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Differential Between Multi-Drug Resistance Pattern of Extended Spectrum β- Lactamases Producing *E. coli* and *K. pneumoniae*

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The current study aimed to determine the prevalence of Extended-Spectrum β-Lactamases (ESBLs) in *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) strains isolated from patients with Urinary Tract Infections (UTIs), to study the association between presence of ESBL enzyme and multi-drug resistance strains and finally, and to investigate the predominant ESBL gene in *E. coli* and *K. pneumoniae*. The strains were examined for the presence of ESBL as a Clinical Laboratory Standards Institute (CLSI) guideline. Among 284 clinical isolates, 52.8% (n = 150) and 47.2% (n =134) were *E. coli* and *K. pneumoniae*, respectively, and 110 strains were ESBL producer, which 68 strains were *K. pneumoniae* and 42 strains were *E. coli*. Significant difference observed between the TEM gene and ciprofloxacin resistant in *E. coli* ($P \leq 0.05$) while no significant difference observed between CTX-M, SHV genes and the other multi-drug resistant *E. coli*. No significant difference observed between CTX-M, TEM, and SHV genes and multi-drug resistant *K. pneumoniae*. In conclusion, spreading of ESBL-producing strains is a concern, as it causes limitations to the antimicrobial agents for optimal treatment of patients. Prevalence of ESBLs was more observed in *K. pneumoniae* than *E. coli*. In addition, TEM gene was more prevalent in *E. coli* and resistance to ciprofloxacin was predominant in *E. coli*.

Key words: Multi-drug Resistant, ESBLs, Urinary Tract Infection.

Antimicrobial resistance is a worldwide concern and responsible for distribution of infections. Resistance to β-lactam antibiotics is associated with the presence of ESBL. Production of ESBLs was found to be one of the most important mechanisms for resistance to β-lactam antibiotics. ESBLs producer bacteria are resistant to the third-generation cephalosporins. During the last two decades, ESBL producing Gram-negative bacilli have emerged as a major problem in hospital settings[1].

In addition, ESBL is mediated resistant to broad-spectrum cephalosporins (e.g., ceftazidime, ceftriaxone, and cefotaxime) and aztreonam[2].

ESBLs are mutant, plasmid-mediated beta-lactamases derived from older, broad-spectrum beta-lactamases (*e.g.*, TEM-1, TEM-2, and SHV-1), have an extended substrate profile, which allows hydrolysis of all cephalosporins, penicillins, and aztreonam[3]. These enzymes are most commonly

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proteins that were produced by *Klebsiella* spp. and *E. coli*. Failure to detect ESBL produced bacteria by routine disk-diffusion tests has been well-documented[4].

In the other hand, UTI is the one of the most common infection diseases in humans. *E. coli* and *K. pneumoniae* are found as an important pathogenic microorganism in UTI.

Resistance to β-lactame antibiotics is mainly mediated by the production of diverse set of enzymes that the most important are ESBLs and Metallo β-lactameses. Studies revealed that by use of the third generation of cephalosprins led to the multidrug resistance and producing of ESBL bacteria.

There are diverse set of enzymes, which are responsible for producing ESBL including TEM, SHV and CTX-M.

In recent years, resistance to different antibiotics has raised dramatically leaving physicians with few therapeutic options. Rates of resistance to antibiotics differ from region to region, in making an appropriate choice of empiric or definitive therapy for UTI; it is useful to avail of information on prevailing levels of antimicrobial resistance among common urinary pathogens.

Treatment of UTI cases is often started empirically. Therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy.

The current study aimed to determine the prevalence of ESBLs in *E. coli* and *K. pneumoniae* strains isolated from patients with UTI, to study association between presence of ESBL and multi-drug resistance bacteria and, to investigate predominant gene responsible for ESBLs production in *E. coli* and *K. pneumoniae*.

MATERIALS AND METHODS

A prospective study was conducted over a period of one year (March 2007 to April 2008 (in Milad hospital in capital of Iran,(the strains collected from in-patients and out-patients. Strains were identified, based on the standard bacteriological techniques and were tested for presence of ESBL enzyme by using the combination disk method recommended by CLSI[5].

Combination disk method

The combination-disk test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, was performed for the detection of ESBL according to the CLSI guidelines. Overnight culture suspension of the 0.5 McFarland of tested bacteria inoculated to Mueller Hinton Agar plate. Cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/ 10 µg) disks were placed 20 mm apart on the agar plate. Similarly, the ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/10 µg) disks were placed 20 mm apart. After incubating overnight at 37°C, a ≥ 5-mm increase in the zone diameter for either antimicrobial agent, which were tested in combination with clavulanic acid, its zone when tested alone, was interpreted as positive for ESBL enzyme[5].

Antibiotic susceptibility testing

Susceptibility of the ESBL producing bacteria to amikacin, cotrimoxazol and ciprofloxacin was determined by the Kirby-Bauer disk diffusion method according to the CLSI guideline[6].

Quality Control

K. pneumoniae ATCC 700603 and *E. coli* ATCC 25922 were positive and negative quality control for presence of ESBL, respectively. *E.coli*

Table 1. Primers used in PCR for identification of ESBLs genes

ESBLs Genes	Primers	References
TEM	Forward 5'-GAGTATCAACATTTCCGTGTC-3' Reverse 5'-TAATCAGTGAGGCACCTTCTC-3'	7
SHV	Forward: 5'–AAGATCCACTATCGCCCAGCAG-3' Reverse 5'-AAGATCCACTATCGC CCAGCAG-3'	7
CTX-M	Forward 5'-ACGCTGTTGTTAGGAAGTG-3' Reverse 5'-TTGAGGCTGGGTGAAGT-3'	7

ATCC 25922 was used for the quality control of the Kirby-Bauer disk diffusion method.

PCR Amplification

PCR analysis for β-lactamase genes of the family blaTEM, CTX-M and SHV was carried out.

The Primers used for SHV, TEM and CTX-M genes are listed in Table 1.

Statistical analysis

Chi-square test by SPSS16 was used with appropriate correction for the observation and for the significant difference between the ESBLs positive strains and multi-drug resistant *K. pneumonia* and *E. coli*. $P \leq 0.05$ was considered significant.

RESULTS

A total of 284 isolates were tested for presence of ESBL during a period of one year. Among 284 strains, 52.8% (n = 150) and 47.2% (n =134) were *E. coli* and *K. pneumoniae*, respectively. Overall, 38.7% (n = 110) of the strains were ESBLs positive, which *K. pneumoniae* with 50.74% (n = 68) positive strains showed higher prevalence than *E. coli* with 28% (n = 42).

Of the 68 ESBL positive *K. pneumoniae* strains, 4 strains harbored TEM gene, 14 strains carried CTX-M gene and 57 strains carried SHV genes as detected by PCR and among 42 ESBL positive *E. coli* strains, 40 strains showed positive results for TEM gene, 6 strains harbored CTX-M gene and 12 possessed SHV gene.

While some ESBLs confer high-level of resistance to all oxyimino-cephalosporins, for other ESBLs, resistance may only be slightly increased or selectively affected in certain β-lactams.

Antibiotic susceptibility pattern of uro-pathogens were as follows:

E. coli: Cotrimoxazole (43%), amikacin (38%) and ciprofloxacin (57.1%).

K. pnemoniae: Cotrimoxazole (38%), amikacin (36.7%) and ciprofloxacin (23.5%).

In *E. coli*, significant difference observed between TEM gene and resistance to ciprofloxacin (*P* d" 0.05) while no significant difference observed between CTX-M, SHV genes and multi-drug resistance strains. No significant difference observed between CTX-M TEM, and SHV genes and multi-drug resistant *K. pneumoniae*.

DISCUSSION

Antibiotic sensitivity pattern of organisms are changing rapidly over a short period. It is especially consistent for developing country where antibiotics are prescribed irrationally not only by the medical practitioners but the antibiotics are also purchased directly from the chemist (Medicine shop keepers) without prescription.

Resistance to extended spectrum cephalosporins is mainly mediated by the production of ESBLs. Recent studies on ESBL producer bacteria in *Enterobacteriaceae,* which were isolated from clinical specimens, showed an increase in the occurrence of ESBL producers[8]. In a similar study by Mathur *et al*, 62% of the *E. coli* and 73% of the *K. pneumoniae* strains were reported to be ESBL producers[9].

In the present study, we observed 28% of the *E. coli* and 50.74 % of the *K. pneumoniae* strains were ESBL producers. In addition, studies also reported that *K. pneumoniae* were more reported as ESBL producers than *E. coli*[10,11].

The findings of current research revealed that SHV was more observed in *K. pneumoniae* while the prevalence of TEM gene in *E. coli* was not comparable. The results showed that the lowest frequency of CTX-M occurred in *E .coli.*

Interestingly, our finding presented multi-drug resistant *E. coli* strains were more than *K. pneumoniae* strains. Ciprofloxacin resistant *E. coli* was predominant and the lowest resistance to ciprofloxacin observed in *K. pneumoniae*. The reason could be explained by gene responsible for ciprofloxacin resistant that it may confer by plasmid harboring TEM gene. In our study, the most active antibiotic against ESBLs producer strains was amikacin and ciprofloxacin was found as a best antibiotic for treatment of *K. pneumoniae* producing ESBL.

ESBL producing organisms, being the commonest nosocomial pathogens, it is essential to detect and treat them as early as possible. Since ESBL producer bacteria is more common among the nosocomial pathogens, early detection will definitely help in controlling hospital infections.

Enterobacteriaceae are the common isolates in most of the laboratories. Now a days, a majority of these isolates are multi-drug resistance.

The control of these multi-drug resistance organisms is a therapeutic challenge.

Study by Ghafourian *et al.*, showed 43.9% of *K. pneumoniae* was ESBL producers in Imam Reza Hospital in Tabriz, which was lower than our report in *K .pneumoniae*. Resistance rate to amikacin, ciprofloxacin and cotrimoxazol in Imam Reza hospital was less than the results, which obtained in our study[12].

In the study a tertiary care hospital in Tehran 77% of *K.pneumoniae* were ESBLs producer[13] while in our study frequency of ESBL positive strains were lower. In a survey by bazzaz *et al*., in 2007 in general hospitals in Iran, 59.2% of isolates were positive for ESBL, our findings in Milad hospital revealed near results to bazzaz *et al.*[14]. The prevalence of ESBL producers among the 131 clinical isolates was found to be 28% for *E. coli* and 50.74% for *K. pneumoniae*. The report of ESBL occurrence among uro-pathogenic *E. coli* and *K. pneumoniae* in India were known to be 40 and 54.54%, respectively[15]. The ESBL positive isolates of *E. coli* and *K. pneumoniae* were found to be multidrug resistance. Our findings is corresponding with Bhowmick and Rashid results[16].

In conclusion, spreading of ESBL-producing strains is a concern, as it causes limitations to the antimicrobial agents for optimal treatment of patients. Prevalence of ESBLs was more among *K .pneumoniae* and TEM was more observed in *E. coli*. Interestingly, Resistance to ciprofloxacin was predominant in *E. coli.*

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