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Antimicrobial Susceptibility and Distribution of TEM and CTX-M Genes among ESBL-producing Klebsiella pneumoniae and Pseudomonas aeruginosa Causing Urinary Tract Infections

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Abstract

***Background*:** Extended spectrum beta lactamases (ESBLs) have been observed in nearly all the species of family *Enterobacteriaceae.* The enzymes are plasmid mediated and are derived from broad-spectrum beta lactamase TEM and CTX- M by a limited number of mutations. This study was undertaken to characterize ESBL producers among *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by PCR, which were initially screened by phenotypic method.

***Materials and Methods*:** A cross-sectional study was performed to evaluate 180 strains (30 *K. pneumoniae* and 150 *P. aeruginosa*) isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections during a period of six months. The prevalence of ESBL producing *K. pneumoniae* and *P. aeruginosa* was evaluated by disk diffusion test and polymerase chain reaction (PCR) by detecting TEM and CTX-M gene.

***Results*:** The results of the study revealed that the prevalence of ESBL producing *P. aeruginosa* and *K. pneumoniae* by disk diffusion test was 13.3% for *P. aeruginosa* and 66.6% for *K. pneumoniae.* Seventy five percent and 65% of *K. pneumoniae* harboured the gene TEM and CTX-M, respectively. Forty five percent of *P. aeruginosa* isolates harboured the gene TEM but none of them demonstrated the gene CTX-M using PCR method.

***Conclusion*:** ESBL producing *P. aeruginosa* and *K. pneumoniae* isolates showed a high prevalence in this study. Therefore it seems that continuous surveillance is essential to monitor the ESBLs producing microorganisms in hospitals and community.

Introduction

Development of microbial resistance to antibiotics is a global concern. Isolation of microbial agents less susceptible to regular antibiotics and appearence of increasing resistant isolates during antibacterial therapy is rising throughout the world. *Klebsiella pneumoniae* is an important pathogen that causes urinary tract infections (UTIs), pneumonia, and intra-abdominal infections in hospitalized immunocompromised patients with severe underlying diseases [1]. Resistance of *K. pneumoniae* to many antibiotics such as extended spectrum cephalosporins due to plasmid mediated enzymes (extended spectrum beta-lactamases (ESBLs) results in treatment failure of infections caused by these isolates [2]. *Pseudomonas aeruginosa* can colonize human body sites, with a preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat; as well as stools. The prevalence of colonization by *P. aeruginosa* in healthy subjects is usually low, but higher colonization rates can be observed following hospitalization, especially amongst subjects treated with broad-spectrum antimicrobial agents special for extended spectrum cephalosporin. Colonization is common in the respiratory tract of mechanically ventilated patients, in the gastrointestinal tract of patients receiving anticancer chemotherapy, and on the skin of burn patients. Also, sinks, mops, disinfectant solutions, respiratory equipment, food mixers and other moist environments can act as reservoirs of *P. aeruginosa* in the hospital setting [3, 4]. Therefore, it is important to find out resistance in antimicrobial agents used for this purpose. There is a problem that occurred among ESBL-producing Enterobacteriaceae which have widely caused the spread of infections worldwide [5-7]. Although, today hundreds variant of ESBLs have been described [8, 9], but the most common of them are derivatives of TEM or SHV enzymes. Also, in recent years non-TEM and non-SHV ESBLs have been reported, mainly the CTX-M enzymes [7-11]. The aim of this study was to evaluate the antimicrobial susceptibility of ESBL producing *K. pneumoniae* and *P. aeruginosa* from hospitalized patients with urinary tract infection (UTI) and the distribution of TEM and CTX-M genes among ESBL-producing *K. pneumoniae* and *P. aeruginosa* causing Urinary Tract Infection.

Materials and Methods

Isolation of bacteria: All 180 strains (30 Klebsiella pneumoniae and 150 Pseudomonas aeruginosa) isolated from urine culture of hospitalized patients (Amir Al-Momenin Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2010- 2011 were evaluated. Isolated bacteria were identified by Gram's stain and standard biochemical tests [12].

Phenotypic detection of ESBLs: ESBLs producing strains detected using single or combined ceftazidime-clavulanic acid disks (MAST Co. UK). This was a combination test for phenotypic confirmatory of ESBLs [13, 14]. Phenotypic detection of ESBLs was defined by an increase ≥5 mm in the inhibition zone around clavulanic acid disk comparing with zone around the disks of without clavulanic acid. For activity of antibacterial assay, bacterial suspensions with concentration of 1.5×10^8 cfu/ml (0.5 McFarland standard) were prepared in nutrient broth. Oxoid combination disk method was used for detection of ESBLs producing organisms. In this method the bacteria were cultured on a Muller-Hinton agar plate, then ampicillin (25 µg), Trimethoprim-Sulfamethoxazol (1.25+23.15), ceftazidime (30 µg), gentamicin (10 µg), nitrofurantoin (30 µg), nalidixin acid (30 µg) and ciprofloxacin (30 µg) disks (Mast, UK) were placed on media in 20-30 mm with other disks. The plates were incubated for 18-24 h at 37ºC [15].

DNA extraction, PCR: The colonies of ESBLs producing organisms were suspended in (Tris+ EDTA) TE buffer and their DNA were extracted by simple boiling [16]. The PCR method for detection of TEM and CTX- M gene was performed as described previously with minor modifications [17]. Briefly, blaTEM and blaCTX-M genes were detected using specific pair of primers (Moosavian and Deiham, 2010) (Table 1). The PCR mixture consisted of 10 pmol of each primers, 1 µl DNA sample (3 µg/µl), 1.5 mM MgCl2, 0.2 mM each dNTP, and 5 U Taq DNA polymerase (Cinagen, Iran) in a total number of 50 µl of PCR reaction. Amplification of TEM gene was performed by following program: initial denaturation at 94ºC for 2 min and 35 cycles of 1 min at 94ºC, 30 sec at 52ºC and 1 min at 72ºC. Five min at 72ºC was considered for the final extension. Then, PCR products were analyzed by agarose gels electrophoresis.

Statistical Analysis: All the experiments and measurement were repeated at least three times. Descriptive statistical analyses were performed using SPSS and Excel 2010 software.

Results

Klebsiella pneumoniae: Twenty (66.6%) out of 30 *K. pneumoniae* isolates were ESBLs producing organisms by disc diffusion.Antibiotic susceptibility of ESBL producing *K. pneumoniae* was evaluated for 6 antimicrobial. However, overall *K. pneumoniae* were resistance to 6 of the agent including ampicillin (65%), gentamicin (30%), trimethoprim-sulfamethoxazol (25%), ciprofloxacin (20%), nitrofurantoin (15%) and nalidixic acid (15%). As was shown in table 2, the most frequent resistance was observed for ampicillin and the least for ciprofloxacin. The amplification of TEM and CTX- M of *K. pneumoniae* revealed that 15 (75%) and 13 (65%) of the isolates harboured those genes and the rest were negative (Table 4).

Pseudomonas aeruginosa: Twenty (13.3%) out of 150 *P. aeruginosa* isolates were ESBLs producing organisms by disc diffusion. Antibiotic susceptibility of ESBL producing *P. aeruginosa* was evaluated for 6 antimicrobial. However, overall *P. aeruginosa* were resistance to 6 of the agent including ampicillin (85%), trimethoprim-sulfamethoxazol (65%), nitrofurantoin (65%), nalidixic acid (65%), gentamicin (25%), and ciprofloxacin (15%). As was demonstrated in table 3 the most frequent resistance was seen for ampicillin, trimethoprim-sulfamethoxazol, nitrofurantoin and nalidixic acid; and the least for ciprofloxacin. The amplification of TEM and CTX- M of *P. aeruginosa* revealed that 9 (45%) of the isolates harboured the TEM gene and none of them demonstrated the CTX- M gene (Table 5).

Table 1. The oligonucleotide primers used for amplification of Beta - lactamase genes

NO	Primers	Sequences
1	TEM	5' ATAAAATTCTTGAAGACGAAA 3' 5' GACAGTTACCAATGCTTAATCA 3'
2	CTX-M	5' TTAATGATGACTCAGAGCATTC 3' 5' GATACCTCGCTCCATTTATTG 3'

Table 2. Percentage of antimicrobial susceptibility of 20 strains of Klebsiella pneumoniae

	Am (%)	Fm (%)	NA (%)	CP (%)	SXT (%)	CAZ (%)	Gm (%)
S	0	80	60	70	70	0	20
I	35	60	25	10	5	0	50
R	65	30	15	20	25	100	30

Gm= gentamicin, CAZ= ceftazidime, SXT= sulfamethoxazol-trimethoprim, CP= ciprofloxacin, NA= nalidixic acid, Fm= nitrofurantoin, AM= ampicillin
S= Sensitive, I= Intermediate, R= Resistant

Table 3. Percentage of antimicrobial susceptibility of 20 strains of Pseudomonas aeruginosa

	Am (%)	Fm (%)	NA (%)	CP (%)	SXT (%)	CAZ (%)	Gm (%)
S	0	60	10	60	20	0	20
I	15	20	25	25	15	0	55
R	85	20	65	15	65	100	25

Gm= gentamicin, CAZ= ceftazidime, SXT= sulfamethoxazol-trimethoprim, CP= ciprofloxacin, NA= nalidixic acid, Fm= nitrofurantoin, AM= ampicillin
S= Sensitive, I= Intermediate, R= Resistant

Table 4. Frequency of TEM and CTX-M genes in ESBLs-positive Klebsiella pneumoniae

	CTX-M gene	TEM gene	Am	Fm	NA	CP	SXT	CAZ	Gm
1	+	+	R	I	S	S	S	R	I
2	+	-	R	I	S	S	S	R	S
3	-	+	I	I	I	S	S	R	S
4	+	+	I	I	R	S	S	R	S
5	+	+	I	I	S	S	S	R	S
6	+	+	R	R	S	S	S	R	I
7	-	-	I	R	S	S	S	R	I
8	+	+	R	I	S	S	S	R	I
9	+	-	R	I	S	S	S	R	I
10	+	+	R	I	S	S	S	R	I
11	-	+	R	I	S	S	S	R	I
12	+	-	R	I	S	S	S	R	I
13	-	+	R	I	R	R	R	R	R
14	+	+	R	S	R	I	R	R	R
15	-	+	I	R	S	S	S	R	I
16	+	+	R	R	S	S	S	R	I
17	+	+	I	I	I	R	R	R	R
18	-	+	R	S	I	R	R	R	R
19	+	+	I	R	I	R	I	R	R
20	-	-	R	R	I	I	R	R	R

Gm= gentamicin, CAZ= ceftazidime, SXT= sulfamethoxazol-trimethoprim, CP= ciprofloxacin, NA= nalidixic acid, Fm= nitrofurantoin, AM= ampicillin
S= Sensitive, I= Intermediate, R= Resistant

Table 5. Frequency of TEM and CTX-M genes in ESBLs-positive Pseudomonas aeruginosa isolates

	CTX-M gene	TEM gene	Am	Fm	NA	CP	SXT	CAZ	Gm
1	-	-	R	R	R	S	R	R	S
2	-	+	R	S	S	S	R	R	I
3	-	-	R	S	R	S	R	R	I
4	-	-	R	S	R	I	S	R	I
5	-	-	R	S	I	R	S	R	R
6	-	+	R	S	R	R	I	R	R
7	-	+	R	R	R	S	R	R	S
8	-	-	I	I	I	I	R	R	I
9	-	+	I	S	R	S	S	R	I
10	-	-	R	R	R	S	R	R	I
11	-	+	R	S	I	I	R	R	R
12	-	-	R	R	R	S	R	R	I
13	-	+	R	S	R	S	R	R	R
14	-	-	R	S	S	R	S	R	I
15	-	-	R	I	I	I	I	R	I
16	-	+	I	I	I	I	S	R	R
17	-	-	R	S	R	S	R	R	S
18	-	+	R	S	R	S	R	R	I
19	-	+	R	S	R	S	R	R	I
20	-	-	R	I	R	S	R	R	S

Gm= gentamicin, CAZ= ceftazidime, SXT= sulfamethoxazol-trimethoprim, CP= ciprofloxacin, NA= nalidixic acid, Fm= nitrofurantoin, AM= ampicillin
S= Sensitive, I= Intermediate, R= Resistant

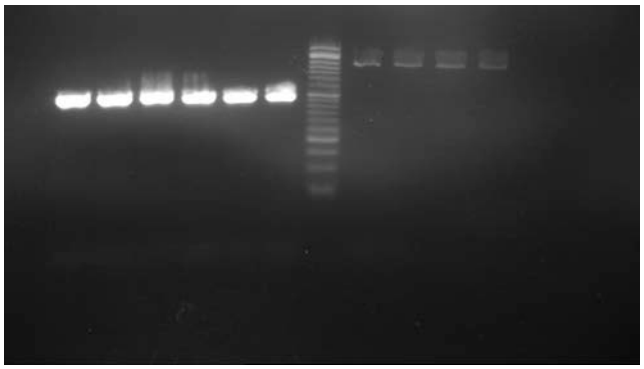

Figure 1. PCR results for TEM and CTX-M genes. Lane numbers 1-6 shows the fragment of CTX-M, lane 7 demonstrates the 50 bp DNA size marker and 8-11 shows the fragment of TEM gene

Discussion

Based on the results of this study, the prevalence of ESBL producing *P. aeruginosa* and *K. pneumoniae* by disk diffusion test was high, 13.3% for *P. aeruginosa* and 66.6% for *K. pneumonia.* 75% and 65% of *K. pneumonia* isolates harboured the gene TEM and CTX-M, respectively and 45% of isolated *P. aeruginosa* harboured the gene TEM by PCR method. Resistance to beta-lactam antibiotics of Gram- negative bacteria isolated from clinical samples has been increased worldwide. Based on the results of this study, the prevalence of ESBL producing *P. aeruginosa* and *K. pneumoniae* by disk diffusion test was high, 13.3% for *P. aeruginosa* and 66.6% for *K. pneumonia.* 75% and 65% of *K. pneumonia*

isolates harboured the gene TEM and CTX-M, respectively and 45% of isolated *P. aeruginosa* harboured the gene TEM by PCR method. The prevalence of the ESBL producing organisms in Taiwan was in the range of 8.5% to 29.8% in *K. pneumoniae* and 1.5% to 16.7% in *E. coli* [18]. In Isfahan, 51% of isolated *E. coli* and 70% of isolated *K. pneumoniae* were ESBLs producing bacteria [19]. The prevalence of the ESBL producing organisms in Tehran were, 21% for *E. coli* and 12% for *K. pneumonia* [20]. Feizabadi showed that 72% of *K. pneumoniae* strains isolated from Tehran hospitals were ESBLs-producing [21]. Other studies demonstrated the prevalence of 57.1 and 55%, for ESBL-producing *K. pneumonia*, respectively based on phenotypic confirmatory test [6, 22]. In another study in Mashhad, the prevalence of ESBLs producing *E. coli* and *K. pneumoniae* was reported 57.5% and 61% and generally the prevalence of ESBLs producing organisms were 59.2% by Kirby-Baure disk diffusion method and the phenotypic disk confirmatory test [23]. In another study, by disk diffusion test, resistance of *K. pneumoniae* to ceftazidime and cefotaxime were 34.7% and 33.5% respectively. The prevalence of blaSHV, blaCTX-M, *bla*TEM among these isolates were 26%, 24.5%, 18%, respectively [24].

A report from Tehran revealed that 69.7% of *K. pneumoniae* isolated from Tehran were ESBL positive and the prevalence of blaTEM, blaSHV, blaCTX-M-I and blaCTX-M-III among these isolates was 54%, 67.4%, 46.51% and 29%, respectively [25]. In another study from Tehran, the prevalence of blaSHV and blaTEM genes for *K. pneumoniae* was reported 67% and 16%, respectively [26].

In summary, the prevalence of ESBLs producing organisms in Zabol is high and most *K. pneumoniae* and *P. aeruginosa* isolates harboured CTX and TEM genes. It seems necessary for clinicians and health care systems to be fully aware of ESBLs producing microorganisms. The ESBLs production monitoring is recommended to avoid treatment failure and suitable infection control in Iran.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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