

Incidence of Beta-Lactamase Enzymes among *Klebsiella pneumoniae* Isolates Causing Urinary Tract Infections in Aliabad, North-East Iran

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ABSTRACT

Aims: In the past decade, drug resistance in Gram negative bacilli has become a serious problem. The production of extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase, and metallo beta-lactamase (MBL) enzymes in *Klebsiella pneumoniae* strains is the mechanism of drug resistance among these commonly isolated Gram negative bacteria from clinical specimens. The aim of this study was to assess the frequency of β -lactamase enzymes, including extended spectrum β -lactamases (ESBLs), metallo- β -lactamases (MBLs), and AmpC beta-lactamases, in *K. pneumoniae* strains isolated from urine samples referred to medical laboratories in Aliabad.

Materials & Methods: A total of 780 urine samples were collected from patients suspected of having UTI from March to June 2017. In positive urine samples, *K. pneumoniae* isolates were identified by biochemical tests. Antibiotic resistance pattern was determined by disk diffusion method, and phenotypic confirmatory test was performed for detecting ESBLs, MBLs, and AmpC BLs producers.

Findings: Out of 378 positive samples for UTI, 97 *K. pneumoniae* strains were isolated. Most of the isolates (more than 90%) were resistant to ampicillin and amoxicillin; however, imipenem and amikacin were effective antibiotics against the isolates. The frequency of ESBLs, MBLs, and AmpC BLs producers was determined as 33.3, 21.3, and 5.1%, respectively.

Conclusions: In this study, 14 isolates were simultaneously positive for ESBL and AmpC BL production, and 2 isolates were co-producer of ESBL and MBL. This finding could have a great impact on the management and treatment of UTI cases. Therefore, detection of beta-lactamases is of great importance for controlling and reducing the spread of ESBL, AmpC BL, and MBL producing strains.

Keywords: Beta-lactamases, Incidence, *Klebsiella pneumoniae*, Urine, Antibiotic.

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Introduction

In recent years, overuse and misuse of beta-lactam antibiotics have led to the rapid emergence of antibiotic resistant bacteria [1], while drug resistance in Gram negative bacilli has become a serious challenge worldwide [2]. The most common resistance mechanism in Gram-negative bacteria is the production of beta-lactamase enzymes that hydrolyze beta-lactam ring of drugs such as cephalosporins and penicillins. Over the past two decades, new beta-lactam antibiotics that are specifically resistant to hydrolyzing by beta-lactamase enzymes have been developed. However, Gram-negative bacteria have developed new strategies to inactivate these novel antibiotics by producing new beta-lactamases, including AmpC β -lactamases, extended-spectrum β -lactamases (ESBLs), and metallo β -lactamases (MBLs). AmpC β -lactamases, which are a type of cephalosporinase, are also partially able to hydrolyze other beta-lactams. These enzymes hydrolyze broad-spectrum cephalosporins while not being inhibited by common inhibitors such as clavulanate [3].

ESBLs are plasmid-mediated enzymes that, in addition to mediating resistance to penicillins, mediate resistance to a wide range of cephalosporins, including third-generation cephalosporin and monobactams [4]. Beta-lactamase inhibitors such as clavulanic acid have inhibitory effects on the function of these enzymes [5]. The rate of ESBL production by the Enterobacteriaceae family members varies worldwide. Among the Enterobacteriaceae members, *Escherichia coli* is the highest ESBL producers, followed by *Klebsiella pneumoniae*. MBLs could hydrolyze a wide range of beta-lactam antibiotics, including penicillins, cephalosporins, carbapenems, cephamycins, but they could not hydrolyze aztroram, and their catalytic activity is

not inhibited by beta-lactamase inhibitors. MBLs are commonly found in *Pseudomonas aeruginosa* and *Acinetobacter* species; however, they have recently been increasing in the Enterobacteriaceae family members like *K. pneumoniae* and *E. coli* that exhibit notable drug resistance [2, 6].

K. pneumoniae is an opportunistic bacterium causing nosocomial and community-acquired infections [7]. It is the second leading cause of urinary tract infections (UTIs) after *E. coli* [8]. Currently, high resistance of *K. pneumoniae* to a broad spectrum of drugs including beta-lactam antibiotics, fluoroquinolones, and aminoglycosides has been reported [7]. As a result, infections caused by *K. pneumoniae* fail to respond to conventional treatments, which in turn increases morbidity, mortality, and health care costs [9].

Epidemiological studies on the antibiotic resistance pattern could help us choose an effective antibiotic for the treatment of infections such as UTIs, which are usually treated with empirical antimicrobial therapy [10].

Objectives: The present study was undertaken to know antimicrobial susceptibility pattern and the frequency of different types of beta-lactamase enzymes (ESBLs, AmpC BLs, and MBLs) in *K. pneumoniae* strains isolated from urine samples in Aliabad, North-East Iran, Golestan province.

Materials and Methods

Sample collection and identification of isolates: This cross-sectional study was conducted on urine samples referred to Aliabad medical laboratories from March to June 2017. Midstream urine samples were collected and evaluated for the presence of leucocytes and/or bacteriuria. It should be noted that a written informed consent was obtained from all patients, and their

data were recorded anonymously. The urine samples were cultured on blood agar and Eosin Methylene blue media (Merck, Germany) with the 0.001-mL loop. After overnight incubation at 37°C, all cultures with a bacterial count of $\geq 10^5$ CFU/mL were considered as positive for UTI and included in the study. Then biochemical tests including gram stain, TSI, IMViC, lysine iron agar, urea, and motility were performed on pure colonies to identify the isolates [11].

Antibiotic susceptibility test: To determine antibiotic sensitivity pattern of the isolates, the Kirby-Bauer disk diffusion method was used according to the CLSI guidelines [12]. The standard strains included in this study were *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 as quality controls. After overnight incubation at 37°C, the results were interpreted by measuring the inhibition zone diameter and comparing with the standards. The susceptibility of the isolates to each antibiotic was interpreted as sensitive (S), intermediate resistant (I), or resistant (R). The antibiotics disks used in this study were purchased from MAST Company.

Detection of Beta-lactamases ESBL: To determine ESBL-producing isolates, ceftazidime (CAZ) or cefotaxime (CTX) resistant isolates were screened for ESBL production by double disk-diffusion test (DDDT). For this purpose, Mueller-Hinton agar plates with the disks containing 30ug of CAZ and CTX, with and without 10ug of clavulanic acid were used. After incubation, the inhibition zones diameter of each isolate were measured on Mueller-Hinton agar plates. If the inhibition zone surrounding at least one combination disk was 5 mm larger than that produced around the corresponding antimicrobial disk without clavulanic acid, the isolate was considered as ESBL producer [12-13].

AmpC: Cefoxitin (30µg) resistant isolates were screened for AmpC β-lactamases

production by boronic acid double disk-diffusion test. For this purpose, two disks containing cefoxitin (30µg) and cefoxitin + boronic acid (30/400µg) were placed on the inoculated Muller-Hinton agar plates. After overnight incubation at 37°C, if the inhibition zone diameter surrounding the cefoxitin + boronic acid disk was 5mm greater than the inhibition zone diameter around the cefoxitin disk alone, AmpC production was considered as positive [14-15].

MBL: Imipenem (10µg) resistant isolates were screened for the presence of MBL by the IMP-EDTA double disk-diffusion test (DDDT). To perform the test, two disks containing imipenem (10µg) and imipenem + EDTA (10µg/750µg) were placed on the inoculated Muller-Hinton agar plates and incubated overnight at 37°C. If the inhibition zone diameter around the imipenem+ EDTA disk was 5mm greater than the inhibition zone diameter surrounding the imipenem disk alone, MBL production was considered as positive [16].

Findings

Sample collection and Identification of Isolates: From a total of 780 urine samples collected from March to June 2017, 378 samples (49.61%) with a colony count of $\geq 10^5$ CFU/mL were considered as positive for UTIs. Among which 270 (71.42%) samples were obtained from female patients, and 108 (28.57%) samples were obtained from male patients. Some personal and health related information about the patients is given in Table 1. The mean age of the patients was 45 years (SD=±20.21). Out of 378 samples, 97 samples (25.6%) were positive for the presence of *K. pneumoniae* strains.

Antibiotic Susceptibility Test: The antibiotic resistant pattern of *K. pneumoniae* isolates to 12 antimicrobial agents is shown in Table 2. Most of the isolates showed high resistance to ampicillin (92.7%), followed

Table 1) Summary of the Patients' Demographic Information

Characteristics	Number	Percent	Total Number
Sex	Male: 108	28.57	378
	Female: 270	71.42	
Pregnancy state	159	58.8	270
UTI history	106	28	378
Diabetes	53	14	378
Symptoms of prostatitis	17	15.74	108
History of antibiotic use in the past year	90	23.8	378
Marital status	306	80.95	378
Median age	45 (SD=±20.21)	-	-

by amoxicillin (90.7%) and tetracycline (63.9%). Most of the strains were sensitive to imipenem and amikacin (88.6%). Also, 68% of the isolates exhibited a multidrug resistance (MDR) phenotype.

β-lactamases Detection: *K. pneumonia* isolates resistant to ceftazidime and cefotaxime were examined for the presence of ESBLs by combined disk assay. Among 46 screened isolates, 39 (84.7%) isolates were found to be ESBL producer. Out of 30 isolates resistant to cefoxitin, 25 (83.3%) isolates were positive for AmpC BL production. These findings were obtained by respective phenotypic confirmatory test of combined disc method. Also, 14 isolates (11.9%) were found to be co-producer of ESBL and AmpC BL. Imipenem resistant isolates were selected for the detection of MBL production. Among which, 6 (6 of 8, 75%) isolates were MBL positive, and 2 isolates (1.7%) were positive for both ESBL and MBL. The frequency of ESBLs, AmpC BLs, and MBLs production within the selected *K. pneumonia* isolates is presented in Table 3. The isolates positive for all three enzymes were 100% resistant to ampicillin and amoxicillin. In addition, MBL-positive isolates were 100%

resistant to tetracycline.

Discussion

The present study was performed to determine antibiotic susceptibility pattern and the frequency of different types of beta-lactamase enzymes (ESBLs, AmpC BLs, and MBLs) in *K. pneumonia* strains isolated from urine samples in Aliabad, North-East Iran, Golestan province. According to the obtained results, 25.6% of the urine samples were positive for the presence of *K. pneumonia*. Most of the isolates showed high resistance to ampicillin and amoxicillin, consistent with similar studies performed in the past [17-20]. The highest bacterial susceptibility was observed to imipenem and amikacin. The susceptibility rate to these antibiotics was 88.6%, which is similar to the finding of a study by Baghani Aval et al. (2018) [21]. However, in other studies conducted in different regions of Iran, dissimilar rates of susceptibility have been reported [17-19]. The prevalence of ESBL-producing *K. pneumonia* strains varies in different regions. It could be as low as 8.3% in Nepal and as high as 58 and 67.2% in India and Egypt, respectively [4, 22-23]. In the present

Table 2) Antibiotic Resistance Pattern of 97 *K. pneumonia* Isolates to 12 Antimicrobial agents

Antimicrobial agents	Resistance No. (%)	Intermediate No. (%)	Sensitive No. (%)
Imipenem (10µg)	8 (8.2)	3 (3)	86 (88.6)
Ciprofloxacin (5µg)	28 (28.8)	9 (9.2)	60 (61.8)
Gentamicin (10µg)	20 (20.6)	6 (6.1)	71 (73.1)
Cefotaxime (30µg)	59 (60.8)	5 (5.1)	33 (34)
Ceftazidime (30µg)	46 (47.4)	8 (8.2)	43 (44.3)
Cefoxitin (30 µg)	30 (30.9)	6 (6.1)	61 (62.8)
Co-trimoxazole (1.25 µg)	29 (29.8)	5 (5.1)	63 (64.9)
Tetracycline (30 µg)	62 (63.9)	2 (2)	33 (34)
Amikacin (30µg)	11 (11.3)	-	86 (88.6)
Nalidixic acid (30µg)	30 (30.9)	3 (3)	64 (65.9)
Amoxicilin (30µg)	88 (90.7)	1 (1)	8 (8.2)
Ampicilin (10µg)	90 (92.7)	2 (2)	5 (5.1)

Table 3) Frequency of ESBLs, AmpC BLs, and MBLs production among total and selected *K. pneumonia* isolates

Enzymes	No. (%) of the Total Isolates	No. (%) of the Selected Isolates
ESBLs	39 of 117 (33.3)	39 of 46 (84.7)
AmpC BLs	25 of 117 (21.3)	25 of 30 (83.3)
MBLs	6 of 117 (5.1)	6 of 8 (75)
ESBLs & AmpC BLs	14 of 117 (11.9)	14 (11.9)
ESBLs & MBLs	2 of 117 (1.7)	2 (1.7)

study, the frequency of ESBLs producing *K. pneumonia* isolates was 33.3%, which is in line with the findings of other studies performed in Iran [18, 24]. Previous studies have shown that the prevalence of AmpC

BL-producing *K. pneumonia* strains differs across different geographical regions. It varies from 0% in Tabriz (northwestern Iran) to 38.6% in Taiwan [25-26]. In the present study, the frequency of AmpC BL producing *K.*

pneumonia isolates was 21.3%; this finding is similar with the result of another study by Kazemian et al. (2019) in Tehran and Ilam, Iran [27]. The prevalence of MBL-producing *K. pneumonia* strains in Iran varies between 0% in Isfahan (central Iran) to 43.4% in Tehran and Ilam, Iran [24, 27]; in this study, the frequency of MBL-producing *K. pneumonia* strains was 5.1%.

Based on the obtained results, the frequency of ESBL-producing *K. pneumonia* strains in this study was 33.3%, which is similar to the results of some Iranian studies, but the frequency distribution of AmpC and MBL enzymes was significantly different from the findings of other research performed in Iran [18, 24, 25, 27]. Currently, carbapenems are the most sensitive and reliable treatment options for infections caused by ESBL, AmpC BL, and MBL producing isolates. However, irrational use of carbapenems may lead to the development of resistant organisms. Therefore, knowledge of these organisms and their detection in different regions are of great importance for controlling their spread and helping physicians choose appropriate treatment. Finally, antibiogram testing prior to antibiotic prescribing by physicians, rational use of antibiotics, and avoiding self-medication are among the inevitable necessary measures.

Conclusion

In this study, the incidence of ESBL, AmpC BL, and carbapenemase production was investigated among the *K. pneumonia* isolates causing urinary tract infections in Aliabad, North-East Iran. In the present study, 14 isolates were simultaneously positive for ESBL and AmpC BL production, and 2 isolates were co-producer of ESBL and MBL. Consequently, this finding could have a major impact on the management of UTI cases inside and outside the hospitals. Therefore, restricting the use of carbapenems and

third-generation cephalosporins along with the application of infection control measures are the most effective means of controlling and reducing the spread of ESBLs, AmpC BLs, and MBLs producing strains.

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