Fecal Carriage of Extended-spectrum Beta-lactamase and AmpC Beta-lactamase-producing Enterobacteriaceae in a Turkish Community

G Hazirolan, I Mumcuoglu, G Altan, BB Özmen, N Aksu, ZC Karahan

Background: Community-acquired infection caused by extended-spectrum beta-lactamase (ESBL)-producing microorganisms has an increasing frequency. Aim: The aim of this study was to determine the fecal carriage of ESBL and AmpC beta-lactamase-producing Enterobacteriaceae in community and to investigate cefotaxime-M (CTX-M) genes among ESBL isolates. Materials and Methods: A total of 1402 fecal specimens which were collected from outpatients included in the study. ESBL screening, ESBL production, and AmpC beta-lactamase detection were performed. Matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) was used for identification of species. Antibiotic susceptibilities of the isolates were detected by disk diffusion method. CTX-M beta-lactamase genes were investigated by polymerase chain reaction. Results: During the study period, a total of 1402 fecal samples were analysed with ESBL screening test and 490 Enterobacteriaceae strains isolated from these samples (Escherichia coli [n = 461, 94.1%], Klebsiella pneumoniae [n = 25, 5.1%], and Enterobacter cloacae [n = 4, 0.8%]). Fecal carriage of ESBL-producing Enterobacteriaceae in the community was 34.3%. AmpC beta-lactamases were detected in 26 (5.3%), and the frequency of CTX-M was found as 96.9%. The resistance rates of the E. coli strains to fluoroquinolones, trimethoprim–sulfamethoxazole, and carbapenems were 31.2%, 33.3%, and 0%, respectively. Conclusion: The relative high prevalence of fecal carriage of ESBL-producing bacteria in community warrants further study in this field including developing policies about antimicrobial use and close monitoring of resistance patterns.

Keywords: AmpC, cefotaxime-M, Enterobacteriaceae, extended-spectrum beta-lactamase, fecal carriage

Date of Acceptance: 20-Nov-2017

Introduction

A number of epidemics related to extended-spectrum beta-lactamase (ESBL)-producing bacteria have been reported in Turkey as well as in other parts of the world.[1-3] ESBL-producing bacteria constitute a significant treatment and dissemination problem, particularly in hospitalized patients.[4] ESBL-producing Enterobacteriaceae have been isolated as the agents of community-acquired infections in the past two decades, and it was shown that 70% of the ESBL-producing Escherichia coli infections were community acquired.[5-7] ESBLs are usually enzymes derived from TEM and SHV; however, cefotaxime-M (CTX-M) is the most frequently reported beta-lactamase in the community-acquired isolates.[8-10] CTX-M beta-lactamases were first determined in the clinical strains of Enterobacteriaceae in Europe and Argentina in the late 1980s, and then, a number of community-acquired and nosocomial CTX-M beta-lactamases were reported in a number of countries all around the world.[9,10] Plasmid-based AmpC beta-lactamases are less frequent than ESBLs, and in contradistinction to ESBLs, they hydrolyze...
cephemycins, and they are responsible for resistance against a wider spectrum of beta-lactam antibiotics.\(^{11,12}\) AmpC beta-lactamases are usually transferred on the same plasmid with ESBL genes. They constitute a significant problem for hospitalized patients as the causative agents of severe infections. However, community-acquired infections and healthy fecal carriers have also been reported.\(^{13-16}\)

The present study was undertaken to determine the prevalence of ESBL and AmpC beta-lactamase producers in the stool samples of the patients who were admitted to outpatient clinics of Ankara Numune Training and Research Hospital in Turkey and to screen ESBL-positive isolates for the presence of genes encoding CTX-M by polymerase chain reaction (PCR).

**MATERIALS AND METHODS**

**Ethical consideration**

The prospective study protocol was reviewed and approved by the Ethical Clinical Committee of Ankara Numune Education and Research Hospital located in Ankara, Turkey (meeting number: 867/2016). The study was performed according to the Declarations of Helsinki (2013).

**Study setting and population**

The study was conducted in Ankara Numune Training and Research Hospital, Ankara, Turkey. A total of 1402 stool samples obtained from outpatients that admitted to Ankara Numune Training and Research Hospital Microbiology Laboratory between January and April 2016 were included in the study. Exclusion criteria were hospitalization or use of antibiotics in the previous 3 months. Single stool sample from each participant was analyzed.

**Phenotypic analysis of extended-spectrum beta-lactamase and AmpC beta-lactamase**

To analyze ESBL, a total of 0.5 g of each stool sample was suspended in 5 mL of sterile saline, and aliquots of 50 μl were streaked onto ceftazidime blue (EMB) agar that contained 1 μg/ml ceftazidime for ESBL screening. The cultures were kept at 37°C in the aerobic environment, for 48 h at maximum. Following species identification of the cultured bacteria by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry, (MALDI-TOF MS, Bruker, Daltonik, Germany), production of ESBL was detected using ceftazidime 30 μg, ceftazidime/clavulanic acid 30 μg/10 μg (Becton Dickinson, NJ, USA), and CTX 30 μg/CTX/clavulanic acid 30 μg/10 μg (Becton Dickinson, NJ, USA) disks, in relation with the recommendations of Clinical and Laboratory Standards Institute (CLSI). AmpC beta-lactamase production was analyzed in relation with recommendations of European Committee on Antimicrobial Susceptibility Testing using cefoxitin (FOX) 30 μg (Becton Dickinson, NJ, USA), and FOX/clavulacin 30/200 μg (Liofilchem, Roseto, Italy) disks. The bacteria suspension at a density of 0.5 McFarland was spread over Mueller-Hinton Agar (MHA, Oxoid, Basingstoke, United Kingdom) plates, and then, the disks were placed. The bacteria were considered positive for ESBL production when the diameter of the inhibition zone around the combination disks was ≥5 mm larger than the disks that did not contain clavulanic acid after incubation at 37°C for 24 h.\(^{17}\) The isolate tested was considered positive for AmpC beta-lactamase when the inhibition diameter around the FOX/clavulacin disk was ≥5 mm greater than the inhibition zone around FOX disc.\(^{18}\) AmpC beta-lactamase was investigated only by phenotypically. Klebsiella pneumoniae ATCC 700603 and E. coli ATCC 25922 standard strains were used as positive and negative controls (NCs), respectively.

**Identification and antibiotic susceptibility tests**

All bacteria reproduced in ESBL screening plates were Gram stained, and they were identified at species level with MALDI-TOF MS (Bruker, Daltonik, Germany). Commercial antibiotic disks (Bioanalyse, Ankara, Turkey) of amoxicillin/clavulamic acid, piperacillin/tazobactam, FOX, CTX, ceftriaxone, cefepime, imipenem, meropenem, ertapenem (ERT), ciprofloxacin (CIP), amikacin (AN), gentamicin (GN), and trimethoprim-sulfamethoxazole (SXT) were used to determine antibiotic susceptibilities of the identified strains. The antibiotic susceptibilities of the strains were determined with Kirby-Bauer disk diffusion method, according to CLSI criteria.\(^{17}\) E. coli ATCC 25922 was used as quality control strain.

**Determination of cefotaxime-M presence**

The DNA extraction of the strains was done with Ribospin™ vRD (GeneAll Biotechnology, Seoul, Korea) kit. The presence of CTX-M gene was determined using PCR method and CTX-MU1 (5'-ATGTGCAGYACCAGTAARGT) and CTX-MU2 (5'-TGTTTARTARGTSCCAGA) primers, as previously described.\(^{19}\) Amplified products were viewed in agarose gel electrophoresis. Obtained band sizes were interpreted by comparing them with DNA molecular weight standard (Thermo Scientific, MA, USA) and NC. Citrobacter freundii 2525 (CTX-M-3) was used as the quality control strain.

**RESULTS**

During the study period, 1402 patients’ stool samples were examined. The mean age of the study population was 47.2 ± 24.5 years. There was growth in ESBL screening disk in 481 (34.3%) of 1402 stool samples.
A total of 490 Enterobacteriaceae members were reproduced from 481 stool samples that showed bacterial growth. Among them, 461 (94.1%) were identified as E. coli, 25 (5.1%) were identified as K. pneumoniae, and 4 (0.8%) were identified as Enterobacter cloacae. The fluoroquinolone resistance rate was 31.2%, and the SXT resistance rate was 33.3% in ESBL-producing E. coli strains. However, none of the reproduced strains showed carbapenem resistance. ESBL-producing strains and their susceptibilities for various antibiotics are presented in Table 1.

Phenotypic AmpC beta-lactamase was detected in 26 (5.3%) of 490 ESBL-positive strains. Among them, 24 were E. coli and two K. pneumoniae. All strains with phenotypic AmpC beta-lactamase were resistant to ceftazidime and CTX and susceptible to ERT [Table 2].

PCR reactions indicated the presence of CTX-M gene in 96.9% of 490 strains that were phenotypically positive for ESBL. Figure 1 has showed gel electrophoresis of CTX-M-positive strains. CTX-M gene was determined in 98% (452/461) of E. coli strains, 84% (21/25) of K. pneumoniae strains, and 50% of (2/4) E. cloacae strains [Table 2].

**DISCUSSION**

Several rates have been reported for fecal carriage of ESBL-producing Enterobacteriaceae members in hospitalized patients, outpatients and in the community. Fecal carriage of ESBL-producing Enterobacteriaceae members increased particularly in the past two decades, and this has become a significant problem all around the world.[20-22] Developing countries are affected more from this condition. Frequent intercontinental trips made it a global problem.[5] K. pneumoniae and TEM and SHV enzymes are the most common among the nosocomial ESBL-producing Enterobacteriaceae strains, and E. coli and CTX-M enzyme are the most frequent among ESBL-producing Enterobacteriaceae strains.[20]

In our study, we found fecal carriage rate for ESBL-producing Enterobacteriaceae as 34.3% in the community. E. coli strains were the most frequent ones among ESBL-producing Enterobacteriaceae members. This pathogen is the most common Gram-negative bacterial species, widespread in the community, and health-care settings. The predominance of E. coli strains among ESBL-producing Enterobacteriaceae members confirms the results of previous studies.[6,21-30] Our fecal carriage rate for ESBL-producing Enterobacteriaceae is quite high when compared to the rates reported from France (6.0%), Switzerland (5.8%), Spain (5.06%), Japan (6.4%), Tunisia (7.3%), Germany (6.4%), the Netherlands (9.5%), and Libya (13.4%). On the other hand, this rate is smaller than the rates reported in China (50%), Egypt (63%), and Thailand (65.7%).[6,21-30]
In our study, Azap et al.\textsuperscript{[31]} determined fecal ESBL-producing Enterobacteriaceae carriage rate as 15.2% among 795 outpatients in 2006, and Küçükbasmaç\textsuperscript{[32]} reported fecal carriage rate as 21.3% in 150 patients who were not hospitalized or given antibiotics in 2009. In 2013, Tukenmez et al.\textsuperscript{[33]} determined fecal ESBL-producing Enterobacteriaceae carriage rate as 18.7% in males who were scheduled for transrectal prostate biopsy. In our study, we studied fecal ESBL-producing Enterobacteriaceae carriage in a larger study population (1402 participants) and determined a higher fecal ESBL-producing Enterobacteriaceae carriage rate (34.3%) compared to previous studies performed in Turkey.

The most frequent community-acquired ESBLs are CTX-M beta-lactamases, and the most frequent gene family among them is CTX-M-15.\textsuperscript{[34]} Ahmed et al.\textsuperscript{[21]} determined CTX-M gene in all (100%) ESBL-producing \textit{E. coli} strains isolated from stool samples of the Libyan community. Valenza et al.\textsuperscript{[26]} found CTX-M gene in 95.2% of ESBL-producing \textit{E. coli} strains in Germany. Lonchel et al.\textsuperscript{[2]} determined CTX-M beta-lactamase in 97% of fecal ESBL-producing \textit{E. coli} strains in Cameroon. Reports from our study setting are scarce, and CTX-M enzyme was reported in 89.4%–99% of community-acquired fecal ESBL-producing isolates.\textsuperscript{[32,33]} In our study, CTX-M enzyme was found positive in 96.9% of the ESBL-producing Enterobacteriaceae, in fecal carriers. Our results indicated that the rate of CTX-M enzyme was high in ESBL-producing Enterobacteriaceae members in the community.

Although extended-spectrum cephalosporin resistance usually appears with ESBL, AmpC beta-lactamase is another beta-lactamase that hydrolyzes cephalosporins. Simultaneous presence of ESBL and AmpC beta-lactamase may prevent accurate determination of ESBL phenotype. Although AmpC beta-lactamases are less frequent compared to ESBL, they present resistance against a wider spectrum of cephalosporins.\textsuperscript{[35]} In our study, we found fecal carriage rate of AmpC beta-lactamase-producing \textit{E. coli} as 5.3%. Those data have been presented for the first time because no studies up to date have investigated fecal carriage of AmpC beta-lactamase in Turkish population. Ahmed et al.\textsuperscript{[21]} reported rate of fecal AmpC beta-lactamase carriage as 6.7% in \textit{E. coli} strains in the community in Libya; Reuland et al.\textsuperscript{[27]} found a rate as 1.3% in the Netherlands. Bassyouni et al.\textsuperscript{[36]} reported a rate of fecal AmpC beta-lactamase carriage as 3% in the health care workers in Egypt. Garrido et al.\textsuperscript{[22]} found the rate of fecal AmpC beta-lactamase carriage as 0.59% in Spain. The rate of fecal AmpC beta-lactamase carriage found in our study is similar to the rates reported from developing countries. We could not show the presence of AmpC beta-lactamase genotypically in our study, and this is a limitation of our study.

CTX-M beta-lactamase genes are widely known to be carried on a plasmid linked to mobile genetic elements that are utilized as vehicles for resistance genes horizontal movement. Unfortunately, high resistance to fluoroquinolones, aminoglycosides, and SXT is also seen in addition to resistance to penicillins and cephalosporins in \textit{E. coli} strains that carry community-acquired CTX-M enzyme-positive ESBLs.\textsuperscript{[2,20,31]} In our study, we did not find resistance to AN in \textit{E. coli} strains that carried CTX-M enzyme-positive ESBLs; however, CIP resistance rate was 31.2%, and SXT resistance rate was 33.3%.

In our country, CIP resistance was reported as 17%–39%, and AN resistance was reported as 1%–86% in the fecal isolates of the community.\textsuperscript{[31,32]} No AN resistance was determined in fecal ESBL Enterobacteriaceae carriers in Canada, the United Kingdom, and Cameroon; however, the resistance rates for GN, CIP, and SXT were higher than the rates found in our study.\textsuperscript{[2,37,38]}

In the present study, we determined the prevalence of ESBL-producing Enterobacteriaceae in individuals who were not hospitalized or administered antibiotics in the previous 3 months. The high ESBL prevalence and resistance to CIP and SXT would cause problems particularly in community-acquired urinary tract infections, in which the pathogenic microorganism originates from the gastrointestinal flora. We found high rates of ESBL and AmpC-producing isolates, most of them originated from CTX-M, and those high rates could threaten public health. The use of wide-spectrum cephalosporins must be reduced in humans and animals, and regular epidemiological surveillance studies must be performed to decrease fecal carriage of those isolates and therefore the infections that may arise due to them.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.


REFERENCES


