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Full Length Research Paper

Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *klebseilla pneumonia* isolated from urine in an Iranian 1000-bed tertiary care hospital

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Betal-lactam antibiotics are the most frequently prescribed antibiotics. Emerging resistance to these antibiotics among gram-negative bacilli limited their utility. This study was done to determine the frequency of extended spectrum beta-lactmase (ESBLs) producing *Escherichia coli* and *Klebsiella pneumonia*e isolated from urine in Milad Hospital of Tehran. This study was done on 735 urine gram-negative bacilli isolates including 620 strains of *E. coli* and 115 strains of *K. pneumoniae* in microbiology laboratory of Milad Hospital in Tehran, Iran. ESBIs resistance was detected in 132 (21%) of isolates of *E. coli* and 18 (12%) in those with *K. pneumonia*e. Of 150 patients which had positive ESBLs isolates, 104 were outpatients and 46 others were hospitalized. Nearly 80% ESBIs isolates were resistant to co-trimoxazole and nitrofuantion was the most effective antibiotic against ESBLs producing isolates. Our study revealed that there is a high frequency isolates of ESBIs producing strains of *E. coli* and *K. pneupmia*e in both community and hospital. This has a significant implication for patients' management. Further drug resistance surveillance in our hospitals and molecular characteristics of ESBIs isolates in our country is necessary.

Key words: Extended spectrum beta-lactamase (ESBLs), *Escherichia coli*, *Klebseilla pneumonia*, urinary tract infections.

INTRODUCTION

Extended spectrum β lactamase (ESBLs) are enzymes conferring broad resistance to penicillin, cephalosporin and monobactam but not to carbapenem (Mehrgan et al., 2008; Kader et al., 2006). ESBIs are often plasmid mediated and most are members of TEM-1, TMH-2 and SHV-1 family's enzymes. These enzymes are produced by Enbterobacteriaceae mainly by *Escherichia coli*, *Klebsiella pneumonia*e and *Klebsiella oxytoca*. They have been detected in other gram-negative bacilli such as Salmonella species, Proteus spp., *Pseudomonas aeruginosa* and other Enteobactteiaceae (Astal et al., 2004; Vandana et al., 2009; Shah et al., 2004; Bhattacharya et al., 2006). In 1983, the first ESBL-producing organism was isolated in Germany. Thereafter, such organisms were reported in the United States following outbreaks of infections caused by these pathogens. In the recent years, the importance of such ESBL–mediated infections has been increasingly reported worldwide (Aminzadeh et al., 2008; Khanfar et al., 2009; Mumtaz et al., 2007).

The ESBL producing bacteria are increasingly causing urinary tract infections (UTI) both in hospitalized and outpatients. The increase of drug resistance among these organisms has made therapy of UTI difficult and has led to greater use of expensive broad spectrum antibiotics such as third generation of cephalosporin. Detection of

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Table 1. Prevalence of ESBL producing organism among outpatient and inpatients.

Organism	In patients (ESBLs)	Out patients (ESBLs)
E. coli	44 (33%)	88 (66%)
k. pneumoniae	6 (33%)	12 (66%)

ESBLs using conventional antimicrobial susceptibility methods and delay in the detection and reporting of ESBL production by gram-negative bacilli is associated with prolonged hospital stay, increase morbidity, motility and health care costs. (Mehrgan et al., 2008). With reports on high prevalence of ESBLs production in *E. coli*, *K. pneumoniae* and paucity of information especially on the uropathogens, this study was done to determine the frequency of ESBLs producing *E. coli* and *K. pneumonia*e isolated from urine in Milad Hospital of Tehran (Table 1).

MATERIALS AND METHODS

Between March and June 2009, a total of 11308 urine samples were processed for significant bacteruria in the Microbiology Department of Milad Hospital from patients clinically suspected to have UTI. Milad hospital is a 1000-bed and non-university affiliated hospital .This study was done on 735 urine gram-negative bacilli isolates including 620 strains of *E. coli* and 115 strains of *K. pneumoniae*. Medical and demographic data of the patients were collected using patients files. Data recorded were as follows: Demographic (age gender) presence of urinary catheter admission ward. Fresh mid-stream urine was collected aseptically in sterilized bottles or disposable sterile plastic bags submitted to clinical microbiology laboratory. The specimens received were inoculated onto blood and MacConkey agar. All plates were incubated at 37°C for 24 h. Significant isolates were identified as species level using conventional bacteriological methods (Mahon et al., 2007). Only one isolate per patient was included in the study. Microbial sensitivity test were performed on the Muller–Hinton agar plates with disk diffusion method as recommended by clinical laboratory standards institute (CLSI, 2006).

Production of ESBLs in our study among *E. coli* and *K. pneumoniae* isolates was determined by the combination disk technique using antibiotic disks containing ceftazidim (30), cefotaxim (30 µg) and cefpodoxim (30 µg) either alone or in combination with clavulanic acid (10 µg). Susceptibility testing to other antibiotics was performed by disk diffusion methods as recommended by clinical laboratory standard institute (CLSI). The following antibiotic disks (MAST diagnostic Group UK) were used: Tetracycline (30 µg), Amikacin (30 µg), Nalidixic acid (30 µg), Gentamycin (10 µg), Nitrofurantoin (300 µg), Co-trimoxazol (1.25/ 23.75 µg). Oflaxacin (5 µg), Cefotaxime (30 µg), Ceftizoxime (30 µg), Cephalothin (30 µg), Ceftriaxone (30 µg) Ceftazidime (30 µg) and Carbenicillin (100 µg).The susceptibility testing results were interpreted according to the recommendation of CLSI. The quality control check for routine susceptibility testing was performed once weekly using the reference strains *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853. ESBLs positive *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603 were used to check the results of disks that contain a combination of beta-lactam antibiotics and a beta-lactamase inhibitor. All data were analyzed using SPSS for Windows (SPSS Inc., Chicago, IL).

RESULTS

During our study period, of 11308 urine sample submitted to microbiology laboratory for culture, 1020 pathogen were isolated. Of 1020 pathogen isolated, uropathogenes isolates and gram-negative bacilli with 735 isolates were the leading causative of UTI. In our study, 620 strains of *E. coli* and 115 strains of *K. pneumoniae* were isolated from urine specimens of patients admitted to Milad Hospital of Tehran. Of 150 patients which had positive ESBLs, 104 were outpatients and 46 were hospitalized (Table 1).

There was a high frequency of ESBL-positve *E. coli* isolation and 21% isolates of *E. coli* were ESBL positive, while 12% isolates of *K. pneumoniae* were positive for ESBLS. Nearly 80% ESBL-producing *E. coli* isolates were resistant to co-trimoxazole. Resistance to tetracycline and nalidixic acids among *E.coli* isolates were 79 and 99.91%, respectively. While that for *K. pneumoniae* was 50 and 45%. Resistance to ofloxacin, gentamycin and amikacin were 70, 50 and 54% for *E. coli* and 28, 84 and 78% for *K. pneumoniae*, respectively. Nitrofuantoin was the most effective antibiotic against ESBL producing isolates and only 20% ESBL producing isolates of *E. coli* and 33% ESBLs positive strains of *K. pneumoniae* were resistant to nitrofurantoin (Table 2).

DISCUSSION

During the past decade, ESBLs producing gram-negative bacilli especially *E. coli and K. pneumoniae* have emerged as serious pathogens both in hospital and community acquired infections worldwide. Recent studies reveled that patients with infection such as septicemia with ESBLs producing organisms had significantly higher fatality rate than those with non-ESBL isolates (Mehrgan et al., 2008). The occurrence of ESBL among clinical isolates vary greatly world wide and geogra-phically and are rapidly changing over time (Babypadmini et al., 2004). In our study, the incidence of ESBL producing *K. pneumoniae* was 12%. The highest isolation rate of ESBLs-producing *K. pneumoniae* has been reported from the Latin America (54.4%), the Western Pacific (24.6%) and Europe (22.6%). The frequency of ESBL producing *E. coli* in this area was reported to be 8.5, 7.9 and 5.3%, respectively (Aminzadeh et al., 2008).

There is a limited data from Iranian laboratories re-garding prevalence of ESBLs produced in urine isolates. Studies from various part of our country have reported the prevalence of ESBL producing among clinical isolates

Table 2. Comparison of frequency drug resistance among ESBL producing isolates of *E. coli* and *K. pneumoniae*.

***E. coli* (n = 132)%**		***K. pneumonia* (n = 18)%**
Tetracycline	79	50
Amikacin	54	78
Nalidixic acid	85	45
Gentamycin	50	84
Nitrofurantoin	20	67
Co-Trimoxazol	80	45
Ofloxacin	70	28
Ceflizoxime	99.91	99.95
Cefazolin	99.99	100
Cefalotine	99.99	100
Ceftizoxime	99.98	99.95
Ceftazidime	88	100
Carbenicillin	99.98	99.95
Ampicillin	99.99	100

of *E. coli* as varying from 45.2 to 67.2% (Aminzadehet al., 2008; Mehrgan et al., 2008). This figure has been reported for *K. pneumonia*e from 44.4 to 52% (Feizabadi et al., 2006; Aminzadeh et al., 2008). Similar finding have been reported from other countries. For example, in a study by Ullah and co-w0rkes in Pakistan, 56.9% isolates of *E. coli* were ESBL positive (Ullah et al., 2009) and in study from India, nearly 40% urinary isolates of *E. coli* and *K. pneuoniae* were ESBL positive (Babypadmini et al., 2004). Resistance to other antibiotics among ESBLs producing *E. coli* and *K. pneumoniae* was prevalent. In this study, the prevalence of resistance to fluroro-quinolones such as ofloxacin by ESBLs producing organisms was nearly 70%. The most effective antibiotic against ESBL producing *E.* coli was nitrofurantion. In our study, 80% ESBLs producing isolates of *K. pneumonae* were resistant to ofloxacin. Other orally prescribed antibiotics such as co-trmoxazole were not effective against ESBL producing organism and approximately 85% of these organisms were resistant to co-trmoxazole. Resistance of both ESBLS producing *E. coli* and *K. pneumoae* to ampicillin was 100% (Table 2).

In conclusion, our study showed that ESBLS production was prevalent in *E. coli* and *K. pneumoniae* urine isolates. In addition, the majority of theses organisms were resistant to other common antibiotics used for treatment of UTI. Further drug resistance surveillance in our hospitals and molecular characteristics of ESBLs isolates in our country is necessary. This study is important for strict antibiotic policy implementation in hospitals to estimate the impact of higher drug resistance in bacteria and to take steps for reducing this resistance.

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