted for culture from nonhospitalized patients were processed by conventional procedures using commercially prepared media (Bio-Media Unlimited Ltd., Toronto, ON, Canada). Isolates of *E. coli* (*n* = 5,532) were identified by conventional methods. Duplicate isolates from the same patient were excluded from the study.

Antimicrobial Susceptibility Testing: Suspensions of test organisms for susceptibility testing by standardized disk diffusion were prepared to a concentration equivalent to 0.5 McFarland standard and were inoculated onto Mueller-Hinton agar plates (PML Microbiologicals, Mississauga, ON, Canada), using antimicrobial disks (Oxoid Canada, Ottawa, ON, Canada). Disk zone diameters were interpreted according to CLSI interpretive criteria.

ESBL Testing: In accordance with CLSI guidelines, isolates were initially screened for ESBL production, followed by confirmatory testing if warranted. An isolate was confirmed as an ESBL producer by the Phenotypic Confirmatory Test or by the Double Disk Synergy Test.

AmpC Phenotype: If the isolate expressed no synergy with clavulanic acid, was nonsusceptible to cefoxitin (30 µg), and had a zone size for aztreonam (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), or ceftriaxone (30 µg) that was ≤ 27,≤ 27, ≤ 22, or ≤ 25 mm, respectively, this was considered indicative of the AmpC phenotype.

Profile Analysis: Antimicrobial susceptibility profiles were analyzed for eight oral antimicrobials tested: fosfomycin (200 µg), ampicillin (10 µg) , cephalothin (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), norfloxacin (10 µg), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg).

RESULTS & DISCUSSION

Table 1: Susceptibility testing results of study strains of AmpC phenotype (*n* = 35) vs ESBL-producing (*n* = 42) *E. coli* against eight oral antimicrobial agents

Antimicrobial Agent	Susceptible AmpC	Susceptible ESBL	Intermediate AmpC	Intermediate ESBL	Resistant AmpC	Resistant ESBL	Total
Fosfomycin	34(97%)1	41(98%)	0(0%)	0(0%)	1(3%)	1(2%)	77
Ampicillin	0(0%)	0(0%)	0(0%)	0(0%)	35(100%)	42(100%)	77
Cephalothin	0(0%)	0(0%)	0(0%)	0(0%)	35(100%)	42(100%)	77
Ciprofloxacin	30(86%)	9(21%)	0(0%)	1(2%)	5(14%)	32(76%)	77
Nitrofurantoin	35(100%)	41(98%)	0(0%)	0(0%)	0(0%)	1(2%)	77
Norfloxacin	30(86%)	9(21%)	0(0%)	1(2%)	5(14%)	32(76%)	77
Tetracycline	17(49%)	14(33%)	0(0%)	0(0%)	18(51%)	28(67%)	77
Trimethoprim/ sulfamethoxazole	27(77%)	13(31%)	0(0%)	1(2%)	8(23%)	28(67%)	77

[1] Number of isolates with in vitro response to the tested antimicrobial agent (% value rounded to the nearest integer).

FIGURE 1: PERCENT DISTRIBUTION OF PATIENTS BY AGE GROUP

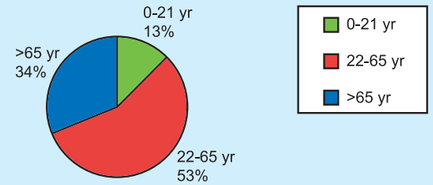

FIGURE 2: SUSCEPTIBILITY TO NON-BETA-LACTAMS

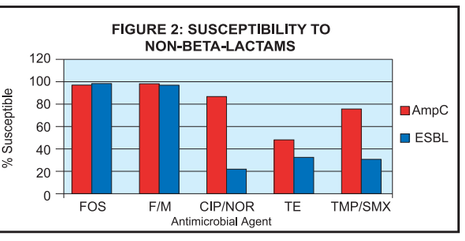

Table 2: Frequency of resistance of AmpC phenotype vs ESBL-producing *E. coli* against six oral non-β-lactams

Number of Isolates	AmpC *n* = 35	ESBL *n* = 42
R to 0 or only 1 non-β-lactam[1]	25 (71%)	4 (9%)
R to 2, 3, or 4 non-β-lactams	10 (29%)	38 (91%)

[1] Non-β-lactams: ciprofloxacin, fosfomycin, nitrofurantoin, norfloxacin, tetracycline, and trimethoprim/sulfamethoxazole.

A total of 5,532 *E. coli* urinary isolates were screened for ESBL production using CLSI guidelines. Of these, 77 were confirmed as ESBL (*n* = 42), or expressed the AmpC phenotype (*n* = 35).

The mean age of patients with AmpC phenotype or ESBL-positive organisms was 51.8 years, with a range of <1 to 91 years. The percent distribution of patients by age group is shown in Figure 1. More isolates were recovered from females (0 - 21 years, *n* = 8; 22-65 years, *n* = 36; > 65 years, *n* = 24) than from males (0-21 years, *n* = 2; 22-65 years, *n* = 5; > 65 years, *n* = 2). The high female to male ratio is consistent with urinary isolation rates encountered in routine practice, and is consistent with our data from previous studies.

Table 1 summarizes the antimicrobial test profiles of the 77 isolates. Both fosfomycin and nitrofurantoin were significantly more active (p < 0.001) *in vitro* than the other antimicrobials tested in this study against AmpC phenotype as well as ESBL producing isolates.

ESBL- and AmpC-producing *E. coli* strains are often multidrug resistant. The *in vitro* resistance rate of ESBL-producing *E. coli* isolates in this study was however significantly higher than that of the AmpC phenotype-expressing isolates against ciprofloxacin (p < 0.001), norfloxacin (p < 0.001), and trimethoprim -sulfamethoxazole (p < 0.001) (Figure 2). Furthermore, as shown in Table 2, AmpC phenotype expressors were significantly less likely than ESBL producers to be resistant to multiple (≥ 2) oral non-β-lactams tested in this study (p < 0.001). To our knowledge, this is the first report describing a statistically significant difference between these two types of resistant organisms by virtue of their *in vitro* response to oral non-β-lactams.

CONCLUSIONS

- *E. coli* isolates harboring ESBLs are significantly more likely than AmpC phenotype-expressing isolates to be resistant to multiple (≥ 2) oral non-β-lactam agents tested in this study.
- Ciprofloxacin, norfloxacin, and trimethoprim-sulfamethoxazole are associated with higher likelihood of resistance in ESBL-producing than in AmpC phenotype-expressing *E. coli.*
- Based on these susceptibility test results, fosfomycin and nitrofurantoin may be useful in the treatment of uncomplicated UTIs caused by multidrug-resistant *E. coli* with ESBLs or expressing the AmpC phenotype.

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