Full Length Research Paper

Identification of extended-spectrum-beta-lactamases(ESBLs), metallo-beta-lactamases (MBLs), Amp-C and KPC β -lactamases among *Klebsiella pneumoniae* isolated from adults and pediatric patients in Iran

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Klebsiella pneumoniae is one of the major bacteria that cause acute infections. β -lactamases are main defensive mechanisms in bacteria against drugs. The aim of this study was to determine antibiotic resistance patterns and detection of extended-spectrum-beta-lactamase (ESBL), MBL, Amp-C and KPC β -lactamases among *K. pneumoniae* strains isolated from adults and infants. This descriptive study was done on 83 *K. pneumoniae* isolated from two hospitals. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion and broth microdilution methods according to Clinical Laboratory Standards Institute (CLSI) guidelines. ESBLs, MBL, Amp-C and KPC producing strains were detected by phenotypic confirmatory test, combination disk diffusion test (CDDT), Amp-C detection kit and Modified Hodge test, respectively. From 83 *K. pneumoniae* strains, 48 (57.5%), 3 (3.5%), 23 (28%) and 5 (6%) were ESBL, MBL, Amp-C and KPC positive, respectively. In this study, Fosfomycin and Tigecycline were more active than other antibiotics. The results demonstrated that incidence of ESBLs, Amp-C, MBLs and KPC was critical especially in infants. Therefore, detection of drug resistance patterns in *K. pneumoniae* and β -lactamases producing isolates is important for the prevention and control of these infections.

Key words: *Klebsiella pneumoniae*, infants, extended-spectrum-beta-lactamase (ESBL), Amp-C, MBLs, KPC.

INTRODUCTION

Klebsiella pneumoniae is an opportunistic pathogen and a common cause of nosocomial infections such as pyogenic liver abscesses, meningitis, endophthalmitis, pneumonia, septicemias, urinary tract infections and soft tissue infections. Suitable options have been restricted for the treatment of infections with increase of multi-drug

resistant (MDR) bacteria especially, *K. pneumoniae* nosocomial isolates (García-Sureda et al., 2011). It has been a very big problem treating infants infections due to MDR organisms (Roy et al., 2011). In some countries, many infant deaths occur as a result of MDR bacteria. Enzymes are major defensive mechanisms in some bac-

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teria against drugs (Drawz et al., 2010). The most clinically important enzymes are KPC-enzyme (Ambler class A), MBL enzymes (class B), Amp-C (class C) and ESBLs (class A), identified in *K. pneumoniae* as a source of nosocomial outbreaks in hospitals (Poirel et al., 2011; Thomson et al., 2010). ESBLs are currently reported as a major problem of drug resistance in *K. pneumoniae* (Ruiz de Alegria et al., 2011). ESBLs are enzymes that hydrolyze cephalosporins, penicillins and aztreonam and encoded by mobile elements such as plasmids. ESBL producing bacteria are usually resistant to drugs. Since ESBL genes are transmissible to other bacteria, it is necessary that ESBLs be tested for other bacteria in hospital and long-term care facility patient groups where ESBLs are encountered. Bacteria including both ESBL and AmpC enzymes are becoming more common (Thomson et al., 2010).

AmpC β -lactamases constitute a group of enzymes widely spread in *Klebsiella pneumoniae*. They inactivate cephalosporins such as ceftazidime, cefotaxime and cefpodoxime. Plasmid-mediated AmpC enzymes, which originated from chromosomal AmpC of variety organisms, have been reported since the 1980s (Mammeri et al., 2010). Carbapenem-hydrolyzing enzymes belonging to Ambler classes A, B, and D have been reported in the world among *Klebsiella pneumoniae*. Most of those bacteria are MDR. *K. pneumoniae* possessing the KPC β -lactamases is now spreading in some medical centers in USA and is being increasingly reported worldwide (Landman et al., 2009). KPC-producing isolates of *K. pneumoniae* and other bacteria have spread rapidly in the world especially in New York. Other reports have been described in other areas, including China, Greece, Denmark, Argentina, Hungary, Brazil, Colombia, Finland, France, Italy, Germany, Sweden, Puerto Rico, Norway and the United Kingdom (Chen et al., 2011a). These strains typically possess an underlying ESBLs or Amp-C enzymes (Landman et al., 2009), which are a molecular class A serine enzyme belonging to functional group 2f, that can hydrolyze carbapenems, penicillins, cephalosporins and aztreonam. These enzymes can be inhibited by clavulanic acid and tazobactam (Fontana et al., 2010). The metallo- β -lactamases, which have been reported in the 1960s, were detected in some countries. In the 1990s, with the spread of enzymes encoding metallo- β -lactamases located on mobile elements such as plasmids, these β -lactamases increased in *K. pneumoniae* such as New Delhi metallo-beta-lactamase (NDM), Imipenemase (IMP), Australia imipenemase (AIM), Seoul imipenemase (SIM), Verona integron-encoded-metallo- β -lactamase (VIM), German imipenemase (GIM), Dutch imipenemase (DIM) and São Paulo metallo- β -lactamase (SPM) types (Cornaglia et al., 2011).

Recently, NDM-1 emerged as a global threat because the bacteria which possess this metallo- β -lactamase are resistant to almost all β -lactam antibiotics, aminoglycosides, fluoroquinolones and other classes of drug agents. The NDM-1 gene is located on plasmid or chromosome. The

rapid emergence of NDM-1 has been related to transmissible plasmids which can transfer among different isolates; subsequently, NDM-1 can spread throughout the world (Kus et al., 2011; Diene et al., 2011; Liang et al., 2011; Ong et al., 2011). So far, in Iranian studies on different bacteria, the emphasis has been on identification of Ambler class A and class D and also three reports on Ambler class B beta-lactamases (Fallah et al., 2011). The aim of this study was to determine antibiotic resistant patterns and detection of beta-lactamase types in *K. pneumoniae* isolated from hospitalized patients in Mofid Children and Taleghani hospitals, Tehran, Iran during 2012 year.

MATERIALS AND METHODS

Bacterial isolates

From October 2011 to May 2012, 83 non-duplicate and non-consecutive *Klebsiella pneumoniae* that consisted of males 27 (32.53%), females 14 (16.86%) and infants 42 (50.60%) were collected from hospitalized patients in Mofid Children and Taleghani hospitals, Tehran, Iran. The isolates were stored at -20°C in trypticase soy broth containing 20% glycerol.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of each *Klebsiella pneumoniae* isolates was determined by the Kirby-Bauer disk diffusion method (Mast Group, Merseyside, UK) on Mueller Hinton agar (Merck, Germany) and interpreted as recommended by Clinical Laboratory Standards Institute (CLSI, 2012) or FDA breakpoints (tigecycline) guidelines. Disks of Penicillins [Piperacillin (PIP, 100 μ g), Ampicillin (AMP, 10 μ g)], Beta lactam/beta lactamase inhibitor combinations [Piperacillin/Tazobactam (PTZ, 100/10 μ g)], Cepheims [Ceftazidime (CAZ, 30 μ g), Cefotaxime (CTX, 30 μ g), Cefepime (FEP, 30 μ g), Ceftriaxone (CRO, 30 μ g), Cefpodoxime (CPD, 30 μ g)], Monobactams [Aztreonam (ATM, 30 μ g)], Carbapenems [Imipenem (IPM, 10 μ g), Meropenem (MEM, 10 μ g), Doripenem (DOR, 10 μ g), Ertapenem (ETP, 10 μ g)], Aminoglycosides [Gentamicin (GEN, 10 μ g), Amikacin (AK, 30 μ g)], Tetracyclines [Tetracycline (TE, 10 μ g)], Fluoroquinolones [Ciprofloxacin (CIP, 5 μ g)], inhibitors [Trimethoprim-sulfamethoxazole (TS, 2.5 μ g)], Fosfomycins [Fosfomycin/Trometamol (FOT, 200 μ g)], Tigecycline (TGC, 15 μ g) and were used and *Escherichia coli* ATCC25922 was used as a control strain.

Minimum inhibitory concentration (MIC)

Antimicrobial drug susceptibilities were determined according to the guidelines of the CLSI by broth microdilution. Muller-Hinton broth (Merck, Germany) with distinct dose of imipenem, meropenem, Cefepime, Ampicillin, Piperacillin/Tazobactam, Cefotaxime, Ceftriaxone and Ceftazidime (GLAXO England Co and Himedia) was prepared. After shaking, 0.1 ml of diluted drug was added to each well of 96-well microtiter plates. Microbial suspensions were adjusted to 0.5 MacFarland and diluted in 1:10 with broth to yield 10^7 CFU/ml and to each well, 0.005 ml of the bacterial inoculums was seeded. Control line with no bacterial inoculation and *Escherichia coli* ATCC25922 was simultaneously maintained. Microplates were incubated at 37°C for 18 to 24 h. The lowest concentration of the drugs that produced no visible bacterial growth

was reported as the MIC.

Phenotypic detection of MBL

Combination disk diffusion test (CDDT) was performed for identification of MBLs by imipenem, meropenem, doripenem and ertapenem (Mast Group, Merseyside, UK) alone and in combination with EDTA.

The inhibition zones of the Imipenem and Imipenem + EDTA, Meropenem and Meropenem + EDTA, Doripenem + EDTA, Ertapenem and Ertapenem + EDTA discs were compared after 18 h of incubation in air at 37°C. A zone diameter difference between the discs and discs + EDTA ≥ 7 mm was interpreted as a positive result for MBL production (Galani et al., 2008).

Phenotypic detection of ESBL

Detection of ESBLs was tested for all the isolates by Combination Disk Diffusion Test (CDDT) containing Ceftazidime (CAZ) and Cefotaxime (CTX) with CAZ 30 μ g + CA 10 μ g and CTX 30 μ g + CA 10 μ g per disc (Mast Group, Merseyside, UK). The zone of inhibitions was compared for the CTX, CAZ discs with that of the CAZ 30 μ g + clavulanic (CA) 10 μ g and CTX 30 μ g + CA 10 disc. An increase in zone diameter of ≥ 5 mm in the presence of clavulanic acid indicated the presence of ESBL in the test organism. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC700603 were used as negative and positive controls for ESBL production, respectively.

Phenotypic detection of KPC

Identification of KPC enzyme was performed for all the *K. pneumoniae* by Modified Hodge Test. 0.5 McFarland dilution containing *E. coli* ATCC 25922 was prepared in 5 ml of broth and then diluted in 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of Mueller Hinton broth (Merck, Germany). A lawn of the 1:10 dilution of *E. coli* ATCC 25922 was streaked to a Mueller Hinton agar (Merck, Germany) plate and allow to dry for 4 to 5 min. 10 μ g Ertapenem susceptibility disc (Mast Group, Merseyside, UK) was placed in the center of the test area.

In a line, test bacteria were streaked from the edge of the disc to the edge of the plate; and incubated for 18 to 24 h at 37°C. After 18 to 24 h of incubation, they were examined for a clover leaf-type indentation at the intersection of the test bacteria and *E. coli* ATCC25922, within the zone of inhibition of the Ertapenem susceptibility disc.

Phenotypic detection of Amp-C

The detection kit for Amp-C was developed by mast group containing 3 discs-labeled A, B and C. 1: Disc A contains Cefpodoxime and an Amp-C enzyme inducer. 2: Disc B contains Cefpodoxime, an Amp-C enzyme inducer and an ESBL enzyme inhibitor. 3: Disc C contains Cefpodoxime, an Amp-C enzyme inducer, an ESBL enzyme inhibitor, and an Amp-C enzyme inhibitors. ESBL enzyme inhibitor is included the disc set (discs B and C) for identification of the existence of Amp-C enzyme when the bacteria are also co-producing ESBL enzyme. Bacteria were tested employing on plates of Mueller Hinton agar (Merck, Germany).

The discs were located on the inoculated agar surface and incubated at 37°C for 18 to 24 h, and after incubation the zones of inhibition were measured; zones with a ≥ 5 mm increase in diameter around disc C compared with both discs A and B were considered positive for Amp-C existence (Halstead et al., 2012).

Statistical analysis

Our study is descriptive and experimental; therefore to analyze the results, we used MINITAB16 software. P.value and confidence intervals are <0.05 and 95%, respectively.

RESULTS

Clinical data, susceptibility testing, and screening for ESBL, MBL, Amp-C and KPC producers

The isolates were 45 (54.21%) of Taleghani and 38 (45.79%) of Mofid Children hospitals and belonged to males 27 (32.53%), females 14 (16.86%) and infants 42 (50.60%). The number of *K. pneumoniae* strains isolated from patients was 83 followed by 20 (24.09%), 7 (8.43%), 4 (4.81%), 0 (0.0%), 4 (4.81%), 4 (4.81%) and 44 (53.01%) in the age group >60 years, 50 to 59 years, 40 to 49 years, 30 to 39 years, 20 to 29 years, 8 to 19 years and 0 to 7 years, respectively. The source of studied isolates was as follows: Pediatric unit, 38 (45.7%); outpatient, 13 (15.6%); intensive care units (ICUs), 9 (10.8%); surgery, 7 (8.4%); NICU, 4 (4.8%); bone marrow transplant unit, 3(3.6%); hematology, 2(2.4%); endocrine, 2(2.4%); digestion, 2(2.4%); orthopedic 1(1.2%) and others, 2(2.4%).

Bacterial isolates were recovered from different clinical specimens: urine, 33 (39.7%); blood culture, 27(32.5%); wound, 7(8.4%); sputum, 7 (8.4%); Intra-abdominal, 4 (4.8%); cerebrospinal fluid, 1(1.2%) and other samples, 4 (4.8%). Table 1 summarizes the clinical data of 48 isolates of ESBL-producing *K. pneumoniae* collected from Mofid Children and Taleghani Hospitals, Tehran, Iran. Antimicrobial drug-resistance patterns of 83 *K. pneumoniae* isolates were ATM (49%), MEM (20%), GEN (29%), CIP (46%), AK (12%), CAZ (46.7%), IMI (20%), CTX (50%), FEP (36.15%), TE (33%), AMP (65%), PIP (50%), CRO (49%), CPD (57%), TGC (5%), DOR (20%), ETP (21%), PTZ (22%), TS (50%) and FOT (3%) (Table 2) and MIC isolates are shown in Table 3. The Combination Disk Diffusion Test (CDDT) was applied for detection ESBLs in 83 *Klebsiella pneumoniae* isolates using Ceftazidime and Cefotaxime alone and in combination with clavulanic acid that 48(57.5%) isolates were positive for production of ESBL that 28(62.3%) belonged to Taleghani Hospital and 20(52.7%) to Mofid Children hospital (Figure 1). Combination disk diffusion test showed that 3(3.5%) of 83 isolates of *K. pneumoniae* collected from Mofid Children and Taleghani Hospitals were MBL positive (Figure 2).

Amp-C screening by Mast Group kit showed that 23 (28%) of isolates were Amp-C positive that belonged to Taleghani Hospital 14(31.3%) and 9(23.7%) to Mofid Children Hospital (Figure 3). Modified Hodge Test (MHT) was applied to detect KPC in 83 *K. pneumoniae* that 5(6%) of isolates were KPC positive (KPC-positive isolates were 3(6.7%) of Taleghani Hospital and 2(5.3%)

Table 1. Distribution of ESBL, MBL, Amp-C and KPC-producing *K. pneumoniae*

Strain ID	Hospital	Age	Sex	Sample	Date of isolation	Ward	ESBL	MBL	AmpC	KPC
K1	A	80	M	Sputum	12.10.2011	ICU	+	-	+	+
K2	A	53	F	Urine	11.10.2011	Surgical	+	-	+	-
K4	A	79	M	Sputum	23.10.2011	ICU	+	-	+	-
K5	A	63	M	Sputum	01.11.2011	Orthopedic	+	-	+	-
K7	A	Newborn	F	Unknown	07.11.2011	NICU	+	-	+	-
K9	A	43	M	Wound	07.11.2011	Surgical	+	-	+	-
K12	A	52	M	Blood	07.11.2011	ICU	+	-	+	-
K13	A	83	M	Wound	10.11.2011	ICU	+	-	+	+
K16	A	Newborn	F	Blood	01.12.2011	NICU	+	-	-	-
K17	A	49	M	Urine	05.12.2011	Digestion	+	-	+	-
K20	A	60	M	Intra-abdominal	28.01.2012	Digestion	+	-	-	-
K21	A	61	F	Blood	02.02.2012	Hematology	+	-	-	-
K22	A	Newborn	M	Sputum	04.02.2012	NICU	+	-	-	-
K23	A	48	F	Sputum	09.02.2012	ICU	+	-	+	-
K24	A	73	F	Urine	11.02.2012	Surgical	+	-	-	-
K27	A	75	F	Urine	12.02.2012	Outpatient	+	-	-	-
K30	A	60	M	Intra-abdominal	17.02.2012	ICU	+	-	-	+
K33	A	80	M	Sputum	19.02.2012	ICU	+	-	-	-
K34	A	Newborn	F	Urine	20.02.2012	NICU	+	-	-	-
K35	A	45	F	Blood	21.02.2012	Outpatient	+	+	+	-
K36	A	20	F	Sputum	22.02.2012	Bone marrow	+	-	-	-
K38	A	Unknown	F	Unknown	06.12.2011	Outpatient	+	-	-	-
K40	A	85	M	Urine	05.12.2011	Outpatient	+	+	+	-
K41	A	15	M	Intra-abdominal	05.12.2011	Outpatient	+	-	-	-
K42	A	58	F	Urine	05.12.2011	Outpatient	+	-	-	-
K43	A	22	M	Wound	12.02.2012	Surgical	+	-	+	-
K44	A	66	F	Urine	12.02.2012	ICU	+	-	+	-
K45	A	68	F	Urine	12.02.2012	Outpatient	+	-	-	-
K46	B	Infant	M	Urine	02.05.2011	Pediatric	+	-	+	-
K47	B	Infant	M	Blood	03.05.2011	Pediatric	+	-	+	-
K49	B	Infant	M	Urine	06.05.2011	Pediatric	+	-	-	+
K50	B	Infant	F	Urine	07.05.2011	Pediatric	+	-	-	-
K51	B	Infant	M	Blood	09.05.2011	Pediatric	+	-	+	-
K57	B	Infant	F	Urine	05.06.2011	Pediatric	+	-	-	-
K58	B	Infant	F	Urine	13.06.2011	Pediatric	+	-	+	-
K60	B	Infant	M	Urine	18.06.2011	Pediatric	+	-	-	-
K64	B	Infant	F	Blood	05.07.2011	Pediatric	+	-	-	-
K66	B	Infant	M	Blood	14.07.2011	Pediatric	+	-	-	-
K69	B	Infant	F	Urine	05.08.2011	Pediatric	+	+	+	-
K70	B	Infant	M	Urine	09.08.2011	Pediatric	+	-	+	-
K71	B	Infant	F	Urine	12.08.2011	Pediatric	+	-	-	-
K72	B	Infant	F	Urine	14.08.2011	Pediatric	+	-	+	+
K75	B	Infant	M	Blood	28.08.2011	Pediatric	+	-	+	-
K76	B	Infant	M	Blood	06.09.2011	Pediatric	+	-	-	-
K78	B	Infant	M	Blood	15.09.2011	Pediatric	+	-	+	-
K79	B	Infant	M	Urine	16.09.2011	Pediatric	+	-	-	-
K81	B	Infant	F	Urine	26.09.2011	Pediatric	+	-	-	-
K83	B	Infant	M	Blood	02.10.2011	Pediatric	+	-	-	-

A, Taleghani Hospital; B, Mofid Children Hospital; M, male; F, female; +, positive; -, negative.

Table 2. Antimicrobial susceptibility testing results of 83 isolates of *Klebsiella pneumoniae* collected from Mofid Children and Taleghani Hospitals, Tehran, Iran.

Antibiotic	Resistant no. (%)	Intermediate no. (%)	Sensitive no. (%)
Aztreonam (10 µg)	49(59)	3(3.6)	31(37.3)
Meropenem (10 µg)	20(24)	2(2.4)	61(73.5)
Gentamicin(10 µg)	29(35)	3(3.6)	51(61.5)
Ciprofloxacin (30 µg)	46(55.5)	4(4.8)	33(39.7)
Amikacin (30 µg)	12(14.4)	4(4.8)	67(80.7)
Ceftazidime (30 µg)	46(55.4)	3(3.6)	34(41)
Imipenem (10 µg)	20(24)	2(2.4)	61(73.5)
cefotaxime(30 µg)	50(60.2)	2(2.4)	31(37.3)
Cefepime (FEP, 30 µg)	30(36.15)	9(10.8)	44(53.05)
Tetracycline(TE, 10 µg)	33(39.7)	3(3.6)	57(68.6)
Ampicillin (AMP, 10 µg)	65(78.3)	2(2.4)	16(19.2)
Piperacillin (PIP, 100 µg)	50(60.2)	0(0.0)	33(39.7)
Ceftriaxone (CRO, 30 µg)	49(59)	2(2.4)	32(38.5)
Cefpodoxime (CPD, 30 µg)	57(68.6)	3(3.6)	23(27.7)
Tigecycline (TGC, 15 µg)	5(6.02)	25(30.1)	53(63.8)
Doripenem (DOR, 10 µg)	20(24)	1(1.2)	62(74.6)
Ertapenem (ETP, 10 µg)	21(25.3)	2(2.4)	57(68.6)
Piperacillin/Tazobactam (PTZ, 100/10 µg)	22(26.5)	5(6.02)	56(67.4)
Fosfomycin/Trometamol (FOT, 200 µg)	3(3.6)	12(14.4)	68(82)

Table 3. Microbiological activities of various antimicrobial agents against 83 *K. pneumoniae* isolates.

Antibiotics	MIC(µg/ml)		
	Range	50%	90%
Meropenem	0.25-256	1	32
Imipenem	0.25-256	1	16
Ceftazidime	1->256	64	>256
Ceftriaxone	0.5->256	16	>256
Cefepime	0.5->256	16	>256
cefotaxime	0.5->256	16	>256
Piperacillin/Tazobactam	0.25-256	4	128
Ampicillin	2->256	256	>256

of Mofid Children Hospital (Figure 4). β -Lactamases content showed in *K. pneumoniae* alone or together (Table 4).

DISCUSSION

Effective use of microbiology laboratories is essential for controlling the spread of multi-drug resistant (MDR) pathogens and thereby decreasing the need to use drugs. Infection control is very beneficial to patients and also decreases mortality and morbidity rate in the world. The experiment in diagnostic microbiology is vital for detecting many resistant bacteria. Many bacteria may not be recognized because they have false resistance or

susceptibility in conventional tests. This can lead to patients receiving unsuitable drugs and contribute to the spread of the bacteria to other patients. Therefore, the identification of such "Cryptic" resistance is so vital. Due to the fact that susceptibility assays may be unreliable, beneficial assays are required to detect the resistant mechanisms involved. These mechanisms are AmpC β -lactamases, extended-spectrum-beta-lactamases (ESBLs) and carbapenemases of molecular classes A and B especially KPC and NDM-1 (Thomson et al., 2010). This study exhibits that Fosfomycin and Tigecycline were more active than the other antibiotics. Leone et al. (2012) study showed that of 136 *K. pneumoniae*, 37.1% were MDR and ESBL producers and *K. pneumoniae* strains were resistant to fluoroquinolones,

Figure 1. ESBL screening by CDDT showed that 48(57.5%) of isolates were ESBL positive. A CAZ disc, B CAZ 30µg+CA 10 µg disc, C CTX disc and D CTX 30µg+CA 10 µg disc.

Figure 2. MBL screening by CDDT that 3(3.5%) of isolates were MBL positive. A MEM(10 µg) disc, B MEM(10 µg) +EDTA(10µL), C IMI(10 µg) disc, D IMI (10 µg) +EDTA(10µL), E DOR(10 µg) disc, F DOR(10 µg) +EDTA(10µL), G ETP(10 µg), H ETP(10 µg) +EDTA(10µL) disc and I EDTA disc.

gentamycin and 71% were resistant to imipenem. This study shows that 48 (57.5%), 3 (3.5%), 23 (28%) and 5 (6%) were ESBL, MBL, Amp-C and KPC positive, respectively. ESBL-producing *K. pneumoniae* were obtained from 24 adults and 33 pediatric patients in two hospitals.

This study confirms the relevant role of ESBL-KP as a pathogen in neonatal units. In the Taleghani Hospital, of 28 positive isolates, eight of ESBL-producing strains were

Figure 3. AmpC screening by Mast Group kit showed that 23(28%) of isolates were Amp-C enzyme positive. Disc A contains cefpodoxime and an Amp-C enzyme inducer. Disc B contains cefpodoxime, an Amp-C enzyme inducer and an ESBL enzyme inhibitor. Disc C contains cefpodoxime, an Amp-C enzyme inducer, an ESBL inhibitor, and an Amp-C enzyme inhibitors. 1 CAZ disc, 2 CTX disc, 3 CAZ 30µg+CA 10 µg disc, 4 CTX 30µg+CA 10 µg disc and 5 TGC disc.

Figure 4. KPC screening by MHT that 5 (6%) of isolates were KPC positive. (1) Positive control, (2) negative control, (3) clinical isolate, positive result, (4) *E. coli* ATCC 25922, (5) ETP disc.

related to ICU ward and four of ESBL-producing *K. pneumoniae* were detected in NICU ward and two isolates have simultaneously ESBL + Amp-C and KPC enzymes.

Table 4. Number and percent β -lactamases present in *Klebsiella pneumoniae* collected from Mofid Children and Taleghani Hospitals, Tehran, Iran.

β -Lactamases content	Taleghani no. (%)	Mofid Children No. (%)	Total no. (%)
ESBL	28(62.3)	20(52.7)	48(57.5)
MBL	2(4.4)	1(2.6)	3(3.5)
AmpC	14(31.3)	9(23.7)	23(28)
KPC	3(6.7)	2(5.3)	5(6)
ESBL + MBL	2(4.4)	1(2.6)	3(4)
ESBL + AmpC	11(24.4)	12(31.6)	23(28)
ESBL + KPC	3(6.7)	2(5.3)	5(6)
ESBL + MBL + AmpC	2(4.4)	1(2.6)	3(3.5)
ESBL + AmpC + KPC	2(4.4)	1(2.6)	3(3.5)
ESBL + MBL + KPC	0(0)	0(0)	0(0)
ESBL + MBL + KPC + AmpC	0(0)	0(0)	0(0)

In a research in Spain, the results revealed that 133 adults and 29 pediatric patients were infected with ESBL-producing *K. pneumoniae* (Ruiz de Alegría et al., 2011). The wide use of drugs might facilitate the spread of antibiotic resistance, and thus control of beta-lactamases; especially, ESBL-producing *K. pneumoniae* within infant units should be considered as a precedence. Shigemoto et al showed that of 2,113 isolates of *K. pneumoniae*, 52 were ESBL-positive. Among the ESBL-positive *K. pneumoniae*, five isolates were also MBL positive (Shigemoto et al., 2012). In this study, 2 (4.4%) of adults and 1 (2.6%) of infants were MBL positive and 14 (31.3%) of adults and 9 (23.7%) of infants were Amp-C positive. The prevalence of Amp-C in Iran and the entire world is unknown, because laboratories have problem detecting this resistant mechanism accurately. Rodriguez-Bano et al showed that 100 patients were infected with plasmid-mediated Amp-C beta-lactamases including 17 Amp-C-producing *K. pneumoniae* (Rodríguez-Baño et al., 2012).

A study showed that 127 (49.8%) of the test isolates (*Proteus*, *E. coli* and *K. pneumoniae*) were Amp-C positive (Tan et al., 2009). In this study, 3 (6.7%) adults and 2 (5.3%) infants were KPC positive. In a research in China, the results revealed that 70.6% (77/109) of *K. pneumoniae* were KPC positive and 59.6% (65/109) of isolates were ESBL + KPC positive (Chen et al., 2011b). A study showed two patients with multidrug-resistant KPC-carbapenemase-producing *K. pneumoniae* urinary tract infections (Virgincar et al., 2011).

Our study is the first on infants that harbour types KPC and MBL as well as on ESBL + AmpC + KPC and ESBL + MBL + AmpC in adults and children of Iran. Microbiology laboratories must be able to identify resistant bacteria in a timely manner, especially those that have false susceptibility *in vitro* to antibiotics that may be considered for therapy of infected adults and infants.

Bacteriological excellence is needed more than ever,

and it is vital that ESBLs enzymes, AmpC and carbapenemases be promptly and accurately detected.

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