Characterization of β-Lactamases from Urinary Isolates of Escherichia coli in Tehran

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ABSTRACT

Background: Knowledge of antimicrobial resistance patterns in E. coli, the predominant pathogen associated with urinary tract infections (UTI) is important as a guide in selecting empirical antimicrobial therapy. Methods: To describe the antimicrobial susceptibility of E. coli associated with UTI in a major university hospital in Tehran (Iran), seventy-six clinical isolates of E. coli were studied for susceptibility to β-lactam antibiotics by the disc diffusion method and Minimal Inhibitory Concentrations determination. Results: All isolates were resistant to ampicillin, amoxicillin and oxacillin. Resistance to the other tested antibiotics was shown to be 93.4% to cefradine, 76.3% to carbenicillin, 47.3% to cefazoline, 50% to cefalexin and 32.8% to cephalothin while 1.3% expressed resistance to cefoxitine, and 2.6% were resistant to cefotizoxime and ceftriaxone. Two isolates (2.4%) harbored extended spectrum β-lactamases (ESBL) shown by the double disc diffusion method. Substrate hydrolysis by ultraviolet spectroscopy showed that 87.4% harbored penicillinases, 9% produced cephlosporinases and 3.6% degraded both substrates. Clavulanic acid inhibited enzyme activity in 82.9%, of which 78.95% was penicillinases (group IIa) and 3.95% was cephalosporinases (group IIb) of the Bush classification system. The rest of the isolates (6.58%) were placed in group IV β-lactamases. No group III β-lactamase was found, as EDTA inhibited none of the enzymes. DNA amplification by polymerase chain reaction using specific primers for ampC, TEM and SHV type β-lactamases for all of the isolates showed that 47 organisms (60%) carried the TEM gene and 18 isolates (24%) harbored bla TEM and ampC genes. About 26% of the organisms harbored SHV type enzymes. Conclusion: These results indicate that E. coli can possess a variety of β-lactamases that are responsible for β-lactam resistance. Iran. Biomed. J. 11 (2): 95-99, 2007

Keywords: Urinary, β-lactamase, TEM, SHV, Amp C, E. coli

INTRODUCTION

Urinary Tract Infections (UTI) are the second most common infections present in community practice [1]. Members of Enterobacteriaceae, specifically, E. coli are the main causes of urinary infections [2]. Extensive use of β-lactams in veterinary medicine and human practice is believed to be associated with selection of resistance in both pathogenic and nonpathogenic isolates of E. coli [3]. More than two hundred β-lactamase enzymes are recognized which are classified into 4 main groups and 8 subgroups [4-7].

The resistance of Enterobacter spp. to β-lactam antibiotics is most frequently mediated by production of TEM, SHV and AmpC β-lactamase [8]. In the last decade, production of plasmid-mediated ESBL which hydrolyzes a wide range of the most recently developed cephalosporins, has been recognized as an additional important emerging mechanism of resistance among members of the family Enterobacteriaceae including clinical isolates of E. coli [9-11]. The first plasmid mediated β-lactamases (TEM-1) was described in E. coli in 1960 and within a few years, it was found in many different genera of Gram-negative bacteria [9].

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AmpC family of β-lactamases occurs both chromosomally and plasmid-mediated in E. coli and plasmid encoded AmpC β-lactamases are found to be responsible for global outbreaks [12, 13]. We studied 76 urinary isolates of E. coli for their susceptibility to 12 β-lactam antibiotics. Preferred substrate hydrolysis was performed to determine the class of β-lactamases. DNA amplification of β-lactamase types TEM, SHV and AmpC genes was carried out by PCR using type specific primers of blaTEM, ampC and SHV genes for all of the isolates.

MATERIALS AND METHODS

Bacteria. Seventy-six clinical isolates of E. coli were selected from a collection of urinary Enterobacteriaceae from the Bacteriology Laboratory of Vali-E-Asr Hospital in Tehran (Iran) [14]. E. coli ATCC 25922 was used as a control for antibiotic susceptibility tests. K. pneumoniae 57-1 carrying plasmid mediated SHV gene, E. coli MK148 carrying the ampC gene and E. coli harboring pTEM were used as positive controls for DNA amplification by PCR [15].

Antibiotic susceptibility. The antibiotic susceptibility of bacteria was initially carried out by the disc diffusion method according to the NCCLS recommendations [16]. The antibiotic discs were ampicillin (10 µg), amoxicillin (25 µg), carbenicillin (100 µg), cefalexin (30 µg), cephalothin (30 µg), cefazoline (30 µg), cefradine (30 µg), oxacillin (1 µg), ceftazidime (30 µg), ceftriaxone (30 µg), ceftizoxime (30 µg) (Padtan Teb, Tehran, Iran) and amoxicillin-clavulanic acid (20/10 µg, Difco, USA). Minimum Inhibitory Concentrations (MIC) of the isolates was determined for ampicillin, ceftazidime, cefotaxime, ceftriaxone, cefepime and imipenem by the microdilution broth method using the NCCLS standard procedure [17].

Screening for ESBL production. The double disc synergy test was used to screen for ESBL production [1]. Cefotaxime (30 µg), ceftriaxone (30 µg) and ceftizoxime (30 µg) were placed on Mueller Hinton agar plates adjacent to amoxicillin-clavulanic acid discs (20/10 µg). ESBL production was inferred when cephalexin inhibition zones expanded by the clavulanate.

Substrate hydrolysis. Relative hydrolysis rates of benzylpenicillin and cefaloridine were evaluated by UV spectroscopy. β-lactamase activity was determined by measuring the decrease in optical density of a 0.1 mM solution of cefaloridine (255 nm) or benzylpenicillin (240 nm). Enzymes were called penicillinase if the relative rate of benzylpenicillin hydrolysis was approximately 30% higher than that of observed for cephalexin, or cefalexinase if cephalexin was hydrolyzed at least 30% faster than penicillin [3, 18].

DNA amplification. Plasmid DNA extraction was carried out using a rapid alkaline lysis method [19]. The oligonucleotide primers used for the PCR assays were; 5’-ATAAAAATTTGGAAACGAAA3’ and 5’-GTCAGTTACCATGCTAATC-3’ for TEM, 5’-TGTTTTACATTGTTATCCG-3’ and 5’-GGTAGGGCCAGTGCT-3’ for SHV and 5’-ATGCACAACGCAATCC-3’ and 5’-GGTGAGGGTAGTTGCGATTGG-3’ for AmpC β-lactamases [20-22]. blaTEM and SHV primers were synthesized at the National Research Center for Genetic Engineering and Biotechnology, Iran and ampC primer was synthesized at Faza Pajooh (Tehran, Iran). Reactions were carried out in a Technie DNA thermocycler (Germany) in 25 µl mixtures containing 10 mM Tris-HCl (pH 8.3), 1 mM EDTA, 1.5 mM MgCl2, 200 µM of each deoxyribonucleoside triphosphate, 2-10 µM of oligonucleotide primers and 1 u of Taq DNA polymerase (Fermentas, Lithuania). Following a 4-min incubation time at 94°C, 35 cycles were run with the following temperature profile for each cycle: 94°C for 1 min, the proper annealing temperature for each primer (58°C for blaTEM, 59°C for ampC and 52°C for SHV) for 1 min and 72°C for 1 min. An additional 5-10 min incubation time was also carried out at 72°C. PCR experiments for amplification of the SHV gene failed to produce a single DNA product regardless of numerous standardization strategies. Therefore, presence or absence of the desired fragment was determined on the basis of comparing the resulting bands with a positive control as well as DNA size markers (Fig. 1).

RESULTS

Antibiotic susceptibility. Disc diffusion results are shown in Figure 2. All clinical isolates were resistant to ampicillin, amoxicillin and oxacillin. Resistance was shown to be 93.4% to cefradine, 76.3% to benzylpenicillin and cephalexin respectively.

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carbenicillin, 40.7% to amoxicillin-clavulanate, 47.3% to cefazoline, 50% to cephalaxin, 32.8% to cephalothin, 1.3% to ceftizoxime and 2.6% resistance to cefotaxime and ceftriaxone each MIC results generally confirmed the data obtained by disc diffusion. All 76 isolates were resistant to penicillin (≥1024 mg/l), but they showed the same pattern of susceptibility to imipenem (≤1 mg/l) and cefepime (≤1mg/l), drugs not used to treat urinary infections in Iran. Two isolates carried ESBL, one of them was resistant to cefotaxime and ceftriaxone, but both of them were sensitive to 4th generation cephalosporins with MIC values of 0.5 mg/l (Fig. 2).

**Classification of β-lactamases.** Figure 3 shows classification of the β-lactamases based on relative substrate hydrolysis and enzyme inhibition by clavulanic acid and EDTA according to the classification scheme of Bush [3, 4]. All organisms produced β-lactamases shown by the colony iodometric test [1]. Inhibition by clavulanic acid occurred for 82.9%, among which 78.95% was penicillinas (group IIa), and the rest was cephalosporinases (group IIb). The 6.58% of the isolates not inhibited by clavulanic acid was placed in group IV β-lactamases. None of the enzymes was inhibited by EDTA, suggesting that no class III β-lactamase was present (Fig. 3).

![Fig. 1. PCR amplification of Amp C type β-lactamase in some E. coli isolates. The amplified fragment is 1100 bp long. M, Molecular size marker (100 bp DNA ladder), co+ (positive control), co- (negative control).](image1)

![Fig. 2. Susceptibility patterns of 76 E. coli isolates to 12 antimicrobial agents.](image2)

![Fig. 3. Distribution of β-lactamase types of 76 E. coli isolates in Tehran by the Bush classification method.](image3)
**PCR amplification of TEM, SHV and ampC gene.** PCR products for blaTEM, ampC and SHV genes were 850, 1100 and 800 base pairs, respectively. Of 76 *E. coli* isolates, 47 (60%) carried blaTEM. Twenty four percent of the organisms (18/76) carried ampC gene. It should be mentioned that all of these organisms harbored blaTEM too. Approximately 26% of the isolates carried the SHV β-lactamase (Fig. 1, 4, 5) and two ESBL producers carried all 3 genes. As a matter of fact, there was no correlation between presence of β-lactamase genes with antibiotic susceptibility profiles or MIC.

**DISCUSSION**

Our results on antibiotic susceptibility patterns, β-lactamase types and ESBL production in 76 urinary isolates of *E. coli* from a Tehran University Hospital (Iran) basically agreed with the numerous surveys carried out around the world. The majority of the 76 clinical urinary isolates were resistant to penicillins and the first generation cephalosporins. Conversely, most of the strains were sensitive to the 3rd generation cephalosporins with only 1.3 % resistance to cefotizoxime and 2.6 % to cefotaxime and ceftriaxone antibiotics. In a survey conducted on 311 urinary and fecal isolates of *E. coli* from South east of Iran using disc diffusion, similar susceptibility patterns were observed with the 3rd generation cephalosporins [23]. However, they reported 69.6 % sensitivity to cefradine, whereas we found that 93.4% of our isolates were resistant to this antibiotic.

In a previous study performed on 50 clinical isolates of *E. coli* from the same health center in Tehran, a similar susceptibility pattern for the 3rd generation cephalosporins and 100% cefradine resistance were observed [13]. Production of ESBL among *Enterobacteriaceae* is more often seen in *E. coli* and *Klebsiella spp.* [12]. We found two isolates which produced ESBLs (2.7%): One of them was resistant to the third generation cephalosporins (cefotaxime and ceftriaxone). Relative substrate hydrolysis rates showed that the majority of the isolates carried penicillinases, most of them belonged to class-IIa of the Bush classification, common among *Enterobacteriaceae* [6, 24]. This may stress the point that the physiological conditions under which β-lactamase genes are expressed are extremely important and determine the outcome of clinical infections.

PCR amplification results on all of the isolates showed presence of TEM β-lactamases in 60% of organisms. We also showed that the SHV gene was present in 20 isolates (26%). This is in agreement with other studies showing that the most successful plasmid encoded β-lactamases, in terms of clinical significance, are the members of the TEM- and SHV- families [9]. We also demonstrated the presence of the ampC gene in 24% of the studied organisms. Two ESBL producer isolates carried all three β-lactamases genes.

In conclusion, these studies are essential for clinicians need to be aware of resistance rates observed in clinical isolates. Different prescription policies may influence the rates and patterns of resistance.

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