Original Article

Activity of Fosfomycin Against Extended-Spectrum-β-Lactamase-Producing *Klebsiella pneumoniae* and *Escherichia coli* in Maharaj Nakorn Chiang Mai Hospital

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ABSTRACT

Extended-spectrum β-lactamases (ESBLs), which are found predominantly in *Klebsiella pneumoniae* (ESBL-KP) and *Escherichia coli* (ESBL-EC), can hydrolyze all penicillins, cephalosporins, and monobactams except carbapenems and cephamycins. They are usually resistant to multiple classes of antibiotics including aminoglycosides, quinolones, tetracyclines, and chloramphenicol. In this study, we evaluated the activity of fosfomycin, an antibiotic with a unique mechanism of antibacterial action, against ESBL-producing isolates. In the year 2003, all clinical isolates of *K. pneumoniae* and *E. coli* collected from patients admitted to Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand were tested as ESBL producers by the double-disc synergy test. Three hundred and fifty-nine (21.1%) isolates of *K. pneumoniae* and 398 (13.0%) isolates of *E. coli* produced ESBLs. Forty-three isolates of ESBL-KP and 37 of ESBL-EC were randomly selected to test antimicrobial susceptibility by the disc diffusion method against amikacin, fosfomycin, cefoxitin, imipenem, and levofloxacin. The minimum inhibitory concentration (MIC) of fosfomycin and levofloxacin was also determined by the E-test. All these ESBL producers were susceptible to cefoxitin and imipenem. ESBL-KP and ESBL-EC were susceptible to fosfomycin at 88.4 percent and 97.3 percent, respectively. For the MIC_{50} and MIC_{90} of fosfomycin, ESBL-EC (0.7 and 1.8 μg/ml) were more susceptible than ESBL-KP (~16.0 and 32.0 μg/ml).

In conclusion, although imipenem was the most active drug for ESBL-KP and ESBL-EC, cefoxitin and fosfomycin should be considered for fufure clinical studies. (*J Infect Dis Antimicrob Agents 2005; 22:121-26.*)

INTRODUCTION

Extended-spectrum β-lactamases (ESBLs), which are found predominantly in *Klebsiella pneumoniae* and *Escherichia coli*, can hydrolyze all penicillins, cephalosporins, and monobactams,[1,2] but they do not affect cephamycins (e.g. cefoxitin or cefmetazole) or

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carbapenems (e.g. imipenem or meropenem). ESBLs are derived by the point mutation from the common TEM (Temoniera) and SHV (Sulhydryl variable) β-lactamases, and encoded by large plasmids that are easily transferred between bacterial species. They may carry other drug-resistant genes for aminoglycosides, chloramphenicol, tetracyclines, and fluoroquinolones. In addition, some isolates of ESBL producers were susceptible *in vitro* to cephalosporins that were found to be non-efficacious *in vivo*.[3,4] Fosfomycin, a bactericidal antibiotic, inhibits the first step of cell wall synthesis, and has a broad spectrum of antibacterial activity against most bacteria isolated from patients with lower urinary tract infections. A cross-resistance with other antimicrobials has been uncommon.[5] In this study, we evaluated the activity of fosfomycin against ESBL-producing *K. pneumoniae* (ESBL-KP) and *E. coli* (ESBL-EC).

MATERIALS AND METHODS

All clinical isolates of *K. penumoniae* and *E. coli* were collected from patients admitted to Maharaj Nakorn Chiang Mai Hospital Chieng Mai, Thailand in the year 2003. They were identified by the standard microbiological methods, and tested as ESBL producers by the double-disk (DD) synergy method, as described by Coudron et al.[6] Mueller-Hinton agar was inoculated by the standardized bacterial suspension of overnight incubation, as recommended for the standard disk susceptibility tests.[7] Disks (Oxoid) containing 30 μg of cefotaxine and 30 μg of ceftazidime were placed 15 mm (edge to edge) from a 20/10 μg of amoxicillin-clavulanic acid disk. After overnight incubation, an enhanced zone of inhibition between one of either drug and an amoxicillin-clavulanic acid disk was interpreted as presumptive evidence of the presence of an ESBL.

Forty-three isolates of ESBL-KP and 37 isolates of ESBL-EC were randomly selected for testing antimicrobial susceptibility by the disk diffusion method against amikacin, fosfomycin, cefoxitin, imipenem and levofloxacin. Susceptibility was also tested by the E-test (AB Biodisk) to determine the minimum inhibitory concentration (MIC) of fosfomycin and levofloxacin as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). After the entire agar surface was inoculated with the ESBL producers, a fosfomycin strip was applied on the left, and a levofloxacin strip on the right, with the MIC scale facing upward. After overnight incubation, the MIC (μg/ml) was read where the ellipse intersected the scale. Susceptibility percentage of ESBL producers could be resolved from the MIC interpretive standard of fosfomycin and levofloxacin for Enterobacteriaceae at susceptible points (< 64 mg/ml and < 2 μg/ml, respectively).[8] The MIC_{50} and MIC_{90} values is the MIC value when 90 and 50 percent of bacterial isolates are inhibited, and are obtained by plotting the MIC values of each of all isolates (43 ESBL-KP and 37 ESBL-EC) against the cumulative percentage of bacterial isolates inhibited by this concentration.

RESULTS

Three hundred and fifty-nine (21.1%) of 1,702 isolates of *K. pneumoniae* tested were found to be ESBL producers, and ESBL-EC were detected in 398 (13.0%) of 3,060 isolates (Table 1). ESBL-KP were recovered most frequently from sputum (25.7%), followed by urine (23.9%), fluid (22.9%), pus (21.4%), and blood (11.6%) specimens. ESBL-EC was found most frequently in sputum (25.4%) followed by pus (21.4%), fluid (16.8%) urine (12.6%), and blood (5.5%) (Table 2).

Table 3 shows the percentage of ESBL-KP (43 isolates) and ESBL-EC (37 isolates) susceptible to five antimicrobial agents by the disc diffusion method. All of these ESBL producers were susceptible to cefoxitin

and imipenem, followed by fosfomycin (88.4% and 97.3%), amikacin (44.2% and 78.4%), and levofloxacin (60.5% and 27.0%) for ESBL-KP and ESBL-EC, respectively. The MIC_{50} of fosfomycin againsts ESBL-KP and ESBL-EC was 12.0 µg/ml and 0.7 µg/ml, respectively. The MIC_{90} of fosfomycin againsts ESBL-KP and ESBL-EC was 32.0 and 1.8 µg/ml, respectively, (Table 4, Figure 1, and Figure 2). According to the MIC breakpoints, ESBL-KP and ESBL-EC were susceptible to fosfomycin at 90.7 percent and 100 percent, respectively. For levofloxacin, the percentage of susceptibility was 65.1 percent and 28.6 percent, respectively, (Table 4).

DISCUSSION

Since 1983, when *Klebsiella* species producing a plasmid-mediated ESBL was first reported in Germany, a significant increase in the ESBL rate has been reported from all parts of the world.[9] In the Asia-Pacific area (1998-1999), the ESBL-producing rate in *K. pneumoniae* and *E. coli* was 25.2 percent and 10.1 percent, respectively.[10] The western Pacific and European areas

Table 1. The prevalence of ESBL-producing *K. pneumoniae* and *E. coli* in Maharaj Nakorn Chiang Mai Hospital.

Bacteria	No. tested	ESBL producer (%)
K. pneumoniae	1,702	359 (21.1)
E. coli	3,060	398 (13.0)

Table 2. ESBL-producing *K. pneumoniae* and *E. coli* isolated from clinical specimens of Maharaj Nakorn Chiang Mai Hospital.

Specimen	*K. pneumoniae*		*E. coli*	
	No. tested	ESBL producer (%)	No. tested	ESBL producer (%)
Blood	112	11.6	237	5.5
Fluid	140	22.9	191	16.8
Sputum	499	25.7	181	25.4
Pus	215	21.4	327	21.4
Urine	543	23.9	1,824	12.6
Total	1,509	23.1	2,760	14.1

Table 3. The percentage of ESBL-producing *K. pneumoniae* (ESBL-KP) (43 isolates) and *E. coli* (ESBL-EC) (37 isolates) susceptible to five antimicrobial agents determined by the disc diffusion method.

Antimicrobial agents	% Susceptibility	
	ESBL-KP	ESBL-EC
Amikacin	44.2	78.4
Fosfomycin	88.4	97.3
Cefoxitin	100	100
Imipenem	100	100
Levofloxacin	60.5	27.0

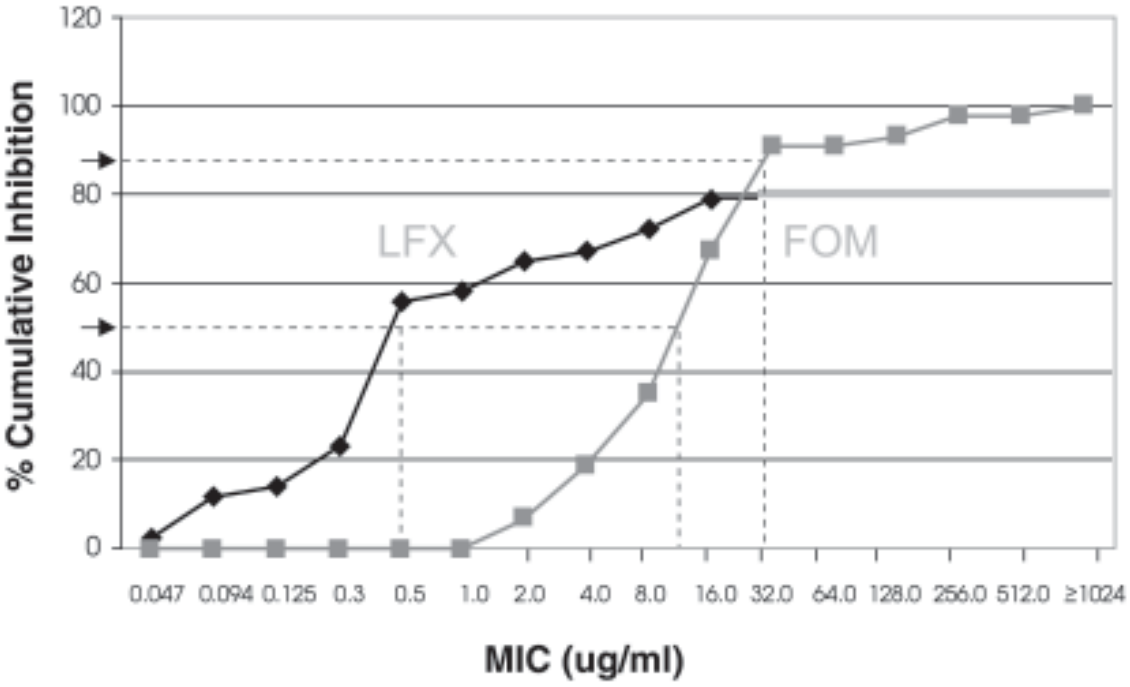

Figure 1. **Percentage cumulative inhibition of fosfomycin (FOM) and levofloxacin (LFX) against ESBL-producing *K. pneumoniae.* The MIC_{50} of FOM and LFX was 12.0 μg/ml and 0.5 μg/ml, respectively. The MIC_{90} of FOM and LFX was 32.0 μg/ml, and >32.0 μg/ml, respectively.**

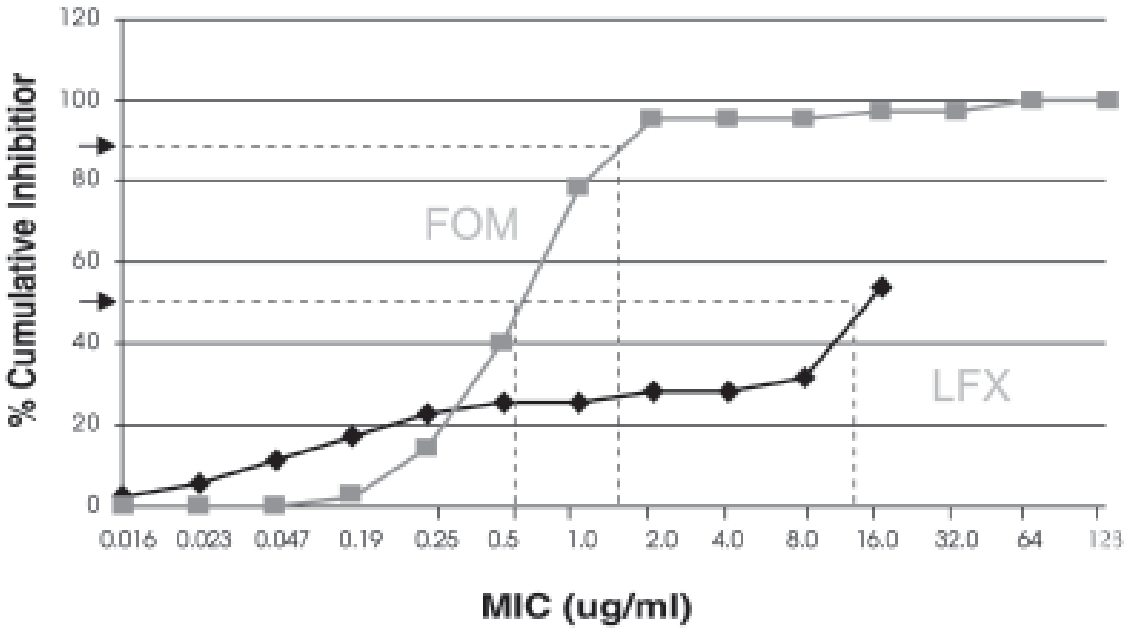

Figure 2. **Percentage cumulative inhibition of fosfomycin (FOM) and levofloxacin (LFX) against ESBL-producing *E. coli,* the MIC_{50} of FOM and LFX was 0.7 μg/ml and 16.0 μg/ml, respectively. The MIC_{90} of FOM and LFX was 1.8 μg/ml, and >32.0 μg/ml, respectively.**

Table 4. The MIC of fosfomycin and levofloxacin against ESBL-producing *K. pneumoniae* (43 isolates) and *E. coli* (37 isolates) by the E-test.

Bacteria/ antimicrobial agents	MIC (μg/ml)			% Susceptibility
	Range	MIC_{50}	MIC_{90}	
K. pneumoniae				
Fosfomycin	1.5 - >1024	~12.0	32.0	90.7
Levofloxacin	0.047 - >32	~0.5	>32	65.1
E. coli				
Fosfomycin	0.19 - 64	~0.7	~1.8	100
Levofloxacin	0.016 - >32	~16	>32	28.6

showed a prevalence of 24.6 percent and 7.9 percent; and 22.6 percent and 5.3 percent, respectively.[11] In our study, the prevalence of ESBL-KP and ESBL-EC was at 21.1 percent and 13.0 percent, respectively. In Latin America[11] and Bangkok (Siriraj Hospital)[12], the prevalence of ESBL-KP (45.4% and 37%, respectively) was quite high when compared to other areas such as Canada and the USA, Hong Kong, and Taiwan.[11,13,14]

Our study further tested the susceptibility of 43 ESBL-KP and 37 ESBL-EC isolates to fosfomycin and four additional antimicrobial agents by the disk diffusion method. All of these ESBL producers were susceptible to cefoxitin and imipinem. They were also susceptible to fosfomycin (88.4% and 97.3%), and amikacin (44.2% and 78.4%), respectively. Daza et al[15] used the automatic ASM Vitek (BioMerieux) for the antibiotic susceptibility testing of bacterial isolates from urine samples, and detected only seven ESBL-EC isolates from 1,580 *E. coli*. Similar to our study, all isolates of ESBL-KP were susceptible to cefoxitin and imipenem. In addition, 71 percent and 99 percent of *K. pneumoniae* and *E. coli* isolates were susceptible to fosfomycin. Contrary to our results, Daza et al found that all isolates of ESBL-EC were susceptible to amikacin.

Alhambra et al[16] tested the *in vitro* susceptibility to 13 antibiotics against the most common urinary pathogens by the agar dilution method. All isolates of *E. coli* and *K. pneumoniae* were susceptible to amikacin and imipenem. Fosfomycin had an MIC_{90} of >128 μg/ml for all isolates tested. The MIC_{90} of fosfomycin against *E. coli* and *Klebsiella* spp. was 8 μg/ml (a susceptible rate of 97.2%) and >128 μg/ml (a susceptible rate at 71.4%) respectively. In contrast, we found that both ESBL-KP and ESBL-EC were highly susceptible to fosfomycin (90.7% and 100%, respectively) with a lower MIC_{90} (32 μg/ml and 1.8 μg/ml, respectively). The difference may be partly explained by an underutilization of fosfomycin in our hospital.

Kusum et al[17] reported the detection rates of ESBL-KP in sputum, urine, and blood samples as of 30 percent, 23.8 percent, and 18.7 percent, respectively. These results were similar to our study which showed that ESBL-KP isolates were recovered most frequently from sputum (25.7%), followed by urine (23.9%) specimens. They also determined the susceptibility testing by a microdilution automatic method (VITEX system, BioMeriux). Similar to our results, all 70 ESBL-KP isolates were susceptible to imipenem, and the rate of susceptibility to levofloxacin was about 65 percent. Further clinical experience with cefoxitin and fosfomycin should be considered in order to more fully evaluate the potential usefullness of these antibiotics against serious infections due to ESBL producers.

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