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Activity of Fosfomycin in Extended-Spectrum Beta-Lactamases Producing *Klebsiella pneumonae* from Hospital Acquired Urinary Tract Infections

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Abstract

Treatment of hospital acquired urinary tract infections (UTIs) caused by extended-spectrum beta-lactamases producing *Klebsiella pneumonae* is a major problem. This organism expresses a high level of resistance to many groups of antibiotics. Fosfomycin is an agent which is recommended for treatment of UTIs caused by ESBLs producers. The aim of this study is to determine the sensitivity pattern of ESBLs producing urinary *K. pneumonae* to antimicrobial agents including fosfomycin in patients of MUHs and determine the prevalence of fosfomycin resistance mediated by plasmid mediated fosfomycin modifying enzymes *fosA*, *fosB* and *fosA*3. Methods: *Klebsiella pneumonae* urinary isolates were collected from patients with hospital acquired UTIs in Mansoura University Hospitals (MUHs). The susceptibility pattern was determined by Kirby Baur method. Isolates resistant to extended spectrum cephalosporins were tested for ESBLs production by double disc diffusion method. Fosfomycin resistance was determined by broth dilution method. Isolates resistant to fosfomycin were tested for *fosA*, *fosB* and *fosA*3 by PCR. Results: A total of 128 ESBLs producing *K. pneumonae* isolates were collected. The highest sensitivity was to imipenem (94.5%). The lowest was to trimethoprime-sulphamethoxazole (21.8%). Co-resistance of ESBLs isolates with fosfomycin was 23.2%. Eighteen fosfomycin resistant isolates (18/30) were positive to *fosA*. Conclusion: ESBLs producing urinary *Klebsiella pneumonae* express moderate sensitivity to fosfomycin. Resistance is mainly mediated by plasmid mediated fosfomycin modifying enzymes *fosA*.

Keywords

***Klebsiella pneumonae*, Extended-Spectrum Beta-Lactamases, Fosfomycin, Urinary Tract Infection, Plasmid Mediated Resistance**

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1. Introduction

Urinary tract infection (UTI) is one of the most common hospital-acquired infections (HAIs) especially in developing countries [1]. It represents about 40% of all hospital acquired infections and is mainly catheter associated [2]. Urinary catheterization for one week or more is associated with development of bacteriuria in at least 25% of patients with daily risk of 5% - 7% [3] [4].

Klebsiella pneumonia is an important cause of these infections. Production of extended-spectrum beta-lactamases (ESBLs) by *K. pneumonae* restricts the therapeutic options for treatment of infections they cause [5].

Therefore, treatment of ESBLs producing organisms which are resistant not only to cephalosporins but also to other agents like quinolones and aminoglycosides represents a major problem [6]. Co-resistance to these agents is very common, because the resistance genes are located on mobile genetic elements such as plasmids and transposons [7].

This rapid increase in the antibiotic resistance necessitates the implementation of alternative treatment strategies. With the limited availability of novel antimicrobial agents, the reevaluation of older antibiotic agents may show a ray of hope.

Among older antibiotics, fosfomycin appears to be an attractive alternative because of its broad spectrum of activity both against Gram-positive and Gram-negative bacteria and its wide distribution in tissues while retaining high serum level [8] [9].

Fosfomycin was discovered in Spain in 1969 from cultures of *Streptomyces*. It acts by inhibiting the formation of peptidoglycans during the bacterial cell wall synthesis. This antibiotic is frequently active against multidrug resistant and extremely resistant *Enterobacteriaceae* [9], and in particular against ESBLs producing *Klebsiella pneumonia* (ESBLs-KP) [10]. However, many studies showed that fosfomycin resistance increases in areas where it is used widely [11]. Many mechanisms were emerged; the most important is plasmid mediated fosfomycin modifying enzymes [12].

Although fosfomycin is known since more than four decades, few data are available about the effectiveness of this antibiotic against the uropathogens and in its role against ESBLs producing organisms in Egypt.

The current study was therefore performed to provide an insight into the antimicrobial activity of fosfomycin and other common antimicrobials against ESBL-KP isolates from hospital acquired UTI. Also, the role of plasmid mediated *fosA*, *fosB* and *fosA*3 in resistance to fosfomycin in ESBLs producing urinary *Klebsiella pneumonae*.

2. Methods

This study protocol was approved by Ethical committee in faculty of medicine, Mansoura University.

This study was conducted at microbiology department, faculty of medicine, Mansoura University. A prospective study was performed in Mansoura University hospitals between March 2014 and December 2015. Samples were collected from patients hospitalized for longer than 72 hours and developed UTI diagnosed according to the criteria described by the Centers for Disease Control/National Health Service Network (CDC/NHSN) and International Nosocomial Infection Control Consortium (INICC) guidelines [13] [14]. Samples were inoculated on cystine lactose electrolyte deficient (CLED) agar (Oxoid Ltd., Basingstoke, UK) using calibrated loop (10 µ). Cultures were incubated for 24 h at 37˚C, and they were considered positive if colony count $\geq 10^5$ CFU/ml. *Klebsiellia pneumonae* was identified using the usual identification methods (colony morphology, Gram stained film) and API 20 E (bioMerieux, Marcy-l'Etoile, France) [15] [16]. Antibiotic sensitivity testing was done by standard Kirby-Bauer disk diffusion method, on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK). All *Klebsiellia pneumonae* that were resistant to 3rd generation cephalosporins were tested for ESBLs production by double disc diffusion test. Sensitivity testing of fosfomycin was done by broth microdilution test; the broth was supplemented with glucose-6-phosphate (Sigma Aldrich) to a final concentration of 25 mg/L as per standard CLSI guidelines [17]. Isolates with intermediate sensitivity were considered as resistant.

Fosfomycin resistance by modifying enzymes was detected by PCR. Plasmid extraction was performed by the alkaline lysis method [18]. Primers used are shown in **Table 1**. The reactions were performed according to protocol described before [19] using a thermal cycler (MJ Research PTC-100). The amplification conditions were: 94˚C for 5 min, 35 cycles of denaturation at 93˚C for 40 s, annealing at 57˚C for 40 s, elongation at 72˚C for 40 s and a final extension of 3 min at 72˚C.

Statistical analysis was computed on Statistical Package for Social Sciences (SPSS, version 16.00; Chicago, IL, USA). Mean, standard deviation was used for descriptive data. The comparison of categorical data was per-

formed using the Chi-square test (χ^2). The difference between groups was considered statistically significant if p values were <0.05.

3. Results

A total of 128 ESBLs producing *Klebsiella pneumonia* were collected from patients with health care associated UTIs in MUHs. Females represent 62.5% (80/128) which was to some extent more than male patients 37.5% (48/128). Patients age range (18 - 72 years), the mean age was 41.7 with standard deviation 15.3 (**Table 2**). In the current study, all penicillins, cephalosporins, and aztreonam were reported as resistant in ESBLs producers according to CLSI recommendations [14]. The most effective antibiotics against ESBLs producing *Klebsiella pneumonae* were imipenem (94.5%), piperacillin-tazobactam (77.3%) and cefoperazone-sulbactam (76.6%). Fosfomycin sensitivity was 76.6% (98/128). The least sensitivity was to trimethoprim-sulphamethoxazole (21.8%) and ampicillin-sulbactam (24.2). Regarding fosfomycin resistant isolates, the most effective antibiotics were imipenem (98%), cefoperazone-sulbactam (80.6%), and piperacillin-tazobactam (74.5%) (**Table 3**).

Only 18 fosfomycin resistant isolates harboring fosfomycin resistant gene *fosA* (60%). No resistant isolates carries *fosA*3 or *fosB* genes.

No relation was found between resistance to fosfomycin and resistance to other drugs except imipenem and cefoperazone-sulbactam (P value < 0.05).

4. Discussion

There is a continuous increase in the antibacterial resistance of most bacterial species according to National Nosocomial Infections Surveillance (NNIS) data [23]. The resistance reached to a serious situation that multidrug resistant ESBLs producing bacteria causing severe UTI have been reported in hospital acquired infections [24] [25].

Beta-lactam/beta-lactamase inhibitor combination antibiotics may be effective as a therapy for extended-spectrum beta-lactamases producing *Enterobacteriaceae* [26].

The current study found that beta lactam-beta lactamase inhibitors combinations generally showed lower sensitivity against ESBL producers *Klebsiella pneumonae* except for piperacillin-tazobactam (77.3%) and cefoperazone-sulbactam (76.6%).

This low sensitivity is agreed with other reports by Jiang *et al.* [27] who found sensitivity to ampicillin-clavunlante and piperacillin-tazobactam were 40% and 60% respectively. Furthermore, other study by Chen *et al.* [28] reported the sensitivity to piperacillin-tazobactam in ESBLs producing *Klebsiella pneumonae* was (74.6%).

Table 1. Primer sequences of fosfomycin-modifying enzymes.

Gene	Primer	Size	Reference
fosA-F	5′-ACATATGCTGCAATCACTCAA-3′	434	[20]
fosA-R	5′-GTGATCAAGCCTCGTCTGAGG-3′		
fosB-F	5′-ATATGATCAAAGGAATAAATC-3′	434	[21]
fosB-R	5′-CATATGAAAATTCATATGAGG-3′		
*fosA*3-*F*	5′-GCGTCAAGCCTGGCATTT-3′	282	[22]
*fosA*3-*R*	5′-GCCGTCAGGGTCGAGAAA-3′		

Table 2. Patients' characters.

Sex	NO (%)
Male	48 (37.5)
Female	80 (62.5)
Age	
Mean ± SD (min-max)	41.7 ± 15.3 (18 - 72)

Table 3. Antibiotic sensitivity pattern of fosfomycin susceptible and fosfomycin resistant ESBLs producing *Klebsiella pneumoniae* isolates.

	Fosfomycin susceptible isolates		Fosfomycin resistant isolates		Total susceptibility No (%)	P value
	S No (%)	NS No (%)	S No (%)	NS No (%)		
Cefoperazone-sulbactam	79(80.6)	19 (19.4)	12 (40)	18 (60)	76.6 (82)	**0.001**
Norfloxacin	42 (42.9)	56 (57.1)	12(40)	18 (60)	54 (42.2)	0.8
Gentanicin	36 (36.7)	62 (57.1)	16 (53.3)	14 (46.7)	52 (40.6)	0.09
Amikacin	53 (54.1)	45 (45.9)	18 (60)	12 (40)	71 (55.4)	0.7
Nitrofurantoin	40 (40.8)	58 (59.2)	10 (33.3)	20 (66.7)	50 (39)	0.5
Trimethoprim-sulphamethoxazole	19 (19.4)	79 (80.6)	9 (30)	21 (60)	37 (21.8)	0.2
Amoxacillin-clavulanic	26 (26.5)	72 (73.5)	13 (43.3)	17 (56.7)	39 (30.4)	0.1
Ampicillin-sulbactam	25 (25.5)	73 (74.5)	6 (20)	24 (80)	31 (24.2)	0.6
Piperacillin-tazobactam	73 (74.5)	25 (25.5)	26 (86.7)	4 (13.3)	98 (77.3)	0.2
Imipenem	96 (98)	2 (2)	25 (83.3)	5 (16.7)	121 (94.5)	**0.008**

S: susceptible; NS: non-susceptible.

This decreased efficacy of beta lactam-beta lactamase inhibitors combinations may be due in the presence of a high bacterial load because of high inoculum infections which overcome the effect of beta-lactamase inhibitors [26] [29].

The ESBLs producing bacteria can acquire co-resistance to other classes of antimicrobial agents, such as fluoroquinolones, trimethoprim-sulphamethoxazole, and aminoglycosides, which are used for UTIs frequently [26]. Low susceptibility of these groups was reported in this study (42.2%, 21.8, 55.4%, and 40.6%) to norfloxacin, trimethoprim-sulphamethoxazole, amikacin and gentamicin respectively. This worldwide alarming with the limitation of newer antibacterial agents necessitates the reevaluation of older agents. One of these agent is; fosfomycin which is available as oral and IV [30]. The importance of fosfomycin for treatment of UTIs has been increased due to increased rate of resistance to other agents, good safety, resistance of anaerobic gut flora, and safe use during pregnancy. It is recommended for treatment of ESBLs producing organisms which become a serious problem in health care [6] [31]-[33]. Few studies encounter the resistance to fosfomycin. To our knowledge, the current study is the first study that reports the fosfomycin resistance in ESBL KP in Egypt. The fosfomycin resistance in this study was 23.4%. This resistance rate is similar to that reported before [34] [35]. This result is higher than other results obtained in other studies [26] [36].

Evaluating co-resistance pattern with other antibacterial agents that are used for treatment of UTIs and ESBLs producing organisms was somewhat helpful in predicting fosfomycin resistance. There is no statistical significance for any specific pattern except for imipenem and cefoperazone-sulbactam. This result is consistent with other reports before [34] [37].

So, it can be used properly for treatment of infections by strains resistant to other antibiotics.

Only 18 resistant isolates carry *fosA* gene, negative result was obtained for *fosA*3 and *fosB*. This result is similar to other reports which find that *fosB* is responsible for the fosfomycin resistance in Gram-positive bacteria like *Staphylococci* and *Enterococci* [21] [38] [39]. In addition, this result may reflect the responsibility of mechanisms other than fosfomycin modifying enzymes for the resistance to fosfomycin in ESBLs producing *Klebsiella pneumonae*. These mechanisms may be chromosomal mutation and fosfomycin degradation [40] [41].

This study has some limitations. First, unavailability of clinical data about patients enrolled in the study. Furthermore, this study represents a report for the sensitivity of fosfomycin *in vitro* rather than *in vivo* whereas clinical response to fosfomycin may be different from laboratory result. So, further studies about *in vivo* results of fosfomycin use for treatment of UTI caused by ESBLs producing *Klebsiella pneumoniae* are recommended and the effect of patients clinical condition on the response to fosfomycin.

5. Conclusion

Lower rate of resistance was reported to fosfomycin in ESBLs producing *Klebsiella pneumonae* (23.4%) compared to other agents like quinolones and aminoglycosides. So fosfomycin is suitable for treatment of UTIs caused by ESBL-KP resistant to other antibacterial agents. Fosfomycin resistance is mediated mainly by plasmid mediated fosfomycin modifying enzymes *fosA*.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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