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HIGH PREVALENCE OF NDM METALLO-β-LACTAMASE AMONG ESBL-PRODUCING *ESCHERICHIA COLI* İSOLATES

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Resistance to β-lactams in *Enterobacteriaceae* has been increasing worldwide. This study aimed to determine the frequency of β-lactamase genes and antibiotic resistance rates of 140 extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates obtained from urinary tract infection in Ordu Province, Turkey. Isolates were identified by classic methods and by automated system. ESBL production was confirmed by double disk synergy test and antimicrobial susceptibility was investigated by disk diffusion method. All isolates were screened for β-lactamase coding genes from three groups (A, B, and D) by polymerase chain reaction. The highest rate of susceptible isolates was observed for imipenem (IPM, 99.3%) and ertapenem (ETP, 97.9%), and the highest rate of resistant isolates was observed for cefuroxime (97.9%), ceftriaxone (97.2%), and cefazolin (90.7%). In our study, $bla_{\text{CTX-M1-like}}$ group was the most prevalent β-lactamase ($n = 109$), followed by bla_{TEM} ($n = 68$), $bla_{\text{CTX-M2}}$ ($n = 22$), and bla_{SHV} ($n = 2$). By contrast to low resistance rate to IPM and ETP, we determined bla_{NDM} in 31 isolates (22.1%). In co-prevalence of $bla_{\text{NDM-1}}$ and ESBL-coding genes, a low carbapenem resistance was determined. We can confirm that $bla_{\text{CTX-M1-types}}$ are still the most frequent β-lactamase coding gene in Turkey. Our study showed the highest prevalence of $bla_{\text{NDM-1}}$ metallo-β-lactamase coding gene in ESBL-producing *E. coli*.

Keywords: ESBL-producing *E. coli*, β-lactamase resistance genes, urinary tract infections

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Introduction

Escherichia coli is known as one of the most predominant organisms causing urinary tract infections (UTIs), which are very common reason for consultation and antibiotic prescription in practice [1]. The number of β-lactamase and especially extended-spectrum β-lactamase (ESBL)-producing strains is increasing around the world. The variants and types of ESBL enzymes have occurred due to many reasons, one of them is selective pressure caused by the common antibiotic use since the 1980s and they are becoming a significant problem for the whole world [2]. ESBL-positive strains are increasingly isolated from the community-acquired urinary system infections in addition to nosocomial infections [3]. ESBL-producing members of *Enterobacteriaceae* are resistant to penicillins, cephalosporins, and aztreonam (ATM). They frequently develop resistance to aminoglycosides, trimethoprim–sulfamethoxazole (SXT), and quinolones [4]. ESBL-producing microrganisms, such as *Klebsiella pneumoniae*, *E. coli*, *Morganella morganii*, *Serratia marcescens*, *Shigella dysenteriae*, *Enterobacter* spp., *Salmonella, Proteus, Citrobacter,* and *Pseudomonas aeruginosa* have been reported from many countries [5].

Currently, more than 450 ESBL variant β-lactamase enzymes with different structures have been reported and the most important enzymes of them are TEM, SHV, CTX-M, PER, GES, VEB, TLA, BES, and OXA β-lactamases. The ESBLs are grouped into four classes as A, B, C, and D enzymes. Class B enzymes are Zn-metallo enzymes, whereas enzymes of classes A, C, and D are active site serine enzymes. TEM, SHV, and CTX-M enzymes are in the class A ESBLs. While the TEM and SHV enzymes are the major types, the CTX-M-type enzymes have emerged among these organisms and CTX-M producing strains have a worldwide dissemination. CTX-M-type β-lactamases have been classified into five groups 1, 2, 8, 9, and 25/26 according to their amino acid sequence similarities [6]. While group 1 includes CTX-M-1, -3, -10, -11, -12, -15, -22, -23, -27, -28, -29, -30, -32, -33, -34, -36, -37, and -42, and group 2 includes CTX-M-2, -4, -5, -6, -7, -20, -31, -35, and Toho-1 [7]. The increasing prevalence of TEM, SHV, and CTX-M-type of ESBLs poses an important threat to the clinical use of third-generation cephalosporins for the treatment of serious infections. Determination of the genes encoding for ESBLs by polymerase chain reaction (PCR) and sequencing can provide useful information about their molecular epidemiology and risk factors associated with these infectious diseases.

Africa and the Middle East have experienced a number of outbreaks of ESBL-producing infections. Several studies demonstrated that CTX-M-type ESBLs are more dominant in some countries including China, India, Japan,

Korea, Malaysia, and Taiwan revealing ESBL-producing strains ranges from 30% to 40% [8]. In a meta-analysis from Turkey, a lower than 20% ESBL rate was found [9]. In another study from Hungary, the predominant gene of CTX-M-15 in ESBL-producing isolates was reported [10]. Molecular characterization of ESBLs was examined in *E. coli* isolates from 10 centers between the years 2011 and 2012. The resistance against carbapenems in bacteria is mostly related to carbapenemases such as oxacillinases carbapenem-hydrolyzing class D β-lactamases encoded by bla_{OXA} genes, metallo-β-lactamases (MBLs) of IMP, VIM, and NDM types, and carbapenemases of Ambler class A as KPC and GES [11]. The aim of this study was to determine the prevalence of ESBL-producing *E. coli*, their drug resistance pattern to commonly used antibiotics in medical practice, and β-lactamase coding genes in these multidrug resistant isolates of urine samples taken from patients diagnosed with UTIs.

Materials and Methods

Bacterial strains and antimicrobial susceptibility test

One hundred and forty ESBL-positive *E. coli* isolates were isolated from urine cultures in the Medical Microbiology Laboratory of Ordu Training and Research Hospital between August 2014 and April 2015 and they were described according to Clinical and Laboratory Standards Institute (CLSI) criteria [12]. Identification of isolates taken from adult and pediatric patients attending a variety of clinics was completed by using classic methods and by Vitek-2Compact (bioMérieux, France) automated system. Isolates were stored in glcyerol Luria–Bertani Broth medium at −80 °C until the day of study. Antimicrobial susceptibility tests were performed by disk diffusion method (Oxoid, UK) using ampicillin (AM), sulbactam/ampicillin, amoxicillin–clavulanic acid (AMC), ceftazidime (CAZ), ceftriaxone (CTX), cefuroxime (CXM), cefazolin (CZ), cefepime (FEP), gentamicin (GN), tobramycin, levofloxacin (LEV), ciprofloxacin (CIP), nitrofurantoin (F), tetracycline (TE), ertapenem (ETP), imipenem (IPM), and SXT antibiotic disks and the results were evaluated according to the CLSI guidelines [12].

The detection of ESBLs

Phenotypic ESBL production was confirmed by double disk synergy test. MHA media were seeded with a swab with *E. coli* strains on 0.5 McFarland prepared suspension and left at room temperature for 15 min. An AMC (20/10 μg) disk was placed in the center with CAZ (30 μg/disk), CTX (CRO; 30 μg/disk),

cefotaxime (CTX; 30 μg/disk), and ATM (30 μg/disk) disks placed around the edges 25–30 mm from the center. Results were assessed after 18–24 h incubation at 35 °C. This phenotypic ESBL screening test is based on the demonstration of a synergy image between AMC and cefotaxime, CAZ, and ATM, according to the guidelines of the CLSI. *E. coli* ATCC 25922 was used for control strain.

Multiplex PCR for detection of bla_{OXA} and $bla_{CTX\text{-}M}$ genes

Genomic DNAs were purified from overnight bacterial culture grown in 3 mL of Luria–Bertani broth using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. Multiplex PCR was used to detect $bla_{\text{OXA-23-24-51 and 58-like}}$ and $bla_{\text{CTX-M-1/M-2}}$ genes using primers listed in Table I. PCRs were performed in a final volume of 50 μL and included 5 μL of genomic DNA, 20 pM of each primer, 10 μL of 10X polymerase activity buffer, 3 μL of 25 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphates (dNTPs), and 1.5 U of *Taq* Polymerase (Fermentas Thermo Fisher Scientific Inc., Waltham, USA). PCR amplification was performed using initial denaturation at 94 °C for 3 min followed by 30 cycles of 25 s at 94 °C, 40 s at 52 °C, and 50 s at 72 °C for bla_{OXA} genes and initial denaturation at 95 °C for 2 min followed by 30 cycles of 1 min at 95 °C, 1 min at 55 °C, and 1 min at 72 °C for $bla_{\text{CTX-M-1/M-2}}$ genes with a final extension of 5 min at 72 °C. All PCR results were analyzed on 1% agarose containing 0.5 mg/L ethidium bromide, subsequently visualized under UV light and evaluated according to their molecular size.

PCR amplifications of ESBL and MBL resistance genes

The primers and PCR amplification conditions used to detect ESBLs and MBLs genes are shown in Table I. The reactions were performed in 50 μL final reaction volume using 5 μL of genomic DNA, 20 pM of each primer, 5 μL of reaction buffer, 3 μL of 25 mM $MgCl_2$, 200 μL of dNTPs, and 1 U Go *Taq* Polymerase (Fermentas Thermo Fisher Scientific Inc., Waltham, USA). The results were run as above and evaluated according to their molecular size and PCR results of control groups, which were defined as β-lactamase gene carrier bacteria in the earlier studies [18–20]. Positive PCR products except $bla_{\text{OXA-23-24-51-58}}$, $bla_{\text{CTX-M1/M2}}$, bla_{TEM}, and bla_{SHV} were sent to Macrogen Inc., Seoul, Korea, to sequence by using the same primers used in PCR reactions. Sequencing results were analyzed using the alignment search tool BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and the multiple sequence alignment program CLUSTALW (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

Table I. Primers used in the amplification of selected genes

Primers	5′→3′	Amplicon size (bp)	T_m(°C)	References
bla_{TEM}	F, AGTATTCAACATTTYCGTGT R, TAATCAGTGAGGCACCTATCTC	847	56	[13]
bla_{SHV}	F, ATGCGTTATATTCGCCTGTG R, TTAGCGTTGCCAGTGCTC	843	55	[5]
$bla_{CTX\text{-}M1}$	F, GCGTGATACCACTTCACCTC R, TGAAGTAAGTGACCAGAATC	260	–	[14]
$bla_{CTX\text{-}M2}$	F, TGATACCACCACGCCGCTC R, TATTGCATCAGAAACCGTGGG	341	–	–
bla_{GES}	F, ATGCGCTTCATTCACGCAC R, CTATTTGTCCGTGCTCAGGA	863	56	[15]
bla_{VEB}	F, ATTTCCCGATGCAAAGCGT R, TTATTCCGGAAGTCCCTGT	542	55	In this study
$bla_{PER\text{-}2}$	F, ATGAATGTCATCACAAAATG R, TCAATCCGGACTCACT	927	50	–
bla_{KPC}	F, ATGTCACTGTATCGCCGTCT R, TTTTCAGAGCCTTACTGCCC	893	55	[16]
bla_{IMP}	F, CATGGTTTGGTGGTTCTTGT R, ATAATTTGGCGGACTTTGGC	488	56	[17]
bla_{VIM}	F, ATTGGTCTATTTGACCGCGTC R, TGCTACTCAACGACTGAGCG	780	58	–
bla_{NDM}	F, GAGATTGCCGAGCGACTTG R, CGAATGTCTGGCAGCACACTT	497	57	[18]
$bla_{OXA\text{-}51}$	F, TAATGCTTTGATCGGCCTTG R, TGGATTGCACTTCATCTTGG	353	52	[17]
$bla_{OXA\text{-}23}$	F, GATCGGATTGGAGAACCAGA R, ATTTCTGACCGCATTTCCAT	501	–	–
$bla_{OXA\text{-}40}$	F, GGTTAGTTGGCCCCCTTAAA R, AGTTGAGCGAAAAGGGGATT	246	–	–
$bla_{OXA\text{-}58}$	F, AAGTAT TGGGGCTTGTGCTG R, CCCCTCTGCGCTCTACATAC	599	–	–

Results

Isolates were obtained from 140 urine samples from 64 pediatric and 76 adult patients. The study group was comprised of 94 females and 46 males with the mean pediatric age of 11.4 ± 3.8 years and the mean adult age of 38.6 ± 14.8 years. Of these isolates, 18 were from hospitalized patients with 122 from clinic outpatients, 7 (5%) intensive care, 43 (30.7%) pediatric, 23 (16.4%) urology, 18 (12.9%) emergency service, 13 (9.3%) infectious diseases, and 36 (25.7%) from other departments. 140 ESBL-positive urine isolates were susceptible to the following antibiotics: IPM with 99.3% and ETP with 99.7%, while the most resistant isolates were against CXM (97.9%), CTX (97.2%), and CZ (90.7%), respectively. All strains were found to be AM resistant. The susceptibility rates of *E.coli* strains showing multiple drug resistance for all antibiotics tested are shown in Table II.

According to PCR results, $bla_{\text{CTX-M1}}$ was the most prevalent β-lactamase 77.8% (109/140), followed by bla_{TEM} 48.6% (68/140), $bla_{\text{CTX-M2}}$ 15.7% (22/140), and bla_{SHV} 1.42% (2/140). More specifically, we determined bla_{NDM} in 31 isolates (22.1%) and they were all confirmed as $bla_{\text{NDM-1}}$ variant according to the sequencing result. No other β-lactamases were identified among the samples.

Table II. Antibiotic resistance rates of ESBL-producing *E. coli*

Antibiotics	Susceptible, *n* (%)	Intermediate, *n* (%)	Resistance, *n* (%)
Ampicillin	–	–	140 (100%)
Sulbactam–ampicillin	23 (16.4%)	41 (29.3%)	76 (54.3%)
Amoxicillin–clavulanic acid	16 (11.4%)	28 (20.0%)	96 (68.6%)
Ceftazidime	3 (2.1%)	54 (38.6%)	83 (59.3%)
Ceftriaxone	3 (2.1%)	1 (0.7%)	136 (97.2%)
Cefuroxime	1 (0.7%)	2 (1.4%)	137 (97.9%)
Cefazolin	13 (9.3%)	–	127 (90.7%)
Cefepime	5 (3.6%)	112 (80.0%)	23 (16.4%)
Gentamicin	107 (76.4%)	–	33 (23.6%)
Tobramycin	72 (51.4%)	3 (2.1%)	65 (46.5%)
Levofloxacin	63 (45.0%)	–	77 (55.0%)
Ciprofloxacin	59 (42.1%)	–	81 (57.9%)
Nitrofurantoin	109 (77.9%)	24 (17.1%)	7 (5.0%)
Tetracycline	45 (32.1%)	–	95 (67.9%)
Ertapenem	137 (97.9%)	–	3 (2.1%)
Imipenem	139 (99.3%)	1 (0.7%)	–
Trimethoprim–sulfamethoxazole	49 (35%)	–	91 (65%)

Discussion

Characteristics of antimicrobial resistance show differences among regions, hospitals, and even departments. Although wild-type *E. coli* strains are susceptible to commonly used antimicrobial agents in the treatment of UTIs, antibiotic resistance in uropathogenic *E. coli* infections is increasing worldwide. Some factors such as common use and misuse of antibiotics both in hospitals by health professionals and in the community by self-prescription, and inadequate antimicrobial surveillance programs are the reasons for increasing bacterial resistance rates [21]. The susceptibility of ESBL-producing *E. coli* isolates to broad spectrum cefalosporins shows variety. In an earlier study from Turkey, all strains were identified to be susceptible to IPM, meropenem, and amikacin and all isolates were observed to be resistant to cefotaxime and CTX. Susceptibility rates to cefoxitin, ETP, cefoperazone/sulbactam, piperacillin/tazobactam, GN, CIP, FEP, AMC, ATM, and CAZ were 96%, 83%, 63%, 61%, 50%, 41%, 25%, 21%, 20%, and 18%, respectively [22]. In recent years, carbapenem resistance has been encountered in ESBL-producing *E. coli* from Turkey and some studies found 100% susceptibility for meropenem and IPM and varying rates of ETP susceptibility (from 99.2% to 83%) for ESBL-producing *E. coli* strains [23, 24]. In our study, similar to other studies, the isolates were mostly susceptible to IPM at 99.3% and ETP at 99.7%, while they were mostly resistant against cefuroxime-axetil (99.3%), CXM (97.9%), CTX (97.2%), and CZ (90.7%).

TEM and SHV types of ESBL-producing organisms are primarily nosocomial and they are produced by many enteric bacteria. Especially CTX-M type has been increasingly reported worldwide. CTX-M-type ESBL-producing *E. coli* pathogens have become an important cause of community-onset blood stream infections and UTIs [25]. The epidemiological characteristics of infections caused by CTX-M-type ESBLs are different from the TEM or SHV type and they are often isolated from the patients with community-onset infections. A study in America in 2000 found a 25% rate of CTX-M type genes, this rate rose to 69% in 2004 and the most common one was CTX-M-15 followed by CTX-M-16, -8, and -14 [26]. A study in Thailand identified the gene prevalence for CTX-M as 99.3%, TEM as 77%, SHV as 3.8%, VEB-1 as 8.5%, and OXA-10 as 8.1% with PER and GES genes not identified in any isolate [27]. Other studies in the world showed the prevalences of bla_{TEM} and bla_{SHV} genes in *E. coli* were 46.4% and 11.2%, respectively [28], and CTX-M types have been reported as the most frequent ESBLs (65%) among *Enterobacteriaceae* isolates [29].The prevalences of bla_{TEM} and bla_{SHV} were 65.5% and 15%, respectively. In a study of patients with ESBL-producing *E. coli*, 85.4% of isolates contained a CTX-M ESBL gene [25]. Another

study demonstrated that CTX-M-14 was the most common ESBL among *E. coli* isolates, while SHV was the predominant among *K. pneumoniae* isolates. It also showed the co-existence of two or more kinds of ESBL in a single isolate frequently [30].

In Turkey, β-lactamase coding gene rates for $bla_{\text{CTX-M}}$, bla_{TEM}, $bla_{\text{OXA-2}}$, bla_{PER}, bla_{SHV}, and $bla_{\text{OXA-10}}$ groups were found in *E. coli* isolates as 89.5%, 59.2%, 15.8%, 14.5%, 11.8%, and 3.9%, respectively. No bla_{GES} and bla_{VEB} β-lactamase genes were encountered in any isolate and 1.3% of the investigated genes were identified in all the isolates. While bla_{TEM} and $bla_{\text{CTX-M}}$ genes were detected together in 25 isolates, $bla_{\text{CTX-M}}$ and bla_{TEM} genes were detected alone in 20 and 2 isolates. bla_{SHV} genes were not identified alone in any isolate [22]. In a different study from 10 hospitals, $bla_{\text{CTX-M1}}$ was the most prevalent β-lactamase (83.18%), followed by bla_{TEM} (44.09%), $bla_{\text{CTX-M2}}$ (31.81%), and bla_{SHV} (1.81%) [13]. In this study, none of the isolates were found to have bla_{VEB}, bla_{GES}, bla_{PER}, bla_{KPC}, bla_{VIM}, and bla_{IMP} positivity. According to the PCR result, $bla_{\text{CTX-M1}}$ was the most prevalent β-lactamase (77.8%), followed by bla_{TEM} (48.6%), $bla_{\text{CTX-M2}}$ (15.7%), and bla_{SHV} (1.42%). According to these results, more specifically $bla_{\text{NDM-1}}$ type MBL coding gene was determined in 31 isolates (22.1%). To the best of our knowledge, this is the first record showing the highest prevalence of $bla_{\text{NDM-1}}$ in ESBL-producing *E. coli* from Turkey. NDM-type enzymes are carbapenemases and they hydrolyze carbapenems such as IPM, meropenem, ETP, and doripenem. According to the high prevalence of $bla_{\text{NDM-1}}$ variants in isolates, high rate of sensitivity to IPM and ETP for isolates was determined. According to CLSI, Minimal İnhibitory Concentration (MIC) values ≤1 and 0.5 μg/mL are accepted as sensitive for IPM and ETP, respectively and Nordmann reported that isolates from *Enterobacteriaceae* carrying $bla_{\text{NDM-1}}$ have MIC values 0.5→32 for IPM and 0.38→32 for ETP. This means that there are some other factors affecting the resistance against antimicrobials besides carbapenemases [31]. The use of some kind of antibiotics such as fluoroquinolones might facilitate the widespread dissemination of ESBL-producing bacteria in the healthcare setting [32].

The important dissemination of ESBL and carbapenemases-producing *E. coli* has lead to a decrease in therapeutic options. The findings indicate that CTX-M1 type β-lactamases are the most frequent among ESBL-producing *E. coli* isolates in our hospital. Although TEM rapidly grow in Turkey, CTX-M1 is still the most frequent CTX-M type β-lactamases. Updates of trends for regional epidemiological data on the prevalence of the β-lactamase genes and antimicrobial resistance are crucial in order to promote appropriate antimicrobial therapy as well as an effective infection control and clinical care management.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Schito, G. C., Naber, K. G., Botto, H., Palou, J., Mazzei, T., Gualco, L., Marchese, A.: The ARESC study: An international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. Int J Antimicrob Agents **34**, 407–413 (2009).
2. Lautenbach, E., Patel, J. B., Bilker, W. B., Edelstein, P. H., Fishman, N. O.: Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: Risk factors for infection and impact of resistance on outcomes. Clin Infect Dis **32**, 1162–1171 (2001).
3. Cantón, R., Novais, A., Valverde, A., Machado, E., Peixe, L., Baquero, F., Coque, T. M.: Prevalence and spread of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in Europe. Clin Microbiol Infect **14**, 144–153 (2008).
4. Rodríguez-Baño, J., Navarro, M. D., Romero, L., Martínez-Martínez, L., Muniain, M. A., Perea, E. J., Pérez-Cano, R., Pascual, A.: Epidemiology and clinical features of infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* in nonhospitalized patients. J Clin Microbiol **42**, 1089–1094 (2004).
5. Hanson, N. D., Moland, E. S., Hossain, A., Neville, S. A., Gosbell, I. B., Thomson, K. S.: Unusual *Salmonella enterica* serotype Typhimurium isolate producing CMY-7, SHV-9 and OXA-30 β-lactamases. J Antimicrob Chemother **49**, 1011–1014 (2002).
6. Bonnet, R.: Growing group of extended-spectrum β-lactamases: The CTX-M enzymes. Antimicrob Agents Chemother **48**, 1–14 (2004).
7. Eckert, C., Gautier, V., Saladin-Allard, M., Hidri, N., Verdet, C., Ould-Hocine, Z., Barnaud, G., Delisle, F., Rossier, A., Lambert, T., Philippon, A., Arlet, G.: Dissemination of CTX-M-Type β-Lactamases among clinical isolates of *Enterobacteriaceae* in Paris, France. Antimicrob Agents Chemother **48**, 1249–1255 (2004).
8. Antony, S. J.: The changing epidemiology of extended spectrum Beta-lactamases (ESBL) infections of the urinary tract focusing on clinical resistance and therapeutic options. In Nikibakhsh, A. A. (ed): Clinical Management of Complicated Urinary Tract Infection. InTech, Rijeka, Croatia, 2011, pp. 19–22.
9. Aykan, Ş. B., Çiftçi, I. H.: Antibiotic resistance patterns of *Escherichia coli* strains isolated from urine cultures in Turkey: A meta-analysis. Mikrobiyol Bul **47**, 603–618 (2013).

10. Melegh, S., Schneider, G., Horváth, M., Jakab, F., Em dy, L., Tigyi, Z.: Identification and characterization of CTX-M-15 producing *Klebsiella pneumoniae* clone ST101 in a Hungarian university teaching hospital. Acta Microbiol Immunol Hung **62**, 233–245 (2015).
11. Poirel, L., Naas, T., Nordmann, P.: Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrob Agents Chemother **54**, 24–38 (2010).
12. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing. 24th Informational Supplement (M100-S24). CLSI, Wayne, PA (2014).
13. Cicek, C. A., Saral, A., Ozad, A. O., Yasar, E., Cizmeci, Z., Balci, P. O., Sari, F., Firat, M., Altintop, Y. A., Ak, S., Caliskan, A., Yildiz, N., Sancaktar, M., Budak, E. E., Erturk, A., Ozgumus, O. B., Sandalli, C.: Nationwide study of *Escherichia coli* producing extended-spectrum β-lactamases TEM, SHV, and CTX-M in Turkey. J Antibiot **66**, 647–650 (2013).
14. Xu, L., Ensor, V., Gossain, S., Nye, K., Hawkey, P.: Rapid and simple detection of $bla_{\text{CTX-M}}$ genes by multiplex PCR assay. J Med Microbiol **54**, 1183–1187 (2005).
15. Moubareck, C., Brémont, S., Conroy, M. C., Courvalin, P., Lambert, T.: GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. Antimicrob Agents Chemother **58**, 3579–3581 (2009).
16. Schechner, V., Straus-Robinson, K., Schwartz, D., Pfeffer, I., Tarabeia, J., Moskovich, R., Chmelnitsky, I., Schwaber, M. J., Carmeli, Y., Navon-Venezia, S.: Evaluation of PCR-based testing for surveillance of KPC-producing carbapenem-resistant members of the *Enterobacteriaceae* family. J Clin Microbiol **47**, 3261–3265 (2009).
17. Jeon, B. C., Jeong, S. H., Bae, I. K., Kwon, S. B., Lee, K., Young, D., Lee, J. H., Song, J. S., Lee, S. H.: Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing the OXA-23 β-lactamase in Korea. J Clin Microbiol **43**, 2241–2245 (2005).
18. Iraz, M., Düzgün, A. Ö., Sandallı, C., Doymaz, M. Z., Akkoyunlu, Y., Saral, A., Peleg, A. Y., Özgümüş, O. B., Beriş, F. Ş., Karaoğlu, H., Çiçek, A. Ç.: Distribution of β-lactamase genes among carbapenem-resistant *Klebsiella pneumoniae* strains isolated from patients in Turkey. Ann Lab Med **35**, 595–601 (2015).
19. Woodford, N., Ellington, M. J., Coelho, J. M., Turton, J. F., Ward, M. E., Brown, S., Amyes, S. G., Livermore, D. M.: Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents **27**, 351–353 (2006).
20. Cicek, A. Ç., Saral, A., Iraz, M., Ceylan, A., Duzgun, A. O., Peleg, A. Y., Sandallı, C.: OXA- and GESF-type β-lactamases predominate in extensively drug-resistant *Acinetobacter baumannii* isolates from a Turkish University Hospital. Clin Microbiol Infect **20**, 410–415 (2014).
21. El bouamri, M. C., Arsalane, L., Zerouali, K., Katfy, K., El kamouni, Y., Zouhair, S.: Molecular characterization of extended spectrum β-lactamase-producing *Escherichia coli* in a university hospital in Morocco, North Africa. Afr J Urol **21**, 161–166 (2015).
22. Görgeç, S., Kuzucu, Ç., Otlu, B., Yetkin, F., Ersoy, Y.: Investigation of beta-lactamase genes and clonal relationship among the extended-spectrum beta-lactamase producing nosocomial *Escherichia coli* isolates. Mikrobiyol Bul **49**, 15–25 (2015).
23. Kiremitci, A., Dinleyici, E. C., Erben, N., Durmaz, G., Yargic, Z. A., Aybey, A. D., Usluer, G.: In vitro activity of ertapenem and other carbapenems against extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in a tertiary care center in Turkey. Expert Opin Pharmacother **9**, 1441–1449 (2008).

24. Kuzucu, Ç., Yetkin, F., Görgeç, S., Ersoy, Y.: Investigation of the susceptibilities of extended-spectrum β-lactamase producing *Escherichia coli* and *Klebsiella* spp. strains to ertapenem and other carbapenems. Mikrobiyol Bul **45**, 28–35 (2011).
25. Hayakawa, K., Gattu, S., Marchaim, D., Bhargava, A., Palla, M., Alshabani, K., Gudur, U. M., Pulluru, H., Bathina, P., Sundaragiri, P. R., Sarkar, M., Kakarlapudi, H., Ramasamy, B., Nanjireddy, P., Mohin, S., Dasagi, M., Datla, S., Kuchipudi, V., Reddy, S., Shahani, S., Upputuri, V., Marrey, S., Gannamani, V., Madhanagopal, N., Annangi, S., Sudha, B., Muppavarapu, K. S., Moshos, J. A., Lephart, P. R., Pogue, J. M., Bush, K., Kaye, K. S.: Epidemiology and risk factors for isolation of *Escherichia coli* producing CTX-M-type extended-spectrum β-lactamase in a large U.S. Medical Center. Antimicrob Agents Chemother **57**, 4010–4018 (2013).
26. Lewis, J. S., Herrera, M., Wickes, B., Patterson, J. E., Jorgensen, J. H.: First report of the emergence of CTX-M-type extended-spectrum β-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother **51**, 4015–4021 (2007).
27. Kiratisin, P., Apisarnthanarak, A., Laesripa, C., Saifon, P.: Molecular characterization and epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing healthcare-associated infection in Thailand, where the CTX-M family is endemic. Antimicrob Agents Chemother **52**, 2818–2824 (2008).
28. Shahcheraghi, F., Nikbin, V. S., Feizabadi, M. M.: Prevalence of ESBLs genes among multidrug-resistant isolates of *Pseudomonas aeruginosa* isolated from patients in Tehran. Microb Drug Resist **15**, 37–39 (2009).
29. Ben-Ami, R., Rodríguez-Baño, J., Arslan, H., Pitout, J. D., Quentin, C., Calbo, E. S., Azap, O. K., Arpin, C., Pascual, A., Livermore, D. M., Garau, J., Carmeli, Y.: A multinational survey of risk factors for infection with extended-spectrum β-lactamase-producing *Enterobacteriaceae* in nonhospitalized patients. Clin Infect Dis **49**, 682–690 (2009).
30. Lin, C. F., Hsu, S. K., Chen, C. H., Huang, J. R., Lo, H. H.: Genotypic detection and molecular epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a regional hospital in central Taiwan. J Med Microbiol **59**, 665–671 (2010).
31. Nordmann, P., Gniadkowski, M., Giske, C. G., Poirel, L., Woodford, N., Miriagou, V., The European Network on Carbapenemases: Identification and screening of carbapenemase-producing *Enterobacteriaceae*. Clin Microbiol Infect **18**, 432–438 (2012).
32. Füzi, M.: Has the use of fluoroquinolone facilitated the widespread dissemination of methicllin-resistant *Staphylococcus aureus* and extended spectrum β-lactamase-producing *Klebsiella pneumoniae* in the healthcare setting? Acta Microbiol Immunol Hung **61**, 399–405 (2014).