**Original article**

# Frequency of extended-spectrum beta lactamase positive and multidrug resistance pattern in Gram-negative urinary isolates, Semnan, Iran

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## Abstract

**Introduction and objective:** Monitoring of the antimicrobial susceptibilities provides information about pathogenic organisms isolated from patients, and assists in choosing the most appropriate empirical antimicrobial therapy. This study tried to determine frequency of extended spectrum beta lactamase (ESBL) positive and multidrug resistance pattern in Gram-negative uropathogenic bacteria.

**Materials and methods:** In this study, 310 Gram-negative uropathogen were investigated. Combined disk test was used as a screening test for ESBL production. Disk diffusion method was performed for antibiotic susceptibility testing.

**Results:** Isolated bacteria were as follow: 226(72.9%) *Escherichia coli*, 76(24.5%) *Klebsiella pneumoniae*, 3(1%) *Citrobacter* spp. 2(0.6 %) *Proteus mirabilis* and 3(1 %) *Pseudomonas* spp. ESBL production was observed in 88(28.4%) of all isolates, 29.2% of *E. coli* isolates and 28.9% of *K. pneumonia.* The most and least resistance were seen in the case of ampicillin (98.4%) and ceftazidime (24.2%), respectively. Resistance to six antibiotics or more was seen in 104 isolates (33.5%).

**Conclusion:** In the present study, relatively high frequency of ESBL production and multidrug resistance were seen in uropathogens. To avoid treatment failure and choose either empirical or direct therapy by physicians, antimicrobial susceptibility testing and ESBL production monitoring are recommended in patients with urinary tract infection (UTI).

**Keywords:** Uropathogen, Multidrug resistance, Extended-spectrum beta lactamase (ESBL), *Escherichia coli*, *Klebsiella*

## Introduction

Urinary tract infection (UTI) is one of the most frequent causes of illness in humans and common both in the community and hospitalized patients. Most of UTIs are caused by a few genera of bacteria of which *Escherichia coli* is the predominant bacterial agent. *Klebsiella pneumoniae* and other enteric Gram- negative rods are the most common bacterial agents of UTI [1].

Antibiotic resistance in uropathogens is increasing worldwide. It varies according to geographic locations and is directly proportional to the use and misuse of antibiotics [2]. Understanding the impact of drug resistance is of critical importance as the changing rate of antibiotic resistance has a large impact on empirical therapy of UTIs [2]. Some bacteria, especially *E. coli* and *Klebsiella* spp., which are more frequent agents of UTI, show increasing resistance to cephalosporins. These organisms produce extended-spectrum beta-lactamases (ESBL), which are coded by genes located on transferable plasmids.

Resistance to the quinolones, by strains of *E. coli* isolated from urine specimens of outpatients, has also increased [3]. Since the pattern of bacterial resistance is constantly changing, the monitoring of the antimicrobial susceptibilities becomes more important. It provides information on the pathogenic organisms isolated from patients, and assists in choosing the most appropriate empirical antimicrobial therapy. In addition, the continuous survey of antimicrobial resistance is crucial for monitoring changes of antimicrobial resistance [3]. The aim of our study was to determine the frequency of ESBL positive and multidrug resistance pattern in Gram-negative urinary isolates from Semnan, Iran.

## Materials and methods

*Bacterial isolates*

From June 2007 to June 2008 a total of 310 Gram-negative urinary isolates, including 226 *E. coli*, 76 *K. pneumoniae*, three *Citrobacter* spp., two *Proteus mirabilis* and three *Pseudomonas* spp, were collected. Only one isolate per patient was included in the study. Urine specimens were cultured on Blood agar and Eosin Methylene Blue (EMB) agar (Merck, Germany), incubated at 37°C for 24 hrs and suspected colonies were identified by API20E system (BioMerio, France) [4].

*Screening test for ESBL production*

Combined disk method was used in this study as a screening test for ESBL production, using disks of cefotaxime 30mcg and ceftazidime 30mcg with and without clavulanic acid 10mcg (Mast Group Ltd., Merseyside, UK). A culture from 0.5 McFarland suspension of bacteria were prepared on the surface of Muller Hinton agar (Merck, Germany). Phenotypic confirmatory test was performed by comparing the inhibition zone of disks containing cefotaxime or ceftazidime with and without clavulanic acid. In the case of more than 5mm inhibition zone around the disk containing clavulanic acid, the isolates were considered as ESBL positive. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 7006039 (Mast Group Ltd.) were used as negative and positive control respectively [5].

*Antibiotic susceptibility testing*

Disk diffusion method was used according to Clinical and Laboratory Standards Institute [5] recommendations. The nine used disks were amoxicillin-clavulanic acid (AMC) 20/10mcg, ampicillin (AM) 10mcg, tetracycline (TET) 30mcg, trimetoprim-sulfamethoxazole (SXT) 1.25/23.75mcg, nalidixic acid (NA) 30mcg, ciprofloxacin (CIP) 5mcg, gentamycin (GM) 10mcg, cefotaxime (CTX) 30mcg, and ceftazidime (CAZ) 30mcg (Mast Group Ltd.). *E. coli*

ATCC 25922 and *Staphylococcus aureus* ATCC 25923 (Mast Group Ltd.) were used as standard controls.

## Results

Of the 310 specimens, which had $10^5$ bacteria per ml or more in urine culture, 226(72.9%) *E. coli*, 76(24.5%) *K. pneumoniae*, 3(1%) *Citrobacter* spp. 2(0.6 %) *P. mirabilis* and 3 (1 %) *Pseudomonas* spp. were isolated. Using disk diffusion method, resistance rates are shown in table 1. The most and least resistance were seen to ampicillin (98.4%) and ceftazidim (24.2%), respectively. Seven isolates (2.3%) were resistance to one antibiotic, 34 isolates (11%) resistant to two antibiotics, 47 isolates (15.1%) resistant to three antibiotics, 65 isolates (21%) resistant to four antibiotics, 53 isolates (17.1%) resistant to five antibiotics, 19 isolates (6.1%) resistant to six antibiotics, 35 isolates (11.3%) resistant to seven antibiotics, 15 isolates (4.8%) resistant to eight antibiotics and 35 isolates (11.3%) resistant to nine antibiotics (Table 2).

Using combined disk, ESBLs were detected among 88 (28.4%) isolates, of which 66 were *E. coli* and 22 were *K. pneumoniae*. None of other isolates were ESBL producer. ESBL production was observed in 29.2% of *E. coli* isolates and 28.9% of *K. pneumoniae* isolates. Multidrug resistance and ESBL production for the isolated bacteria are shown in table 2.

**Table 1:** Resistance rate for Gram-negative urinary isolates

| Antibiotics | *E. coli* N=226 (%) | *K. pneumoniae* N=76 (%) | *Citrobacter* spp. N=3 (%) | *P. mirabilis* N=2 (%) | *Pseudomonas* spp. N=3 (%) | Total N=310 (%) |
|---|---|---|---|---|---|---|
| AM | 224 (99.1) | 73 (96) | 3 (100) | 2(100) | 3(100) | 305 (98.4) |
| AMC | 217 (96) | 69 (90.1) | 3(100) | 2(100) | 3(100) | 294 (94.8) |
| TET | 153(67.7) | 35 (46) | 3(100) | 2(100) | 3(100) | 197 (63.5) |
| SXT | 150 (63.4) | 41 (53.9) | 2(67) | 2(100) | 3(100) | 198 (63.9) |
| NA | 124 (54.9) | 33 (43.4) | 2(67) | 2(100) | 3(100) | 164 (52.9) |
| CIP | 91 (40.2) | 35 (46) | 2(67) | 2(100) | 1(33) | 131(42.3) |
| GM | 57 (25.2) | 19 (25) | 1(33) | 1(50) | 3(100) | 81 (26.1) |
| CTX | 64 (28.3) | 19 (25) | 0 (0) | 0 (0) | 3(100) | 86 (27.7) |
| CAZ | 54 (23.9) | 18 (23.7) | 0 (0) | 0 (0) | 3(100) | 75 (24.2) |

AMC: Amoxicillin- clavulanic acid, AM: Ampicillin, TET: Tetracycline, SXT: Trimetoprim- sulfamethoxazole, NA: Nalidixic acid, CIP: Ciprofloxacin, GM: Gentamicin, CTX: Cefotaxime, CAZ: Ceftazidime

## Discussion

Our results showed that *E. coli* is the dominant bacterial agent of UTI. This is similar to what is reported by Hosseini-Mazinani *et al*. [6], Akram *et al*. [7] and Kader and Kumar [8]. From 310 isolates in this study 28.7% (Confidence interval (CI: 33.7%, 23.7%) were identified as ESBL producer. 29.2 % (CI: 35.1%-23.3%) of *E. coli* isolates and 28.9 % (CI: 39.1%-18.7%) of *K. pneumoniae* isolates were ESBL producer. Hosseini-Mazinani *et al*. [6] in a similar study in Tehran, Iran have reported that 2.4% of uropathogenic *E. coli* harbored ESBL. Our result is comparatively higher than this

Akram *et al*. [7] conducted a survey on the urinary tract isolates in India and

reported that frequencies of ESBL production were 34.2% and 27.3% in *E. coli* and *K. pneumoniae*, respectively. With regard to our results, these percentages are not show significantly different.

**Table 2:** Multidrug resistance and ESBL production for Gram-negative urinary isolates

| Resistance profile | Number | ESBL Producer | |
|---|---|---|---|
| | | *E. coli* | *K. pneumoniae* |
| AMC, AM, TET, SXT, NA, CIP, GM, CTX, CAZ | 35 | 27 | 6 |
| AMC, AM, TET, NA, CIP, GM, CTX, CAZ | 5 | 4 | |
| AMC, AM, TET, SXT, NA, CIP, CTX, CAZ | 10 | 9 | 1 |
| AMC, AM, TET, SXT, NA, CIP, GM | 20 | | 4 |
| AMC, AM, TET, SXT, NA, CTX, CAZ | 10 | 9 | 1 |
| AMC, AM, TET, SXT, GM, CTX, CAZ | 5 | 5 | |
| AMC, AM, TET, SXT, NA, CTX | 10 | 10 | |
| AMC, AM, TET, SXT, NA, CIP | 9 | | |
| AMC, AM, TET, SXT, NA | 46 | | |
| AMC, AM, TET, CIP, GM | 7 | | |
| AMC, AM, CTX, CAZ | 10 | | 10 |
| AMC, AM, SXT, CIP | 21 | | |
| AMC, AM, SXT, NA | 5 | | |
| AMC, AM, TET, SXT | 15 | 2 | |
| AMC, AM, TET, CIP | 9 | | |
| AMC, TET, SXT, NA | 5 | | |
| AMC, AM, NA | 4 | | |
| AMC, AM, CIP | 15 | | |
| AMC, AM, TET | 16 | | |
| AMC, AM, SXT | 7 | | |
| AMC, AM, GM | 5 | | |
| AMC, AM | 25 | | |
| AM, GM | 4 | | |
| AMC, TET | 5 | | |
| AM | 7 | | |

AMC: Amoxicillin- clavulanic acid, AM: Ampicillin, TET: Tetracycline, SXT: Trimetoprim- sulfamethoxazole, NA: Nalidixic acid, CIP: Ciprofloxacin, GM: Gentamycin, CTX: Cefotaxime, CAZ: Ceftazidime

Kader and Kumar [8] reported that ESBL was detected in 9.6% of uropathogenic *E. coli* and 11.3% in *K. pneumoniae* in Saudia Arabia, which were significantly less than our results. Khurana *et al*. [9] in a survey on urinary tract isolates of family Enterobacteriaceae reported that 24.7% of *E. coli* and 38.5% of *K. pneumoniae* were ESBL producer, these results do not show significant differences in comparison to our results.

Sader *et al*. [10] reported that ESBL production among *E. coli* and *K. pneumoniae* were 8.9% and 48.4%, respectively. Although the percentages of ESBL producing strains of *E. coli* reported in our study is significantly higher than, the ESBL producing *K. pneumoniae* in our study. Drulis-Kawa *et al*. [11] reported that 32.5% of uropathogenic *K. pneumoniae* were ESBL producer which do not show significant differences with our results. Resistance frequency of *E. coli* and *K.*

*pneumoniae* isolates compared with other studies is shown in table 3.

## Conclusion

In present study, relatively high frequency of ESBL production and multidrug resistance were seen in uropathogens, and it seems that this is due to misuse of antibiotics in this area. In order to avoid treatment failure and provide physicians with enough information about choosing empirical or direct therapy, antimicrobial susceptibility testing and ESBL production monitoring are recommended in patients with UTI.

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**Table 3:** Resistance frequency of isolated *E. coli* compared in compare with other studies

| | | This study | Akram *et al*. (7) | Kader *et al*. (8) | Chulain *et al*. (12) | Andrade *et al*. (13) | Shahcheragh *et al*. (14) | Kiffer *et al*. (15) | Randrianirina *et al*. (16) |
|---|---|---|---|---|---|---|---|---|---|
| | *E. coli* | 99.1%, CI:(100%, 97.7%) | - | - | 50% | 53.6% | - | 43.4% | - |
| AM | *K. pneumoniae* | 90.1%, CI:(96.8%, 83.4%) | - | - | - | 74.4% | - | 100% | - |
| | *E. coli* | 96%, CI:(100%, 91%) | - | 36% | - | 1.2% | - | - | 11.5% |
| AMC | *K. pneumoniae* | 96%, CI:(100%, 91.6%) | - | 16% | - | 7% | - | - | - |
| | *E. coli* | 67.7%, CI:(73.8%, 61.6%) | 76% | - | - | - | - | 30.5% | - |
| TET | *K. pneumoniae* | 46%, CI:(57.2%, 34.8%) | 53% | - | - | - | - | 19.8% | - |
| | *E. coli* | 63.4%, CI:(69.7%, 57.1%) | 76% | - | - | 40.4% | - | 33.7% | 69.5% |
| SXT | *K. pneumoniae* | 53.9%, CI:(65.1%, 42.7%) | 53% | - | - | 20.9% | - | 17.7% | - |
| | *E. coli* | 54.9%, CI:(61.4%, 48.4%) | - | - | 6.1% | 29.3% | - | 15.5% | 25.3% |
| NA | *K. pneumoniae* | 43.4%, CI:(54.5%, 32.3%) | - | - | - | 25.6% | - | 15.2% | - |
| | *E. coli* | 40.2%, CI:(46.6%, 33.8%) | 69% | 34% | 5.3% | 21.6% | 42.9% | 11.9% | 16.4% |
| CIP | *K. pneumoniae* | 46%, CI:(57.2%, 34.8%) | 47% | 11% | - | 18.6% | - | 6% | - |
| | *E. coli* | 25.2%, CI:(30.8%, 19.6%) | 64% | 7% | - | 8.4% | 26.7% | 3% | 9.1% |
| GM | *K. pneumoniae* | 25%, CI:( 34.7%, 15.3%) | 53% | 9% | - | 14% | - | 3.3% | - |
| | *E. coli* | 26.3%, CI:( 32%, 20.6%) | 56% | - | - | - | 32.1% | - | - |
| CTX | *K. pneumoniae* | 25%, CI:(34.7%, 15.3%) | 41% | - | - | - | - | - | - |
| | *E. coli* | 23.9%, CI:( 29.4%, 18.4%) | 65% | 10% | - | 1.5% | 30.1% | - | 3.1% |
| CAZ | *K. pneumoniae* | 23.7%, CI:( 33.2%, 14.2%) | 53% | - | - | 4.7% | - | - | - |

AMC: Amoxicillin- clavulanic acid, AM: Ampicillin, TET: Tetracycline, SXT: Trimetoprim- sulfamethoxazole, NA: Nalidixic acid, CIP: Ciprofloxacin, GM: Gentamycin, CTX: Cefotaxime, CAZ: Ceftazidime