Frequency assessment of β-lactamase enzymes in *Escherichia coli* and *Klebsiella* isolates in patients with urinary tract infection

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**Background:** Production of β-lactamase enzymes is the most common and important mechanism of resistance in Gram-negative bacteria. The objective of this study was to assess frequency of three main β-lactamase enzymes, including extended spectrum β-lactamases (ESBLs), metallo-β-lactamase (MBL), and *Klebsiella pneumoniae* carbapenemase (KPC) enzymes in *Escherichia coli* and *Klebsiella* spp. isolated from nosocomial and community urinary tract infections (UTI).

**Materials and Methods:** In a cross-sectional study from March to December 2012, midstream urine samples were obtained from patients suspicious of UTI who were hospitalized or referred to Al-Zahra Hospital, Isfahan, Iran. Samples were cultured and *E. coli* and *Klebsiella* spp. were isolated. Prevalence of ESBLs, KPC, and MBLs producing *E. coli* and *Klebsiella* spp. were studied by double-disk (combined-disk), the modified Hodge test and imipenem-ethylenediaminetetraacetic acid combined disc methods respectively. In addition, their antimicrobial susceptibility patterns determined and resistant to carbapenem drugs confirmed by minimum inhibitory concentrations based on E-test method.

**Results:** A total of 1080 *E. coli* and 484 *Klebsiella* strains were isolated during study period. Among 720 *E. coli* and 384 *Klebsiella* isolates from hospitalized patients, 300 (41.7%) and 198 (51.5%) were ESBLs producers, respectively. In out-patients samples, the rate of ESBLs production was 25% (90/360) and 40% (40/100) in *E. coli* and *Klebsiella* isolates, respectively. Prevalence of MBLs producing in hospital *E. coli* and *Klebsiella* isolates were 0.3% (2/720) and 2.6% (10/384), and for KPC data were 1.4% (10/720) and 48.4% (186/384), respectively. No MBLs and KPC producing isolate was seen in non-hospital *E. coli* and *Klebsiella* isolates except for one non-hospital KPC producing *Klebsiella* isolate. **Conclusion:** The result of our study showed high prevalence of ESBLs and KPC, but low prevalence of MBLs in cultured bacteria from urine samples of patients with acute UTI. In addition, KPC was the main carbapenem resistance mechanism in *Klebsiella* and *E. coli* isolates.

**Key words:** Antibiotic resistance, carbapenem drugs, extended spectrum β-lactamase, *Klebsiella pneumoniae* carbapenemase, metallo-β-Lactamase, urinary tract infection

**INTRODUCTION**

Enterobacteriaceae group is the main cause of bacterial infection¹ in the world and in this family *Escherichia coli* and *Klebsiella* spp. are the most prevalent causes of nosocomial infection.¹,² These pathogens are responsible for a broad spectrum of clinical infections in immune competent or immune compromised people and also, have a key role in epidemics of nosocomial infections in many hospitals.³ Extended-spectrum β-lactamases (ESBLs) represents a major threat among multidrug-resistant bacteria isolates. They have risen to prominence among Enterobacteriaceae isolates in nearly all countries, now not only in the nosocomial but also in the community setting.⁴,⁵ These ESBL producing pathogens are now recognized globally as major causes of nosocomial and community-acquired infections.⁶ The impact of ESBL detection is important both from a therapeutic point of view and for infection control purposes. The first ESBL was detected in Germany in 1983, among different enterobacterial isolates recovered patients hospitalized at intensive care unit.⁸ It was recognized by the producer strains unusual resistance to cefotaxime (CTX) and ceftazidime (CAZ), which was transferable by conjugation to *E. coli*. Very soon afterwards in France, in 1984, *Klebsiella pneumoniae* isolates with an identical phenotype were detected in different hospitals.⁷

Carbapenems, for example, imipenem (IPM) and meropenem (MEM), are often used to treat infections caused by ESBL producing *E. coli* and *Klebsiella*.⁸,⁹ However, carbapenemases enzymes recognize almost all hydrolysable β-lactams, and most are resistant to
inhibition by all commercially viable β-lactamase inhibitors. Four classes are available in this group: Molecular classes A, C, and D include the β-lactamases with serine at their active site, whereas molecular class B β-lactamases are all metalloenzymes with zinc in active-site. *Klebsiella pneumoniae* carbapenemase (KPC) enzymes are belonging to class A carbapenemases that reside on transferable plasmids and can hydrolyze all penicillins, cephalosporins, and carbapenems.[8] The emergence of acquired metallo-β-lactamases (MBLs) has clinical and epidemiological implications and is a matter of particular concern worldwide.[9] The options for treating infections caused by KPCs and MBLs are limited and their epidemiology remains largely unknown in Iran. The aim of this study was to determine the prevalence of ESBL, KPCs, and MBLs producing *E. coli* and *Klebsiella* to reduce the antibiotic therapy failure in Al-Zahra Hospital, Isfahan.

**MATERIALS AND METHODS**

This cross-sectional study was performed in Al-Zahra Hospital (Isfahan University of Medical Sciences) from March to December 2012. The study was approved by the Ethical Committee of Isfahan University of Medical Sciences, Isfahan, Iran (project number: 391327). Midstream urine samples from hospitalized or referred patients with acute urinary tract infection (UTI) were obtained and evaluated for the presence of leukocytes and/or bacteriuria. Samples were cultured on blood agar and Eosin Methylene Blue agar mediums (purchased from Himedia Company, India) and incubated at 35°C for 18-24 h. All patient samples with urine cultures yielding growth of ≥10⁵ CFU/ml *E. coli* and *Klebsiella spp.* were included in the study. The pure isolates identified according to Gram stains and biochemical tests.

**Antibiotic susceptibility test**

Antibiotic sensitivity pattern of isolates to common antibiotics used in the hospital was determined by the Kirby Bauer’s disc diffusion method on Mueller-Hinton agar. Choice of antibiotic disks was determined by Clinical Laboratory Standard Institute (CLSI) guidelines.[10] All hospitalized isolates were tested against ampicillin (AMP 10 μg), piperacillin-tazobactam 100 μg, CAZ 30 μg, CTX 30 μg, IPM 10 μg, MEM 10 μg, ertapenem (ERT 10 μg), amikacin (AMK 30 μg), ciprofloxacin (CIP 5 μg), and nitrofurantoin (NIT 30 μg). All non-hospitalized isolates were tested against AMP 10 μg, CAZ 30 μg, CTX 30 μg, IPM 10 μg, MEM 10 μg, ERT 10 μg, gentamicin 10 μg, CIP 5 μg, trimethoprim/sulfamethoxazole (SXT 1.2 μg), and NIT 30 μg. All antimicrobial disks used for susceptibility testing were obtained from BD BBL Sensi-Disc (Becton Dickinson, Sparks, MD, USA). *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used for quality control as recommended by CLSI. The plates were incubated at 35°C for 18 h. The degree of susceptibility of the test isolate to each antibiotic was interpreted as sensitive (S), intermediate resistant (I) or resistant (R) by measuring the zone diameter of inhibition.[10]

**Phenotypic detection of ESBLs production**

The ESBL-producing were determined by the phenotypic method using screening Kirby-Bauer disk diffusion method. A confirmatory test of ESBL-production was performed with the double-disk (combined-disk) method.[10,11] The zones of inhibition of each isolate were tested on Mueller-Hinton agar plates with the disks containing 30 g of CAZ and CTX alone and in combination with 10 g of clavulanic acid, respectively (all above disk purchased from Mast Company). An organism was classified as having an ESBL producing phenotype if the zone of inhibition produced by at least one combination disk was more than 5 mm larger than that produced by the corresponding antimicrobial disk without clavulanic acid.

**Phenotypic detection of KPC**

All isolates with resistance to IPM and MEM were tested for the production of KPC carbapenemase production by the modified Hodge test (MHT) method.[10,12] The MHT was carried out according to CLSI guidelines using ERT 10 μg disk on Mueller-Hinton agar plates. The presence of a “cloverleaf shaped” inhibition zone after overnight incubation was indicated as a positive test result. For confirmatory of carbapenem resistance minimum inhibitory concentration (MIC) determined using E-test method. *E. coli* ATCC 25922 was used as a carbapenem susceptible strain.

**Phenotypic detection of MBL**

In addition, all isolates with resistance to IPM and MEM were tested for the MBLs production by IPM-ethylenediaminetetraacetic acid (EDTA) combined disc method that in positive strains zone of inhibition of IPM with EDTA (IMP + EDTA) is greater than IMP alone by 5 mm.[9] For confirmatory of carbapenem resistance MIC determined using E-test method. The *Pseudomonas aeruginosa* ATCC 27853 strain was used as negative control.

**Statistical analyses**

Differences between proportions were analyzed using the Chi-square test. The null hypothesis was rejected for values of P < 0.001. Statistical analyses were performed with SPSS version 17 software (SPSS Inc., USA) and Microsoft Office Excel.

**RESULT**

During March 2012 to December 2012 a total of 1564 consecutive urine clinical isolates including 1080 of *E. coli* and 484 of *Klebsiella* spp. were collected. Table 1 shows the
sex and hospitalized and non-hospitalized distribution in patients and the overall prevalence of *E. coli* and *Klebsiella* in the urine samples. Females had higher overall prevalence than the males as shown in Table 1 *(P < 0.001)*.

Resistant to CAZ and CTX among *E. coli* and *Klebsiella* isolates is shown in Table 2. Confirmatory test of ESBL-production in these resistance isolates showed among 720 *E. coli* hospitalized isolates, 300 (41.7%) and among 360 non-hospitalized isolates, 90 (25%) were ESBLs producers. These data for *Klebsiella* were 198 (51.5%) among 384 hospitalized isolates and 40 (40%) among 100 non-hospitalized isolates [Table 3] *(P < 0.001)*.

In addition, resistance to IPM, MEM, and ERT is shown in Table 2 that these isolates were selected for detection of MBL and KPC carbapenemases producing. As shown in Table 3, prevalence of MBL producing in hospitalized *E. coli* and *Klebsiella* spp. were 0.3% (2/720) and 2.6% (10/384), respectively. No MBL producing isolate detected in non-hospitalized *E. coli* and *Klebsiella*. Carbapenem resistance determined using MIC by E-test method.

Prevalence of KPC carbapenemases producing that was confirmed by the MHT in hospitalized *E. coli* and *Klebsiella* spp. were 1.4% (10/720) and 48.4% (186/384) respectively [Table 3]. No KPC carbapenemases producing isolate was seen in non-hospitalized *E. coli*, but one non-hospitalized *Klebsiella* was KPC producer *(P < 0.001)*. Carbapenem resistance determined using MIC by E-test method.

Table 4 shows the susceptibility and resistance percentages of the non-hospitalized and hospitalized *E. coli* and *Klebsiella* isolates. In non-hospitalized *Klebsiella* and *E. coli* isolates, more resistance were seen to AMP and SXT, *(P < 0.001)*. In hospitalized *E. coli* and *Klebsiella* isolates AMK and NIT were effective antibiotics *(P < 0.001)*.

**DISCUSSION**

ESBL is associated with *Enterobacteriaceae* species, mostly *K. pneumoniae*, *E. coli*, and *Enterobacter* spp.[13] Since the 1980s, ESBLs producing Gram-negative bacteria have been isolated in many countries.[14] Many outbreaks caused by such bacteria, especially *E. coli* and *K. pneumoniae* have been reported.[15-20]
Prevalence of ESBL in *K. pneumoniae* differs geographically and was reported as a part of study in 1997-2003 to be 12.3% for North America, 51.9% for South America, 16.7% for Northern Europe, 24.4% for Southern Europe, 58.7% for Eastern Europe, and 28.2% for Asia Pacific region.[21] One of the earliest documented reports of the occurrence of ESBL phenotypes comes from Korea, with 7.5% of *E. coli* and 22.8% of *K. pneumoniae* isolates at Yonsei Medical Center being described as ESBL-positive in 1994.[22] Studies by Kawakami *et al.*[23] from a hospital in Tokyo reported rates of 0.4% and 0.6% in *E. coli* and *K. pneumoniae*, respectively, in 1990, rising to 1.7% and 7.2% in 1995. A survey of seven laboratories in the Kinki region, over 2-month periods in 1998 and 2000, revealed 10% of *E. coli* isolates and 4.0% of *K. pneumoniae* isolates to have an ESBLs phenotype.[24] In another survey in Pakistan by Zaman *et al.*, of 200 nosocomial isolates of *Enterobacteriaceae*, reported a rate of 35%. A comprehensive study and community isolates of *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. revealed ESBL phenotype rates of 41%, 36%, and 50%, respectively.[25] A total of 67.2% of isolates in a study conducted in Tehran, Iran were confirmed as ESBL positive,[26] however, in another study this rate was reported 89.3%.[27] Mirsalehan *et al.* found that 60.6% isolates of *E. coli* were producers of ESBLs.[28] Like other investigation in Iran hospitals, our results showed high ESBLs prevalence in both hospitalized and non-hospitalized *E. coli* and *Klebsiella* isolates.

Carbapenemase-producing bacteria such as KPC and MBLs producing bacteria represents a major threat to human health because of fails in most antibiotic therapy.[29] In recent years, the detection of these enzyme among Gram-negative bacilli has been reported from different countries, including Iran.[30] However, these reports in Iran are very incomplete and need to be more investigated. In a study by Deshpande *et al.*[30] in United States prevalence of carbapenemases among 8885 *Enterobacteriaceae* isolates was only 51 isolates. In addition, study of Bratu *et al.*[31] showed 24% KPC producing among *K. pneumoniae* isolates. High KPC producing prevalence (48.4%) in our study represents a major concern in Isfahan province. Also result of our study showed that KPC is main carbapenem resistance mechanism in *Klebsiella* and *E. coli* isolates and MBLs producing isolates are rare in investigated hospital.

Comparing of antibiotics resistant percentage in non-hospitalized and hospitalized isolates showed that both non-hospitalized and hospitalized isolates were more resistant to AMP. AMK and NIT were the effective antibiotics against hospitalized both *Klebsiella* and *E. coli* isolates.

**CONCLUSION**

Result of our study showed high prevalence of ESBLs and KPC but low prevalence of MBLs in cultured bacteria from urine samples of patients with acute UTI. In addition, KPC was the main carbapenem resistance mechanism in *Klebsiella* and *E. coli* isolates.

**ACKNOWLEDGMENTS**

The authors would like to thank all the staff of the Laboratory of Al-Zahra Hospital for their sincere support. Also they are grateful to Professor Mohsen Janghorbani for his valuable comments on the manuscript. University of Isfahan Medical Sciences was the financial supporter of this project (Project Number: 391327).

**AUTHORS’ CONTRIBUTION**

All authors have contributed in designing and conducting the study. DS, SM, SMF, and AM collected the data and RM and AB did the analysis. All authors have assisted in preparation of the first draft of the manuscript or revising it critically for important intellectual content. All authors have read and approved the content of the manuscript and are accountable for all aspects of the work.
REFERENCES


Source of Support: University of Isfahan Medical Sciences (Project Number: 391327). Conflict of Interest: The authors have no conflict of interest.