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Full Length Research Paper

High prevalence of CTX-M-type beta-lactamase in *Escherichia coli* isolates producing extended-spectrum beta-lactamase (ESBL) and displaying antibiotic co-resistance

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In the present study, we investigated the prevalence of CTX-M-type beta-lactamase in extended spectrum beta-lactamase (ESBL)-producing *Escherichia coli* isolated in our hospital as well as their antibiotic resistance and co-resistance rates. Two hundred nineteen *E. coli* isolated from clinical specimens between 2006 and 2007 were included. Antibiotic susceptibility test was performed using disc diffusion method and ESBL production was determined using a double-disc synergy test. The presence of CTX-M-type beta-lactamase genes was investigated through amplification using specific primers. The prevalence of CTX-M-type beta-lactamase was found 87% in *E. coli* isolates. The isolates displayed high rates of resistance to tested antibiotics: 87% to ampicillin-sulbactam (SAM), 77% to amoxicillin-clavulanic acid (AMC), 76% to co-trimoxazole (SXT), 70% to norfloxacin (NOR), 68% to ciprofloxacin (CIP), and 51% to gentamicin (GN). All isolates were found susceptible to imipenem (IPM), meropenem (MEM) and fosfomycin (FOS). Co-resistance was identified in 96% of isolates, and the most common two co-resistance phenotypes were AMC/SAM/GN/NOR/CIP/SXT (12%) and AMC/SAM/NOR/CIP/SXT (11%). CTX-M-type beta-lactamase was present in *E. coli* isolates at extremely high rates. The empiric therapy with SAM, AMC, SXT, NOR, CIP, and GN may not be adequately effective against certain isolates of *E. coli* due to high rate of resistance.

Key words: *Escherichia coli*, antibiotic resistance, co-resistance, extended spectrum beta-lactamase, CTX-M.

INTRODUCTION

Today, an increase in bacterial resistance against antibiotics has become a major worldwide problem (Denton, 2007). During the past years, increasing rates of infections by extended spectrum beta-lactamase (ESBL)-producing isolates has greatly limited the use of non-carbapenem beta-lactam antibiotics, and thus the importance of carbapenem and non-beta lactam antibiotics in therapy has risen incrementally. The co-transmission of antibiotic resistance genes via plasmids may also compromise the effectiveness of many individual antibiotics. Rising antibiotic resistance rates among clinical isolates have resulted in increased morbidity and mortality and extend periods of hospitalization and, consequently, increased economic costs.

Regarding ESBL, it has been reported that TEM- and SHV-type beta-lactamases are widespread and that CTX-M-type beta-lactamases are very common globally (Isturiz, 2008). In Turkey, data relating to limited numbers of previous studies on the prevalence of CTX-M-type beta-lactamases may constitute a possible threat for antibiotic therapy (Gonullu et al., 2008; Yumuk et al., 2008). Clinically, antibiotics are frequently used empirically in the treatment of infections and the selected treatment may need to be modify according to the

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antibiotic susceptibility findings. Therefore, it may be useful to identify co-resistance rates, as well as ESBL rates in *Escherichia coli* isolates, which could be used as a guide especially in empirical therapy. Here we report the prevalence of CTX-M-type beta-lactamase among clinical isolates of ESBL-producing *E. coli* and the resistance and co-resistance rates to various antibiotics in these isolates.

MATERIALS AND METHODS

Bacterial isolates

Non-duplicate *E. coli* isolates (*n* = 219) collected between January 2006 and December 2007 from the Microbiology Laboratories in Istanbul Medical Faculty (IMF, 1.750 beds) located in the European part of Istanbul, were included into the study. The study was planned retrospectively.

Bacterial identification

Bacterial isolates were identified by conventional methods and API ID 32 GN kit (BioMerieux, France).

Antibiotic susceptibility test

Individual isolates were tested, based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI), by the Kirby–Bauer disc diffusion method for susceptibility to the following antibiotics: amoxicillin-clavulanic acid (AMC, 20/10 µg), ampisilin-sulbactam (SAM, 10/10 µg), cefoperazone-sulbactam (SCF, 75/30 µg), piperacillin-tazobactam (TZP, 100/10 µg), imipenem (IMP, 10 µg), meropenem (MEM, 10 µg), gentamicin (GN, 10 µµg), amikacin (AK, 30 µg), norfloxacin (NOR, 10 µg), ciprofloxacin (CIP, 5 µg), co-trimoxazole (SXT, 1.25/23.75 µg), nitrofurantoin (NIT, 300 µg), fosfomycin (FOS, 200 µg) (CLSI, 2005). The presence of ESBL's in *E. coli* isolates was investigated by a double-disc synergy test with using amoxicillin-clavulanic acid, cefotaxime and ceftazidime. Isolates resistant to two or more antibiotics were classified as co-resistant phenotypes. *E. coli* ATCC 25922 and *E. coli* ATCC 35218 were used as quality-control strains.

Extraction of genomic DNA

Bacterial colonies were suspended in 2 ml centrifuge tubes and then centrifuged at 12,000 g to obtain pellets. Pellets were washed in 750 µl of TE buffer (10 mM Tris HCl, pH 8.0, 1 mM EDTA) and then boiled for 10 min in 500 µl of TE buffer and centrifuged. Supernatants were stored at -20°C prior to subsequent DNA amplification (Nazik et al., 2008).

DNA amplification

The presence of CTX-M-type beta-lactamase genes was investigated through amplification using specific primers (CTX-M-F 5'-ATGTGCAGYACCAGTAARGT-3', CTX-M-R 5'-TGGGTRAARTARGTSACCAGA-3') (Pallecchi et al., 2007). The primers were amplified the 593-bp fragment of CTX-M genes. The controls were included with each group of tested strains. The following conditions were used for polymerase chain reaction (PCR): one cycle of denaturation for 7 min at 94°C; 35 cycles of denaturation for 50 s at 94°C, primer annealing for 40 s at 50°C, and primer extension for 60 min at 72°C; and, finally, one cycle of primer extension for 5 min at 72°C.

Imaging the PCR product

Ten microliters of each amplification product was mixed with 2 µl of loading buffer, and then separated on a 1.5% agarose gel for 45 min in 1x TAE buffer containing ethidium bromide. After electrophoresis, gels were visualized under UV light (304 nm).

Statistical analysis

Statistical analysis was performed using SPSS for Windows Version 11.5 (SPSS, Inc., Chicago, IL, USA). Rates of resistance were compared by Chi-square test. A p-value <0.05 was considered to be statistically significant.

RESULTS

Isolates included in the study were isolated from 86 males and 138 females; 138 were outpatients while 81 were inpatients. Of these 219 patients, 115 (52.5%) were aged 0 to 16 years, and 104 (47.5%) were >16 years. A hundred-seventy-four isolates (79.5%) were derived from urine, and the remainder from various clinical specimens [pus = 20 (9.2%), blood = 13 (5.9%), tracheal aspirate = 5 (2.4%), peritoneal fluid = 4 (1.8%), wound = 3 (1.4%)]. Seventy-three strains (33%) were isolated in 2006, and the remaining 146 (67%) in 2007.

CTX-M-type beta-lactamase was present in 190 of 219 (87%) ESBL producing *E. coli* strains. Additionally the prevalence of CTX-M-type beta-lactamase was increased in 2007 according to 2006 (From 74 to 93.2%, p =0.001). During two-years study period, the high rates of resistance in *E. coli* isolates were detected to SAM (87%), AMC (77%), SXT (76%), NOR (70%), CIP (68%), and GN (51%). In our study, resistance to IPM, MEM and FOS was not encountered and low rate of resistance was detected to AK (7%) . The resistance rates to SCF and TZP was lower than that to SAM and AMC (Figure 1). However, 96% (211/219) of the isolates exhibited co-resistance, with the most common co-resistance phenotypes being AMC/SAM/GN/NOR/ CIP /SXT (12%) and AMC/SAM/NOR/CIP/SXT (11%) (Table 1).

Results obtained from investigation of resistance/co-resistance in *E. coli* isolates that were susceptible or resistant to individual antibiotics are displayed in Table 2. When resistant *E. coli* isolates were tested for resistance to other antibiotics, we found resistance rates: 87% to SAM, 73% to AMC, 68% to SXT, 51% to NOR, 49% to CIP, 34% to GN, 22% to TZP, 11% to SCF, 10.5% to NIT and 7% to AK. ESBL-producing *E. coli* isolates that were resistant to SAM and AMC showed higher rates of resistance to NOR or CIP than isolates susceptible to SAM and AMC (p<0.05).

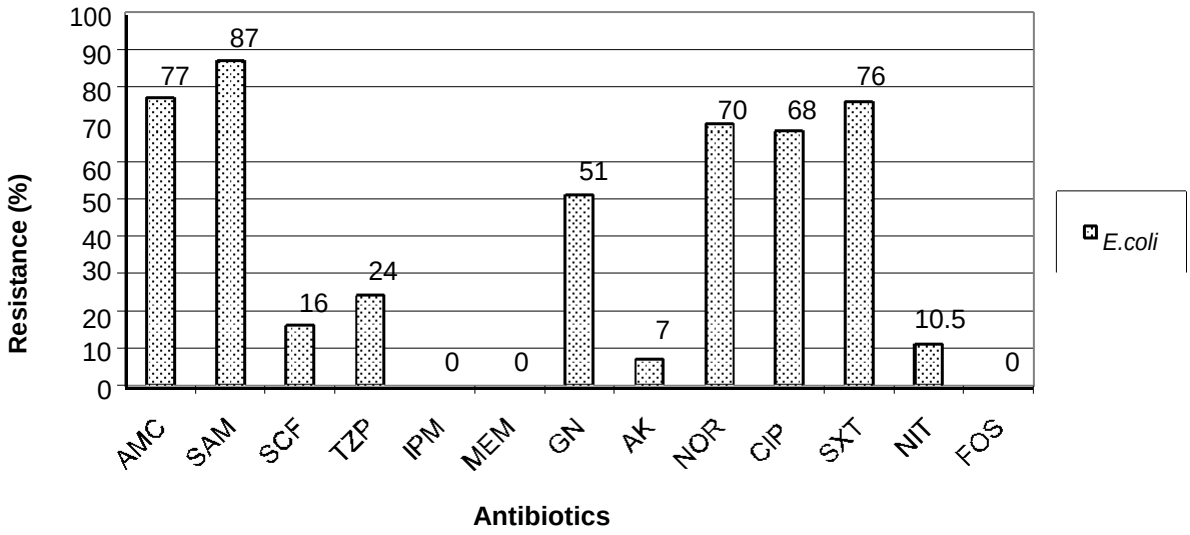

Figure 1. Resistance rates of *E. coli* strains (n = 219) to different antibiotics. AMC: Amoxicillin-clavulanic acid; SAM: Ampicillin-sulbactam; SCF: Cefoperazone-sulbactam; TZP: Piperacillin-tazobactam; IPM: Imipenem; MEM: Meropenem; GN: Gentamicin; AK: Amikacin; NOR: Norfloxacin; CIP: Ciprofloxacin; SXT: Co-trimoxazole; NIT: Nitrofurantoin; FOS: Fosfomycin.

Table 1. Rates of co-resistant phenotypes frequently observed in *E. coli* strains (n = 210).

Co-resistant phenotype	Number of strains (%)
AMC, SAM, GN, NOR, CIP, SXT	25 (12)
AMC, SAM, NOR, CIP, SXT	24 (11)
SAM, GN, NOR, CIP, SXT	11 (5)
AMC, SAM, NOR, CIP	10 (5)
AMC, SAM, GN, NOR, CIP	8 (4)
AMC, SAM, GN, SXT	7 (3)
AMC, SAM, SXT	7 (3)

AMC: Amoxicillin-clavulanic acid; SAM: Ampicillin-sulbactam; SCF: Cefoperazone-sulbactam; TZP: Piperacillin-tazobactam; IPM: Imipenem; MEM: Meropenem; GN: Gentamicin; AK: Amikacin; NOR: Norfloxacin; CIP: Ciprofloxacin; SXT: Co-trimoxazole; NIT: Nitrofurantoin; FOS: Fosfomycin.

DISCUSSION

The worldwide presence of CTX-M-type beta-lactamases in *E. coli* isolates is reported to reach a variable ratio up to 80% (Morosini et al., 2006; Pallecchi et al., 2007). In Turkey, recent studies reported that CTX-M-type beta-lactamases are very common in *E. coli* strains. It was reported that CTX-M-type beta-lactamase was present in 86.8% of community and hospital originated ESBL-producing *E. coli* strains (*n* = 61) (Gonullu et al., 2008). In another study CTX-M- type beta-lactamase was present in 13 (76.5%) of 17 ESBL-producing *E. coli* strains originating from community (Yumuk et al., 2008) . In a more recent study from Turkey, a high prevalence (98%) of CTX -M type beta- lactamases was found in ESBL-positive *E. coli* strains (n = 51) isolated from urinary tract infections (Azap et al., 2010) . In our study, CTX-M-type beta-lactamase was present in 87% of *E. coli* isolates tested. This type of beta- lactamase is common in ESBL-producing *E. coli* isolated in our hospital and this result is comparable to those reported in other studies in our country.

Although, carbapenem resistance in *Enterobacteriaceae* has been rarely reported in past, resistance rates have recently increased. Nonetheless, carbapenem remains the first choice of treatment for infections involving ESBL-producing *E. coli*. It has been estimated that worldwide rate of carbapenem resistance in *Enterobacteriaceae* is nearly 2% (Queenan and Bush, 2007). When national data are taken into account, rates of carbapenem resistance in *E. coli* are estimated to be 0 to 8% (Gunseren et al., 1999; Gur et al., 2008; Ozyurt et al., 2008). However, in the present survey we did not observe resistance to IPM or MEM in any of the *E. coli* isolates tested. The antibiotics AMC, SAM, SCF, TZP, GN, AK, NOR, CIP, SXT, and NIT have been widely used in Turkey to treat a number of diseases, especially infections due to *E. coli*. However, excessive and inappropriate use of these antibiotics risks an increase in antibiotic resistance. In several studies performed in Turkey in the past decade, the following resistance rates have been reported in *E. coli* isolates: 29.2–75% to AMC; 32.7–53% to SAM; 6% to SCF; 10.2–50% to TZP; 3.3–40% to GN; 4–33% to AK; 8.3–63.3% to CIP; 27.9–61.3% to SXT; 6–10% to NIT; and 0.8% to FOS (Aykut Arca and Karabiber, 2007; Aypak et al., 2009; Gunseren et al., 1999; Gur et al., 2008; Kacmaz and Sultan, 2007;

Table 2. Co-resistance in *E. coli* strains (%).

E. coli	AMC	SAM	SCF	TZP	IPM	MEM	GN	AK	NOR	CIP	SXT	NIT	FOS
AMC-S (*n* = 50)	0	56	2	2	0	0	54	2	56	54	90	6	0
AMC-R (*n* = 169)	100	96	20	30	0	0	50	8	74	72	72	12	0
SAM-S (*n* = 29)	24	0	3	7	0	0	52	0	45	38	76	10	0
SAM-R(*n* = 190)	85	100	18	26	0	0	50.5	8	74	72	76	10.5	0
SCF-S (*n* = 184)	73	85	0	14	0	0	54	6.5	73	71	77	10	0
SCF-R (*n* = 35)	97	97	100	77	0	0	34	9	51	49	74	14	0
TZP-S (*n* = 167)	71	84	5	0	0	0	52	4	71	68	77	9	0
TZP-R (*n* = 52)	98	96	52	100	0	0	46	15	67	65	75	15	0
IPM-S (*n* = 219)	77	87	16	24	0	0	51	7	70	68	76	10.5	0
IPM-R (*n* = 0)	-	-	-	-	-	-	-	-	-	-	-	-	-
MEM-S (*n* = 219)	77	87	16	24	0	0	51	7	70	68	76	10.5	0
MEM-R (*n* = 0)	-	-	-	-	-	-	-	-	-	-	-	-	-
GN-S (*n* = 108)	79	87	21	26	0	0	0	5	65	61	75	9	0
GN-R (*n* = 111)	76	86.5	11	22	0	0	100	9	75	74	77.5	12	0
AK-S (*n* = 204)	76	86	16	22	0	0	49.5	0	68	66	76.5	10	0
AK-R (*n* = 15)	93	100	20	53	0	0	67	100	93	87	73	13	0
NOR-S (*n* = 66)	67	76	26	26	0	0	42	1.5	0	0	76	9	0
NOR-R (*n* = 153)	82	91.5	12	23	0	0	54	9	100	97	76.5	11	0
CIP-S (*n* = 71)	68	75	25	25	0	0	41	3	7	0	75	10	0
CIP-R (*n* = 148)	82	93	11.5	23	0	0	55	9	100	100	77	11	0
SXT-S (*n* = 52)	90	86.5	17	25	0	0	48	8	69	65	0	10	0
SXT-R (*n* = 167)	73	87	16	23	0	0	51.5	7	70	68	100	11	0
NIT-S (*n* = 196)	76	87	15	22	0	0	50	7	69	67	76	0	0
NIT-R (*n* = 23)	87	87	22	35	0	0	56.5	9	74	70	78	100	0
FOS-S (*n* = 219)	77	87	16	24	0	0	51	7	70	68	76	10.5	0
FOS-R (*n* = 0)	0	0	0	0	0	0	0	0	0	0	0	0	100

AMC: Amoxicillin–clavulanic acid; SAM: Ampicillin-sulbactam; SCF: Cefoperazone-sulbactam; TZP: Piperacillin-tazobactam; IPM: mipenem; MEM: Meropenem; GN: Gentamicin; AK: Amikacin; NOR: Norfloxacin; CIP: Ciprofloxacin; SXT: Co-trimoxazole; NIT: Nitrofurantoin; FOS: Fosfomycin. S – susceptible; R – resistant.

Korten et al., 2007; Kurutepe et al., 2005; Ozyurt et al., 2008; Sumer et al., 2005; Yilmaz et al., 2009; Yuksel et al., 2006). On the contrary, the following low resistance rates from different locations of the world have been reported: 5.6-26.8% to AMC; 0-1.7% to TZP; 1.9-19.4% to GN; 0.6% to AK; 5.3-32.9% to CIP; 23.5-31% to SXT;, 2.6-10.4% to NIT (Anatoliotaki et al., 2007; Bean et al., 2008; Fedler et al., 2006; Sotto et al., 2001). However, it is reported that these rates are higher in ESBL-producing strains in worldwide (Akyar, 2008; Koksal et al., 2009; Pullukçu et al., 2008; Spanu et al., 2002). In the present study, among the *E. coli* isolates, we determined high rates of resistance to SAM (87%), AMC (77%), and SXT (76%), whereas the lower rates of resistance to AK (7%) (Figure 1). When resistant *E. coli* isolates were analyzed for resistance to other antibiotics, we found resistance rates: 87% to SAM, 73% to AMC, 68% to SXT, and 51% to NOR. According to our results, *E. coli* isolates that have developed resistance to any one of the tested antibiotics suggest that, rates of resistance could be improved at least 51% to AMC, SAM, NOR, and SXT, 49% to CIP, and 34% to GN. Although, CTX-M type beta-lactamases are inhibited by clavulanate and sulbactam, the high resistance to SAM and AMC may result from other additional resistance mechanisms such as production of AmpC type beta-lactamases or porin mutations, which were not investigated, in the present study.

Genes that encode ESBLs are commonly located on plasmids and may increase antibiotic resistance and co-resistance rates between the bacteria. It is well known that various antibiotic resistance genes, including those encoding beta-lactams, macrolides, aminoglycosides, and trimethoprim, are transmitted by plasmids. A recent study demonstrated the transmission of a quinolone resistance gene through a plasmid (Martinez-Martinez et al., 1998). Additionally, it was shown that plasmid-mediated quinolone resistance genes (*qnr*) were more frequent among ESBL-producing bacterial strains (Nazik et al., 2008; Nordmann and Poirel, 2005). According to our previous study on plasmid mediated quinolone resistance genes, 75% (15/20) of the tested quinolone-resistant *Enterobacteriaceae* strains produced CTX-M-type beta-lactamase, which was more frequent than TEM

and SHV type (Nazik et al., 2009). In the same study, nalidixic acid resistance was determined in strains carrying a *qnr* gene. In the present study we observed very high rates of resistance to NOR and CIP (that is up to 70%) (Figure 1). Overall, co-resistance was observed in 96% of tested isolates, with the most common co-resistance phenotypes being AMC/SAM/GN/NOR/CIP/SXT (12%) and AMC/SAM/NOR/CIP/SXT (11%) (Table 1).

Unfortunately, because of high bacterial resistance rates to many antibiotics, therapeutic options appear more limited than ever. FOS, a phosphoric acid derivative discovered in 1969 in Spain, has been widely used in Europe for the treatment of various infections. During the past few years, this drug has entered to use for treatment in our country. In the treatment of non-complicated lower urinary tract infections, FOS can be administered as a single dose. Thanks to its low rate of bacterial resistance, this drug may be a choice in the management of various infections caused by multidrug-resistant bacteria (Schito, 2003; Baylan, 2010). Additionally, fosfomycin resistance rate is unexpectedly low when compared to antibiotics such as NOR, NIT, and SXT that are commonly used to treat urinary tract infections (USIs). In Turkey, FOS resistance rates also were very low in studies performed on ESBL-producing strains of *E. coli*. In a study, 344 ESBL-producing *E. coli* strains were studied, and resistance rates were reported as follows: FOS, 3.5%; CIP, 76.5%; AK, 11%; and SXT, 74.4% (Pullukçu et al., 2008). In another study, 132 ESBL-producing strains of *E. coli* were isolated from patients with USIs (Akyar, 2008). It was demonstrated that all ESBL-producing *E. coli* strains were found to be FOS- susceptible, while rates of resistance to other antibiotics were as follows: GN, 34.8%; AK, 3%; SXT, 68.9%; NIT, 3%; and NOR and CIP, 80.3% each. In a recent study, the FOS resistance rate in 150 ESBL-producing *E. coli* urinary isolates was found 2% (Hosbul et al., 2009). In our study, no resistance to FOS and especially high resistance to flouroquinolones and SXT were detected in *E. coli* isolates tested.

In summary, we showed that a large proportion of the tested isolates was CTX-M-type beta-lactamase-positive. The most effective antibiotics against these isolates were found to be IPM, MEM, FOS, and AK. We observed high levels of resistance to SAM, AMC, SXT, NOR, CIP, and GN, thus we conclude that ESBL producing isolates have developed resistance mechanisms to non beta-lactam antibiotics as well as beta-lactam ones. Thus, empiric therapy with SAM, AMC, SXT, NOR, CIP, and GN may not be adequately effective against certain isolates of *E. coli*.

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