

# Carbapenem Resistance Pattern of Multiple Drug-Resistant and Extended-Spectrum Beta-Lactamase-Positive *Klebsiella pneumoniae* in Isfahan

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**Background:** *Klebsiella pneumoniae* is a producer of carbapenemase (KPC) an emerging pathogen with propensity to malady in weak patients, increasing their mortality rates. Carbapenemase is an enzyme that destroys all beta-lactam antibiotics and is the therapeutic choice for infections with extended-spectrum beta-lactamase (ESBL)-producing organisms. ESBLs are penicillin, narrow spectrum also third-generation cephalosporin, and monobactams hydrolyser and checked by clavulanic acid.

**Objectives:** The present study was performed to separate and identify the carbapenemase resistance pattern of multidrug-resistant (MDR) and ESBL-positive *K. pneumoniae* as well as its prevalence among different wards and various clinical specimens in Isfahan.

**Patients and Methods:** Over 500 different clinical samples were collected from different sections of great teaching hospitals in Isfahan, in which *K. pneumoniae* isolates were identified by IMVIC and urease standard biochemical tests and also were confirmed by determination of the *ureD* Gene. Antimicrobial susceptibility tests were performed as standard disk-diffusion on Mueller-Hinton agar (Merck, Germany) based on the instructions of Clinical Laboratory Standards Institute (CLSI, 2013). Sieving and phenotype conformation of ESBL isolates were performed by double disc synergy test (DDST), and then, the strains identified as ESBL were tested by carbapenem, ertapenem, imipenem and meropenem. Finally, the statistical analyses were performed using the WHONET software version 5.6.

**Results:** Of clinical isolates of *K. pneumoniae*, 142 were confirmed using biochemical methods and then the molecular confirmation was performed by PCR of the *ureD* gene. Of the total isolates, 57% were from males and 43% from females; 120 (84%) of isolates were recognized as MDR. The highest rates of resistance were related to piperacillin (80%), ceftazidime (76%), and cefotaxime (73%). Among these MDR isolates, 101 (71%) were detected as ESBL, using DDST. The ward and the clinical specimen with the most prevalence were ICU with 55 (38.7%) and urine with 61 (42.9%) samples, respectively. The lowest prevalence was related to the neurosurgery ward with 8 (5.6%) samples and the clinical specimen with the lowest prevalence was cerebrospinal fluid (CSF) with 2 (1.4%) samples. The susceptibility patterns to carbapenem agents were as follows: ertapenem 50.7%, meropenem 44.8% and imipenem 35.8%.

**Conclusions:** In this study, the prevalence of carbapenem-resistant *K. pneumoniae* was high in positive ESBL isolates, which can create significant therapeutic problems. According to the resistance pattern of ESBL-positive isolates for carbapenems in this research, ertapenem can probably serve as a suitable therapeutic option for uncomplicated infections by ESBL-producing *K. pneumoniae* instead of imipenem and meropenem.

**Keywords:** *Klebsiella pneumoniae*; Multiple Drug Resistance; Carbapenem

## 1. Background

*Klebsiella pneumoniae*, the producer of carbapenemase, is an emerging pathogen with propensity of causing disease in weak patients with high mortality rates (1). The emergence and universal spread of *Enterobacteriaceae*, the producer of carbapenemase, is an important infection threat in bedridden sensitive patients in hospitals and a serious threat for communities (2, 3). Carbapenemases are the most withering enzymes produced by all beta-lactam antibiotics. All carbapenems like imipenem have broad antimicrobial spectrums (2). Carbapenemases are classified as ambler class A and bush functional group

2f; they differ in metallo-beta-lactamases, which require divalent cations as metal cofactors for their activities (4). Beta-lactamases are hydrolyzing enzymes for beta-lactam antibiotics like penicillin, cephalosporin, carbapenems and monobactams. Third-generation cephalosporins (3GCs) are extended-spectrum cephalosporins, resistant to hydrolysis by beta-lactamases. However, in mid 1980s, new types of beta-lactamases emerged that could also hydrolyze extended-spectrum cephalosporins, which were named "extended-spectrum beta-lactamases" (ESBLs) (5). ESBLs hydrolyze bounded-spectrum penicillins, as well

as third-generation cephalosporins and mono bactams. ESBLs have rates of hydrolysis for ceftazidime, cefotaxime, and aztreonam (amino thiazoleoxime beta-lactam antibiotics), which are inhibited by clavulanic acid. In general, the fourth-generation cephalosporin, cefepime, is less effective against ESBL-producing organisms (5). Moreover, ESBL-producing organisms have resistance to other classes of antibiotics; as a result, treatment options are limited. Resistant organisms are now a major problem in the world (6). Their number is continuously increasing and the therapeutic options are limited. Knowing the prevalence of these organisms is essential and new health policies should be adopted (6). Even though *K. pneumoniae* has intrinsic resistance to ampicillin, it does not show this resistance to extended-spectrum beta-lactam antibiotics.

Many factors can cause bacterial resistance to antibiotics. The bacteria withstanding toward environmental conditions can perform resistant strains which can grow and survive against antibiotics, which may be dangerous. This has led to production of ESBL in *K.pneumoniae* (7). ESBLs are clavulanate-susceptible enzymes which can hydrolyze oxyimino-cephalosporins and monobactams, but cannot hydrolyze cephamycins and carbapenems. The bacteria that produce ESBLs are major medical problems, limiting the antibiotic choice. ESBL determinative isolates are often nosocomial agents (7). Infections with resistant isolates have been emerging as important challenges in medical systems. Antimicrobial resistance is associated with implications including mortality in crescent, length of hospital stay, and care cost increase. In addition, delay in definitive treatment, inferior definitive therapy, and higher virulence of some resistant strains can cause complications for patients (8). Carbapenems are stable to AmpC and ESBLs (9). This group is a suitable therapeutic choice for resistant Gram-negative bacterial infections (9).

## 2. Objectives

The present study was done as separating and identifying of carbapenems resistance pattern from MDR *K. pneumoniae* and positive ESBL and its separation among different wards and among the clinical specimens in Isfahan

## 3. Materials and Methods

### 3.1. Sampling

Over 500 clinical samples including urine, trachea, bronch, blood, sputum, ascetic fluid, cerebrospinal fluid (CSF), abscess, and wound were collected from different wards (infant, internal, ICU, surgery, emergency, etc.) of the chosen educational hospitals in Isfahan, among which, 142 (28%) *K. pneumoniae* isolates were declared by IMVIC biochemical standard tests (indole, methyl red, Voges-Proskauer, and citrate) and urease test. After wards

they were confirmed, as explained before, by determination of the *ureD* gene (243bp), responsible of urea hydrolysis (10). *K. pneumoniae* ATCC 700603 was considered as positive control.

The target gene primers were prepared and lyophilized (10), which are listed below:

*ureD* F: 5'-CCCCTTTTACCCGGAAGAAG-3'

*ureD*R: 5'-GGAAAGAAGATGGCATCCTGC-3'

### 3.2. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility was tested by the standard method, Kirby-Bauer disk-diffusion on Mueller-Hinton agar (Merck, Germany). The primary sieving of *K. pneumoniae* isolates as ESBL producers was performed using antibiotic discs (Mast, England). The performance and interpretation were performed based on the instructions of the Clinical Laboratory Standards Institute (CLSI, 2013). Below is a list of the tested antimicrobial agents: ceftazidime, cefepime, aztreonam, levofloxacin, ticarcillin, cefotaxime, amoxicillin/clavulanic acid, piperacillin/tazobactam, piperacillin, gentamicin, amikacin, and ciprofloxacin. *Escherichia coli* ATCC 25922 was used as negative control.

### 3.3. Screening and Phenotypic Identification of Extended-Spectrum Beta-Lactamases by Double Disc Synergy Test

In primarily sieving of resistant isolates or with low susceptibility more than one, third generation cephalosporin(3GC-ceftazidime, cefotaxime) was recognized as probable ESBL (11). Phenotypic confirmation of double disc synergy test (DDST) was performed. In this test, a pure culture (0.5 McFarland std.) of the tested strains was spread on Mueller-Hinton agar (MHA) plates. Antibiotic disks of ceftazidime and ceftazidime-clavulanic acid were placed with 15 mm distance from each other. The plates were then incubated at 37°C in aerobic conditions. The antimicrobial agents with more than 5 mm increase in diameter for the complex of antimicrobial and clavulanic acid in comparison with the diameter of the antimicrobials agent alonewere considered as ESBL. *E.coli* ATCC 25922 was used as negative control and *K. pneumoniae* ATCC700603 as positive control (10).

### 3.4. Carbapenem-Resistant Strains Screening

The strains recognized as ESBL were tested by carbapenems including ertapenem, imipenem and meropenem. The performance and interpretation were assessed according to the instructions of CLSI 2013 recommendations.

### 3.5. Statistical Analysis

Finally, the statistical analyses were performed by Who net software version 5.6, which analyzed the data based on CLSI 2013.

#### 4. Results

A total of 142 clinical isolates of *K.pneumoniae* were determined with biochemical methods. Afterwards, molecular confirmation was performed by PCR of the *ureD* gene. Analysis for presence of the *ureD* gene demonstrated that all the isolates confirmed as *K. pneumoniae* were positive for the *ureD* gene.

Among the 142 clinical isolates of *K.pneumoniae*, 57% were from males and 43% from females. In this study, MDR *K.pneumoniae* was defined as the resistant isolate to at least three classes of antimicrobial agents (4); 120 (84%) isolates were recognized as MDR. The analysis of the resistance rate between the antibiotics is shown in Figure 1.

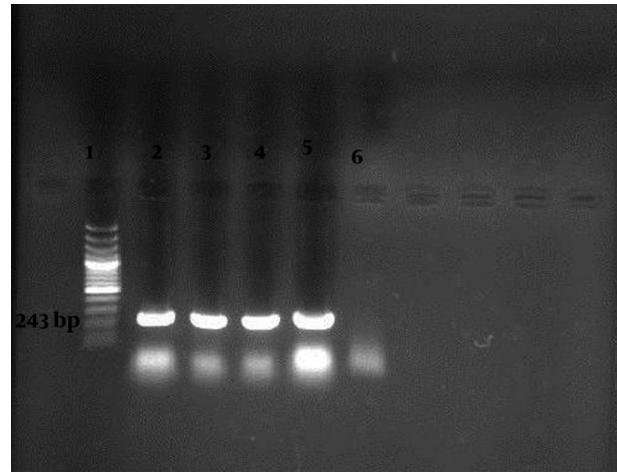
The highest resistance rates were related to piperacillin (80%), ceftazidime (76%) and cefotaxime (73%) and the lowest to ertapenem (47.3%), meropenem (50.8%) and imipenem (58.7%). At the end, determination of ESBL *K.pneumoniae* isolates was performed for 101 (71%) isolates. The results of DDST of separate *K.pneumoniae* strains of pure materials with positive and negative controls re-shown in Figures 2 and 3.

The prevalence rates of the enzyme between various clinical specimens (urine, trachea, brunch, blood, sputum, ascetic fluid, CSF, abscess and wound) and from different wards (infant, Internal, ICU, surgery, emergency, etc.) are shown in Tables 1 and 2.

The highest rates of *K.pneumoniae* was isolated from the

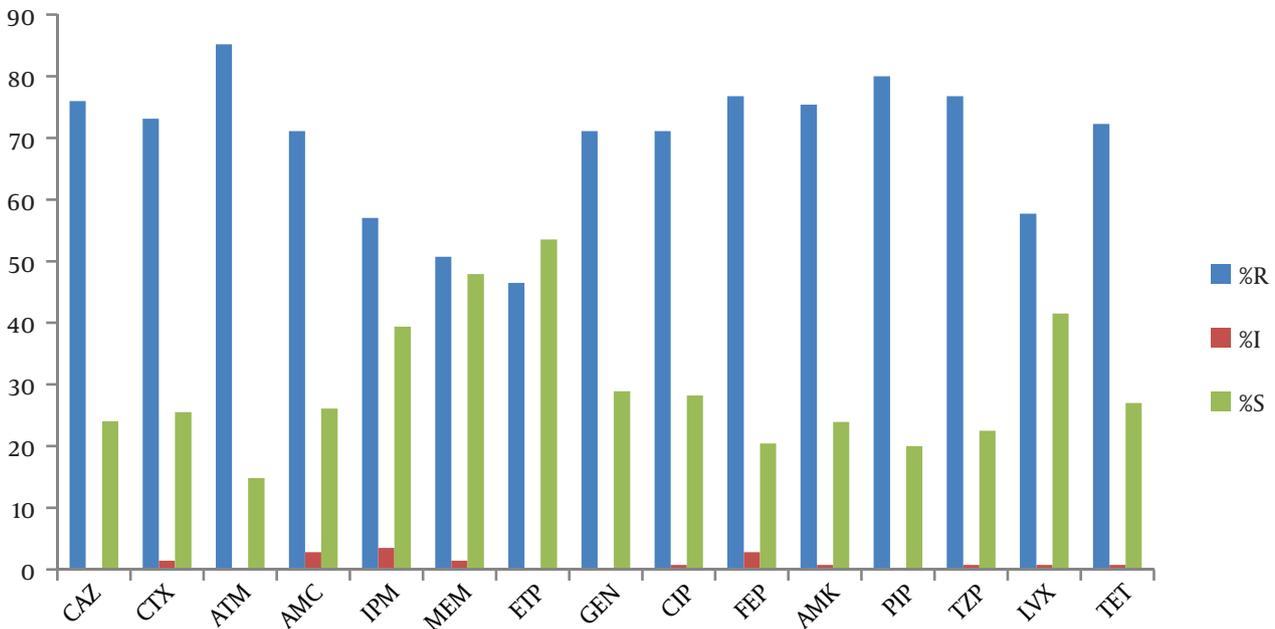
urine samples [61(42.9%)] and the ICU ward [55 (38.7%)]; the lowest included two samples (1.4%) from CSF and 8 (5.6%) from the neurosurgery ward. The susceptibility patterns of carbapenem agents are as follows: ertapenem (50.7%), meropenem (44.8%) and imipenem (35.8%).

Figure 1. Agarose Gel Image of the *ureD* Gene

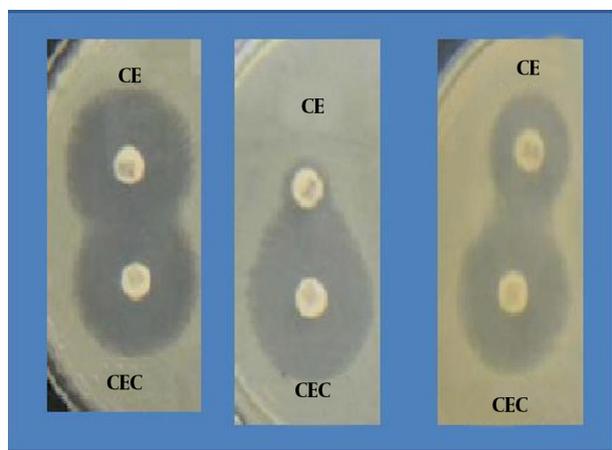


Lane 1, ladder; lane 2, positive control (*K. pneumoniae* ATCC700603); lanes 3-5, test isolates; lane 6, negative control (*E. coli* ATCC 25922)

Figure 2. Pattern of Resistance to Antimicrobial Agents Among All *Klebsiella pneumoniae* Strains



Abbreviations: CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; AMC, amoxicillin clavulanic acid; IPM, imipenem; MEM, meropenem; ETP, ertapenem; GEN, gentamicin; CIP, ciprofloxacin; FEP, cefepime; AMK, amikacin; PIP, piperacillin; TZP, piperacillin/tazobactam; LVX, levofloxacin; TET, tetracycline; R, resistance; S, sensitive; I, intermediate.

**Figure 3.** The Result of Double Disc Synergy Test of *K. pneumoniae* Strains

CE, cefotaxime (30 g); CEC, cefotaxime + clavulanic acid (30 + 10 g); 1, negative control; 2, test isolate; 3, positive control.

**Table 1.** Prevalence of *Klebsiella pneumoniae* Extended-Spectrum Beta-Lactamase Among the Different Clinical Specimens <sup>a,b</sup>

Specimen Type	Number of Patients	ESBL	
		+	-
Abdominal fluid	5 (3.5)	5	0
Abscess	3 (2.1)	1	2
Blood	3 (2.1)	2	1
Bronchial	14 (9.8)	14	0
Catheter	7 (4.9)	7	0
Cerebrospinal fluid	2 (1.4)	2	0
Sputum	4 (2.8)	3	1
Tracheal	28 (19.8)	23	5
Urine	61 (42.9)	36	25
Wound	14 (9.8)	8	6
<b>Total</b>	<b>142 (100)</b>	<b>101 (71)</b>	<b>31 (29)</b>

<sup>a</sup> Abbreviation: ESBL, extended-spectrum beta-lactamase.

<sup>b</sup> Data are presented as No. (%).

**Table 2.** Prevalence of *Klebsiella pneumoniae* Extended-Spectrum Beta-Lactamase Among Various Clinical Wards <sup>a</sup>

Clinical Ward	Number of Isolates (%)	ESBL	
		+	-
Infant	9 (6.3)	4	5
Internal	27 (19)	20	7
ICU	55 (38.7)	45	10
Surgery	27 (19)	16	11
Emergency	16 (11.2)	9	7
Neurosurgery	8 (5.6)	7	1
<b>Total</b>	<b>142 (100)</b>	<b>101 (71)</b>	<b>31 (29)</b>

<sup>a</sup> Abbreviation: ESBL, extended spectrum beta lactamase; ICU, intensive care unit.

**Table 3.** The Carbapenem Resistance Patterns Among the Extended-Spectrum Beta-Lactamase-Positive Isolates <sup>a</sup>

Code	Number	R, %	I, %	S, %
IPM	142	58.7	3.5	37.8
MEM	142	50.8	1.4	47.8
ETP	142	47.3	0	52.7

<sup>a</sup> Abbreviations: IPM, imipenem; MEM, meropenem; ETP, ertapenem; R, resistance; S, sensitive; I, intermediate.

## 5. Discussion

It has been more than two decades that carbapenems have been used as the latest treatment solution for MDR infections caused by *Enterobacteriaceae*. Carbapenemases that can disable carbapenems have been increasingly reported in Asia and Europe and recently in Canada and the United States (12). In 1996, the first isolate of *K. pneumoniae* carbapenemase (KPC) was isolated from a clinical specimen from a hospital in North Carolina in the Intensive Care Antimicrobial Resistance Epidemiology (ICARE) surveillance program (12). Nowadays, carbapenems are the last required therapeutic strategy for treatment of nosocomial infections and increased resistance to this class of antibiotics has left no effective drug in the health care system (13). However, because of such reports, the number of *Enterobacteriaceae* resistant to carbapenems is increasing (14, 15). This type of resistance may be due to decrease of bacteria exterior membrane permeability, overexpression of beta-lactamase enzymes, or carbapenems expression (13, 14).

Carbapenems such as imipenem and meropenem are used as the first treatment strategies for infections by ESBL-producing *Enterobacteriaceae*. Resistance to carbapenem has several combinatory mechanisms: changes in exterior membrane permeability and changes in efflux pump, associated with increase in production of *ampC* beta-lactamases (cephalosporinases) or ESBLs or production of specific carbapenem-hydrolyzing beta-lactamases (carbapenemases) (16). Infection with MDR strains increases the hospital stay and the surgery care costs of the patient. In our study, about 84% (120/142) of all the isolates were declared as MDR. This finding was harmonic with previous studies performed in Iran, Pakistan, Mexico and India, in which MDR *K. pneumoniae* was reported (17). We recognized that the prevalence of ESBL-producing *K. pneumoniae* was 71% (101/142). This rate was similar to neighbor Asian countries, India (66.7%), Turkey (54.7-61%), the United Arab Emirates (41%), Kuwait (31.7%), and Iran (72.1%) and different from Saudi Arabia (25.2%) (17-20). The overall incidence of ESBL production has been different in various geographical regions. It might be due to differences in the types and amounts of antibiotics consumption as well as different sample collection methods. However, it reflects the chronological increase in prevalence of ESBLs in Iran. In this study, the majority of ESBL-producing isolates were achieved from the urine

samples, which was similar to other studies (17, 21). The prevalence of carbapenems resistant to *K.pneumoniae* in this study was between 58.7-47.3 in ESBL-positive isolates that created significant therapeutic problems. Regarding the resistance pattern of ESBL-positive isolates to carbapenems in this research, ertapenem can be considered as a suitable therapeutic switch for infections with ESBL-producing *K.pneumoniae* instead of imipenem and meropenem.

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## Authors' Contributions

Study concept and design: Hossein Fazeli. Acquisition of data: Razie Kamali Dolatabadi and Masoumeh Norouzi. Analysis and interpretation of data: Razie Kamali Dolatabadi and Azade Taraghian. Drafting of the manuscript: Razie Kamali Dolatabadi. Statistical analysis: Razie Kamali Dolatabadi. Administrative, technical, and material support: Razie Kamali Dolatabadi and Azade Taraghian. Study supervision: Hossein Fazeli and Bahram Nasr Isfahani and Sharareh Moghimi.

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