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Investigation of New Delhi metallo-beta-lactamase-1 (*bla*$_{NDM-1}$) gene in carbapenem-resistant *Enterobacterales* strains isolated from a university hospital in Turkey

Merih Simsek[1], Cengiz Demir[1], Esra Seker[2]

[1]*Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Microbiology, Afyonkarahisar, Turkey*
[2]*Afyon Kocatepe University, Faculty of Veterinary, Department of Microbiology, Afyonkarahisar, Turkey*

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Abstract

In this study, the presence of bla$_{NDM-1}$ gene in carbapenem-resistant *Enterobacterales* obtained from various clinical specimen of a University Hospital in Turkey between the years 2014-2019, has been investigated. Carbapenem-resistant *Enterobacterales* were identified using Vitek 2. The presence of Metallo beta lactamase in these bacteria was investigated by phenotypically modified hodge test and genotypically by PCR. Of the 110 carbapenem-resistant *Enterobacterales* isolates, six (5.5%), 17 (15.5%) and 87 (79.0%) were identified as E. cloacae, *E. coli* and *K. pneumoniae* , respectively. While blaNDM-1gene was found in 35 (31.8%) *K. pneumoniae* and three (2.7%) *E. coli* strains, all 38 strains were found positive for blaNDM-1gene by the modified hodge test. It was determined that six E. cloacae strains did not contain the bla$_{NDM-1}$ gene. Multiple antibiotic resistances were detected in carbapenem-resistant *Enterobacterales* strains positive for blaNDM-1. However, 29 *K. pneumoniae* and two *E. coli* strains were identified as resistant to tigecycline. Turkey also risks of the world along with other resistance enzymes and bacteria $_{NDM-1}$ producing the rates must be determined globally and periodically. The fast spread of such resistant through transferable ways should not be overlooked, because enzymatic resistance genes can lead to high mortality hospital outbreaks worldwide.

Keywords: Carbapenemase, bla$_{NDM-1}$ gene, *Enterobacterales*, multidrug resistance, New Delhi metallo beta lactamase-1

Introduction

Beta lactam antibiotics are frequently used in the treatment of infections caused by Gram-negative bacteria. However, in studies conducted today, it has been determined that resistance patterns of *Enterobacterales* species are gradually increasing. Thus, the use of beta lactam antibiotics has gained great importance in the treatment of nosocomial infections. The emergence of Expanded Spectrum Beta Lactamases (ESBLs) and Multi Drug Resistances (MDRs) is the main cause of this problem [1,2].

As a result, it is the first antibiotic group to use carbapenem in the treatment of nosocomial infections caused by Gram-negative bacteria. Because, the effects of these antibiotics on AmpC beta-lactamase and ESBL enzymes, their efficacy on bacteria and broad-spectrum effects are quite high [3-5]. Recently, carbapenem resistant *Enterobacterales* (CRE) strains were listed as one of the most important antibiotic resistance threats in the Disease Control and Prevention Reports [6]. Carbapenemases are beta lactamases that can hydrolyze carbapenems. In addition, the carbapenemase production mechanism of CREs is the most effective mechanism that causes carbapenem resistance [4,7,8].

New Delhi metallo-β-lactamase-1 (NDM-1), encoded by BlaNDM-1gene, was first obtained from a pathogenic and highly infectious multi-drug resistant *Klebsiella pneumoniae* strain [9]. Bacteria that produce NDM-1 can be transmitted from water sources and from person to person, polluted environment and animal foods [10]. NDM-1-producing bacteria have been recognized to be resistant to almost all β-lactam antibiotics (except monobactams), aminoglycosides, quinolones, nitrofurantoin and sulphonamides [8,10-12]. The fact that genes encoding NDM-1 production can often be transferred via plasmids facilitates the worldwide spread of broad-spectrum antibiotic resistance. Thus, the risk of treatment failure and the cost of antimicrobial chemotherapy and hospitalization increase, while the range of therapeutic options decrease [2,7,8].

Recent reports have shown that the plasmid-mediated blaNDM-1gene encoding the NDM-1 is spreading worldwide, mainly in members of the *Enterobacterales*, and this carbapenem resistance

***Corresponding Author:** Merih Simsek, Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Microbiology, Afyonkarahisar, Turkey, E-mail: smerih16@gmail.com

problem will increase in *Enterobacterales* isolates [10,13-15]. In Turkey, the first data related to NDM-1 positive K. pneunoniae have been published in 2012 and the isolation of these isolates from different hospitals has been increasingly reported as well as in other countries (16). However, the investigations in Turkey have generally focused on either K. pneunoniae strains or isolates obtained from limited clinic service [10,17,18]. Therefore, in this study, of University Hospital in Turkey between the years 2014-2019 obtained from various clinical isolates bla_{NDM-1} gene in the presence of the CRE it has been investigated.

Materials And Methods

The identification of bacterial strains

In this study, 1213 *Enterobacterales* isolates (one per patient) obtained from various clinics of a University Hospital between January 2014 and March 2019 were used. The sputum, cerebrospinal fluid, urine, blood, catheter, tracheal aspirate and wound samples obtained to be a single sample from each patient were used for bacterial isolation. These clinics samples were cultured on chocolate agar, Eosin Methylene Blue agar and blood agar. The cultures incubated for 16-48 hours at 37°C. After, each colony was evaluated macroscopically and microscopically. The certain identification of isolates was achieved by using Vitek 2 system (Bio-Mérieux, Hazelwood Inc., USA, MO) system. The strains were kept in tryptic soy broth containing 15% glycerol and at -80°C, until further tests.

Antibiotic susceptibility test

Antibiotic susceptibility tests were performed by using Vitek 2 system (Bio-Mérieux, Hazelwood Inc., USA, MO) system. In this study, the tested antibiotics were imipenem, meropenem, ertapenem, amikacin, gentamicin, cefuroxime, ceftazidime, ceftriaxone, ciprofloxacin, amoxicillin-clavulanic acid, piperacillin-tazobactam, trimethoprim-sulfamethoxazole and tigecycline. Isolates were treated as CRE (imipenem, meropenem, ertapenem) if found to be fully resistant or moderately resistant to one or more of the carbapenems. The data obtained were evaluated according to the European Antimicrobial Susceptibility Test (EUCAST) requirements [19]. In Vitek 2 system, The external (Oneworld Ocuracy Company, Turkey) and internal (Enterococcus faecalis / ATCC 29212, *Staphylococcus sureus* / ATCC 29213 and *Escherichia coli* / ATCC 25922) quality control studies were performed.

Investigation of metallo beta lactamase (MBL) by Modified Hodge test (MHT)

All isolates resistant against one or more carbapenem were analyzed for phenotypic investigation of MBL by using MHT. Thus, the suspension was prepared the 1:10 dilution of 0.5 McFarland's standard with imipenem-susceptible *E. coli* ATCC 25922 and spread onto Mueller Hinton agar plates. The test strains were coated from the center of the plate to the periphery. The plate kept for 15 minutes at room temperature. Imipenem (10 µg) disc was placed in the center of plates were, and plates were incubated for 18-20 hours and at 37°C. After, the cloverleaf-like growth of the indicator strain was agreed to be positive result for MBL screening.

Molecular detection of blaNDM-1resistance gene

DNA extraction and amplification conditions

In this study, optimization studies were performed for NDM-1 gene region. For this purpose, optimal binding temperature for this gene region was optimized and this temperature was determined to be 56°C. After the appropriate binding temperature was determined, DNA was obtained from CRE test strains and control strains by boiling method. Firstly, bacteria colonies were cultured onto blood agar for 24 hours. Suspensions of fresh colonies were then prepared in sterile eppendorf tubes to which 500 µl sterile Tris-EDTA buffer was added. The suspensions were incubated by heating at 95°C for 10 minute using incublock microtube incubators (Denville Scientific, USA) and centrifuged at 4°C, 15000 rpm for 10 min. The supernatant containing bacterial DNA (50 µl) was stored at -20°C for subsequent PCR mixture.

In carbapenem-resistant strains, the amplification of a 726 bp blaNDM-1gene was performed by specific primer set NDM-R (5'-ATC ATG CTG GCC TTG GGG AA-3') and NDM-F (5'-CAA TAT TAT GCA CCC GGT CG-3') [20]. PCR was carried out in final volume 25 µl reaction mixtures containing 12.5 µl SYBR Green qPCR Master Mix (Real Amp SYBR qPCR Master Mix-2X Low ROX), 1 µl Genomic DNA, 1µl (100ng / µL) F primer, 1µl (100ng / µL) R primer, 9.5 µl sterile distilled water. PCR amplification was performed by Real Time PCR (Rotor-Gene Q, QIAGEN, Hilden, Germany). An initial denaturation for 5 min at 94°C, 35 cycles of denaturation for 45 sec at 94°C, annealing for 30 sec at 56°C, extension for 20 sec at 72°C and a final extension for 5 min at 72°C. The presence of all PCR products was examined by agarose gel electrophoresis. *K. pneumoniae* ATCC 700603 and sterile distilled water were used to be positive and negative controls, respectively.

Ethical approval by Afyonkocatepe University local ethical authority was obtained (2019/204).

Results

Bacterial identification findings

A total of 110 (9.1%) isolates were determined to be CRE among 1213 *Enterobacterales* isolates. These isolates are resistant to at least one carbapenem (imipenem, meropenem, ertapenem). Of 110 CRE isolates, 17 (15.5%), six (5.5%) and 87 (79.0%), were determined to be *E. coli*, E. cloacae and *K. pneumoniae* , respectively. In this study, of these strains, 53 (48.2%) were isolated from female patients and 57 (51.8%) from male patients. When the distribution of isolates according to clinics was examined, 57.3% and 42.7% of the isolates belonged to the surgical medicine clinics and the internal medicine clinics, respectively. It was also determined that CRE strains were mostly isolated from blood (36.4%), tracheal aspirate (20.0%), urine (17.3%) and wound (11.8%) samples. Distribution of all CRE strains according to clinical samples and clinics were shown in Table 1.

Antibiotic susceptibility test

According to antibiotic susceptibility test results, the resistance to cefuroxime was found in all of the strains. In addition, high resistance rates against ceftriaxone (97.3%), piperacillin-tazobactam (97.3%), amoxicillin-clavulanic acid (90.9%),

imipenem (85.4%), meropenem (83.6%), ciprofloxacin (76.4%) and ertapenem (70.9%) were detected for tested CRE strains. In the present study, the presence of ESBL was also determined in 88.3% of *K. pneumoniae* and 61.4% of *E. coli*. The antibiotic resistance patterns of all strains for 14 antibiotics were presented in Table 2.

Table 1. Distribution of all CRE strains according to clinical samples and clinics

Clinical samples	**%**
Sputum	7.3
Cerebrospinal fluid	2.7
Urine	17.3
Blood	36.4
Catheter	4.5
Tracheal aspirate	20.0
Wound	11.8
Blood	36.4
Catheter	4.5
Tracheal aspirate	20.0
Wound	11.8
Clinics	**%**
Anesthesiology and Reanimation	19.0
Anesthesia Intensive Care	6.4
Neurosurgery	8.2
Pediatric Health and Diseases	3.6
Internal Intensive Care	5.5
Internal medicine	5.5
Physical therapy and rehabilitation	1.8
General Surgical	10.0
General Surgical Intensive Care	3.6
Chest Diseases Intensive Care	8.2
Gynecology and Obstetrics	2.7
Cardiovascular Surgical	1.8
Nephrology	1,8
Neurology	1.8
Plastic Reconstructive and Aesthetic Surgical	5.5
Neonatal Intensive Care	14.6

Table 2. Antibiotic resistance rates of CRE strains (n = 110)

Antibiotics	***K. pneumoniae* (n=87)**	***E. coli* (n=17)**	***E. cloacae* (n=6)**	**Total (n=110)**
Imipenem	80 (91.9%)	9 (52.9%)	5 (83.3%)	94 (85.4%)
Meropenem	78 (89.6%)	9 (52. 9%)	5 (83.3%)	92 (83.6%)
Ertapenem	67 (77.0%)	6 (35.3%)	5 (83.3%)	78 (70.9%)
Amikacin	52 (59.7%)	12 (70.6%)	2 (33.3%)	66 (60.0%)
Gentamicin	65 (74.7%)	4 (23.5%)	2 (33.3%)	71 (64.5%)
Cefuroxime	87 (100%)	17 (100%)	6 (100%)	110 (100%)
Ceftazidime	85 (97.7%)	16 (94.1%)	6 (100%)	107 (97.3%)
Ceftriaxone	85 (97.7%)	16 (94.1%)	6 (100%)	107 (97.3%)
Amoxicillin-Clavulanic Acid	84 (96.5%)	12 (70.6%)	4 (66.6%)	100 (90.9%)
Piperacillin-Tazobactam	86 (98.8%)	15 (88.2%)	6 (100%)	107 (97.3%)
Ciprofloxacin	73 (83.9%)	7 (41.2%)	4 (66.6%)	84 (76.4%)
Trimethoprim-Sulfamethoxazole	48 (55.2%)	8 (47%)	2 (33.3%)	58 (52.7%)
Tigecycline	39 (44.8%)	5 (29.4%)	1 (16.6%)	45 (40.9%)

Phenotypic detection of MBL by MHT

In this study, MBL production positivity was found to be 71.8% (n=79) in tested 110 carbapenem-resistant isolates by using MHT. In terms of MHT positivity, *K. pneumoniae* (n = 61; 77.2%) had the highest rate, followed by *E. coli* (n = 14; 17.7%) and E. cloacae (n = 4; 5.0%). The phenotypic MBL production for *K. pneumoniae* was shown in Figure 1.

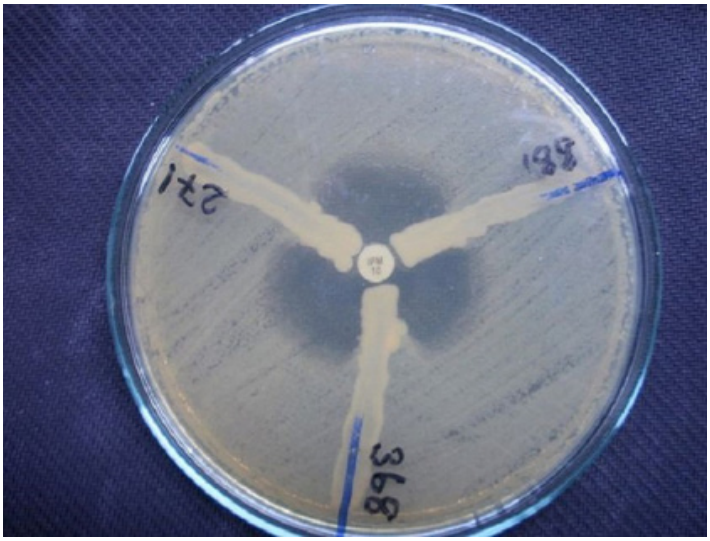

Figure 1. Phenotypic MBL production for *K. pneumoniae*

Detection of blaNDM-1resistance gene

The presence of blaNDM-1gene was detected in 34.5% (n=38) of analyzed 110 CRE strains. While 35 (31.8%) *K. pneumoniae* and three (2.7%) *E. coli* harboured the blaNDM-1gene, this gene was found in none of six E. cloacae strains. These 38 strains were also positive in MHT. Amplification of blaNDM-1gene (726 bp) in strains was shown in Figure 2.

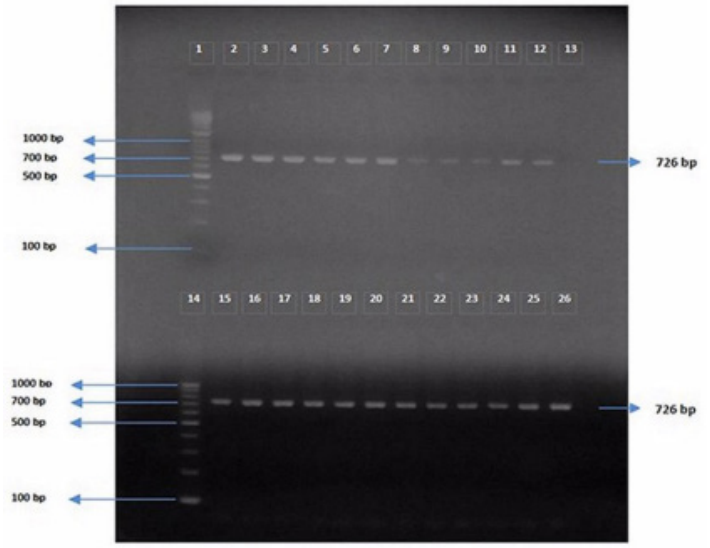

Figure 2. Agarose gel electrophoresis image of PCR amplified product of blaNDM-1gene for *K. pneumoniae* and *E. coli*. Lanes 1 and 14: 100bp DNA ladder (Genesta, GeneAll); Lane 2: positive control (*K. pneumoniae* ATCC 700603, 726 bp); Lanes 3-12 and 15-26: clinical samples; Lane 13: negative control (sterile distilled water)

The characteristics of NDM-1 producing CRE strains

In our study, of 35 NDM-1 producing CR *K. pneumoniae* strains, three, two, three, two, three, four, four, seven and seven were isolated from anesthesiology and reanimation clinic, internal medicine clinic, neurosurgery clinic, cardiovascular surgical clinic, general surgical clinic, chest diseases intensive care unit (ICU), neonatal ICU, anesthesia ICU, internal ICU, respectively. However, three CR *E. coli* strains harbouring the blaNDM-1gene were obtained from general surgical clinic.

While three NDM-1 producing CR *E. coli* was isolated from blood sample, blaNDM-1gene positive CR *K. pneumoniae* strains were obtained from tracheal aspirate (n=7), blood (n=6), urine (n=11), sputum (n=4), catheter (n=3) and wound (n=4) samples.

The multiple antibiotic resistances were another characteristic of blaNDM-1gene positive CRE strains. The antibiotic resistance of blaNDM-1gene positive strains was presented in Table 3.

Table 3. Antibiotic resistance of blaNDM-1gene positive CRE strains

blaNDM-1gene Positive Strains	AMC	TPZ	CXM	CAZ	CRO	ETP	MEM	IPM	AK	CN	CIP	TGC	SXT
K. pneumoniae (n=35)	35	35	35	35	35	35	35	35	28	31	31	29	32
E. coli (n=3)	3	3	3	3	3	3	3	3	2	3	3	2	3

AMC; amoxicillin-clavulanic acid, TZP; piperacillin-tazobactam, CXM; cefuroxime, CAZ; ceftazidime, CRO; ceftriaxone, ERT; ertapenem, MER; meropenem, IMP; imipenem, AK; amikacin, GN; gentamicin, CIP; ciprofloxacin, TIG; tigecycline, SXT; trimethoprim-sulfamethoxazole

Discussion

The present study investigated the presence of blaNDM-1gene in CRE isolates obtained from different clinic samples by PCR and antibiotic resistance of NDM-1 producing strains to various antibiotics commonly used in Turkey.

The recent increase in rates of CRE among health-associated *Enterobacterales* species has been recognized as a major health threat [2,7,8]. In the studies related to CRE prevalence, it was reported that prevalence of CRE was highly dependent on geography and the highest rates were seen in *K. pneumoniae* over other species of the family *Enterobacterales* [8,21,22]. While the prevalence of CRE was found to be between 1.4–4.2% in the United States, this rate was reported to be 1.2%, 5.74%, 5.0%, 0.74% and 2.82% from Lebanon, Malaysia, Taiwan, Saudi Arabia and Turkey, respectively [3,21-25]. Generally, in these studies from different countries, the most commonly isolated CRE species were reported to be E. cloacae, *E. coli* and *K. pneumoniae* [21,22].

In our study, 9.1% (n=110) of total 1213 *Enterobacterales* isolates were determined to be CRE. Of 110 CRE isolates six (5.5%), 17 (15.5%) and 87 (79.0%) were determined to be E. cloacae, *E. coli* and *K. pneumoniae* , respectively. While this CRE isolation rate obtained from our study was higher than the prevalence of CRE reported by other authors, the compatibility was found in terms of isolated strains. The difference between the sample size, sample type, geographical variations and diversity in strains may be the causes of this discrepancy.

It has been determined that some risk factors such as the patient's long stay in the hospital, cephalosporin use, admission to the intensive care unit, diabetes mellitus, presence of central venous catheter, being a transplant patient, and performing surgical procedures are associated with CRE infections [7,26].

In most studies, it has been reported that CRE isolates are generally isolated from pediatric units, internal medicine and surgery units, and intensive care units (ICU) [17,23,26]. The investigations in Turkey were frequently focused on isolates obtained from limited clinic service, particularly ICU [10,17,18]. Different from these studies, we sampled the patients from 16 different clinics and units consisting of surgical medicine clinics and the internal medicine clinics. Our study did not identify specific risk factors for the colonization of CRE, but the results exhibited that most of CRE isolates were from anesthesiology and reanimation clinic (19.0%), neonatal ICU (14.6%), general surgical clinic (10.0%), neurosurgery (8.2%) and chest diseases ICU (8.2%). When the distribution of isolates according to samples was examined, it was noticed that CRE strains were mostly isolated from blood (36.4%), tracheal aspirate (20.0%), urine (17.3%) and wound (11.8%) samples (Table 1).

NDM-1 production encoded by the blaNDM-1gene is currently accepted to be the major β-lactamases of epidemiological and clinical significance such as other groups of carbapenemases (KPC and OXA-48) [2,25,27,28]. In this way, the presence of blaNDM-1, along with other resistance mechanisms, has been agreed a big problem in the treatment of *Enterobacterales*-associated infections. Several studies have described the epidemiological importance of NDM in terms of global spread, dissemination both in the environment and in hospitals. Although it is known that the blaNDM-1gene positivity in CRE isolates was increasingly spread around the world after the first identification in India, the differences on the prevalence rates of this gene appeared according to geographic regions [13-15,25]. Similarly, suggested that some carbapenemases were reported to be more frequent in some regions and emphasized while KPC-type beta-lactamases were prevalent in Greece, Israel, and USA, NDM-1 positive isolates were dominant in far east, India, and Pakistan reported that only one (*Klebsiella oxytoca*) (1.7%) of 57 carbapenemase-producing *Enterobacterales* isolates recovered from four hospitals in Taiwan were NDM-1 producer [1,21]. Similarly, in California, it was reported a carbapenemase-encoding gene was detected in 94/115 of CRE isolates, but only one (0.9%) of these isolates harboured the blaNDM-1gene [23]. In a study conducted in China in 2015, 21 carbapenem-resistant *K. pneumoniae* strains were determined to be blaNDM-1gene positive [12]. In another study from China, reported that among 1735 carbapenem-non-susceptible strains, 54 strains of blaNDM-1positive bacteria were identified, which

consisted of 44 strains of *K. pneumoniae* , eight strains of A. baumannii and two strains of *E. coli* [14,15]. It was revealed that the 126 (86.3%) of 146 NDM-producing *Enterobacterales* isolates collected from 33 general hospitals in South Korea during the period between 2010 and 2015 were positive for blaNDM-1gene. In another study, the blaNDM-1gene positivity was found to be 50.0% and 66.6% in two *E. coli* and three *K. pneumoniae* isolates determined as carbapenemase producers from a children's hospital in Nepal [13]. The different carriage rates of blaNDM-1gene in CRE isolates were reported from Turkey like as from other countries. In Turkey, the first report related to NDM-1 positive K. pneunoniae strain isolated from leukemic patient transferred from a hospital in Iraq was published in 2012 [16]. In 2013, it was reported the NDM-1 positivity was found to be 4.3% (n=4) among 94 carbapenemase-resistant *K. pneumoniae* isolated from in a tertiary university hospital [17]. It was detected the blaNDM-1gene in the six of CRE isolates (3.3%, n=5 *K. pneumoniae* and n=1 E. cloacae) in a tertiary-level reference hospital [25]. In our study, the presence of blaNDM-1gene was detected in 34.5% (n=38) of analyzed 110 CRE strains. While 35 (31.8%) *K. pneumoniae* and three (2.7%) *E. coli* harboured the blaNDM-1gene, this gene was found in none of six E. cloacae strains. This high blaNDM-1gene positivity obtained in our study was remarkable, and this results in Turkey, bla_{NDM-1} gene in patients with no history of contact with foreign exhibited that increasingly more isolated. Also, the most important reasons for this rate increasing may explicable as the use of unnecessary and high-dose antibiotics and the transport of the blaNDM-1gene with plasmids. In 2013, from Turkey, it was reported that the blaNDM-1gene was not detected in any of the 210 CRE clinical isolates by PCR and emphasized the existence of this gene in clinical isolates was not common in Turkey [29]. However, these results we have at our disposal today, the blaNDM-1gene in clinical isolates showed increasingly common in Turkey.

MHT is one of several phenotypic confirmation tests have been described for the detection of CRE. However, many investigators reported that MHT showed poor performance in the detection of MBLs such as NDMs, VIMs and IMPs (26,30,31). In our study, MBL production positivity was found to be 71.8% (n=79) in tested 110 carbapenem-resistant isolates by using MHT. The reason for this high positivity rate can be attributed to the fact that phenotypic methods can capture strains with other resistance mechanisms such as OXA-48, KPC, CTX-M, CMY, AmpC as well as isolates that produce NDM-1. In a study from China, 21 carbapenem-resistant *K. pneumoniae* strains found positive for MBL by MHT were determined to be blaNDM-1gene positive [12]. In another study, it was reported two of 34 *E. coli* isolates and three of 18 *K. pneumoniae* determined as carbapenemase producers by MHT. In the same study, it was determined that between the two MHT positive *E. coli*, one isolate only harboured the blaNDM-1gene, while two among the three MHT positive isolates of *K. pneumoniae* were positive for blaNDM-1gene [13]. In our study, 38 (35 *K. pneumoniae* and three *E. coli*) of 79 strains found to be positive for MBL by using MHT were determined as blaNDM-1gene positive. It was thought that the MHT was not sensitive for detection of NDM-1 activity similar to the other researcher's findings.

Carbapenemase-producing Gram negative bacteria are accepted resistant to all or almost all beta-lactams [1,2,8]. In studies conducted to date, it has been determined that plasmids carrying carbapenemase genes can also carry genes resistant to many antibiotics, and the rate of multiple resistance is quite high among CREs that produce resistance enzymes [14,15,32].

In our study, antibiotic resistance profiles of all isolates were evaluated together. According to the data obtained, the highest resistance rate (100%) was determined against cefuroxime. After, resistance rates against ceftriaxone (97.3%), piperacillin-tazobactam (97.3%), amoxicillin-clavulanic acid (90.9%), imipenem (85.4%), meropenem (83.6%), ciprofloxacin (76.4%) and ertapenem (70.9%) were determined (Table 2). These results were consistent with findings reported by other researchers [8,24,33,34]. Tigecycline, trimethoprim-sulphamethoxazole, amikacin and gentamicin were found to be effective on CRE isolates. Some researchers reported that aminoglycosides are still considered first-line therapy for the treatment of CRE infections and emphasized that gentamicin and amikacin were the most frequently used aminoglycosides in the studies report cases [7,8]. Although tigecycline was seen as the most effective antibiotics on 110 isolates determined to be CRE in our study, in 45 (40.9%) of isolates were determined the resistance to tigecycline. Similar to our findings, many studies have reported that tigecycline are the most effective antimicrobial agents against CRE [21,35,36]. While some investigators emphasized that they also found resistance rates to these two antibiotics [11,25]. In recent years, it was reported that CRE, and more particularly *K. pneumoniae* , have started to develop resistance against these drugs and the efficiency of two antibiotics has decreased on monotherapy treatment [7,8].

In many studies, it was reported that blaNDM-1gene positive CRE strains were mostly isolated from ICUs [17,23,26,25]. Similarly, in our study the origin of 35 NDM-1 producing *K. pneumoniae* strains was determined to be anesthesiology and reanimation clinic (n=3), internal medicine clinic (n=2), neurosurgery clinic (n=3), cardiovascular surgical clinic (n=2), general surgical clinic (n=3), chest diseases intensive care unit (ICU) (n=4), neonatal ICU (n=4), anesthesia ICU (n=7) and internal ICU (n=7). However, three CR *E. coli* strains harbouring the blaNDM-1gene were obtained from general surgical clinic. While three NDM-1 producing *E. coli* was isolated from blood sample, blaNDM-1gene positive *K. pneumoniae* strains were obtained from tracheal aspirate (n=7), blood (n=6), urine (n=11), sputum (n=4), catheter (n=3) and wound (n=4) samples.

As reported in many studies and in our study, the strains carrying the blaNDM-1gene were determined to be resistant againist many other drugs also beta-lactam antibiotics [11,25,27]. All of *K. pneumoniae* strains (n=35) harbouring the blaNDM-1gene exhibited resisttance to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, ceftazidime, ceftriaxone, ertapenem, meropenem and imipenem. Similarly, the resistance to amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, ceftazidime, ceftriaxone, ertapenem, meropenem, imipenem, gentamicin, ciprofloxacin and trimethoprim-sulfamethoxazole was determined in all of blaNDM-1gene positive *E. coli* strains (n=3). In addition, 29 *K. pneumoniae* and two *E. coli* strains were resistant to tigecycline. This finding was remarkable for us. Because, in general, bacteria containing NDM-1 β-lactamase are seem to test susceptible to tigecycline by the researchers [21,36].

Conclusion

Neurosurgeons and neuroscientists from Turkey contributed many papers for autonomic nervous system dysfunction following SAH. More studies are required for investigating this subject.

Conflict of interests
The authors have no conflicts of interest to declare

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Ethical approval
Ethical approval by Afyonkocatepe University local ethical authority was obtained (2019/204)

References

1. Bush K. Alarming β-lactamase-mediated resistance in multidrug-resistant *Enterobacterales*. Curr Opin Microbiol. 2010;13:558-64.
2. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infec Dis. 2016;3:15-21.
3. Kandeel A. Epidemiology of carbapenemase producing *Enterobacterales* in a general hospital. J Microbiol Infec Dis. 2015;5:57-62.
4. Ye Y, Xu L, Han Y, et al. Mechanism for carbapenem resistance of clinical *Enterobacterales* isolates. Exp Ther Med. 2018;15:1143-9.
5. Lim CL, Spelman D. Mortality impact of empirical antimicrobial therapy in ESBL and AmpC producing *Enterobacterales* bacteremia in an Australian tertiary hospital. Infec Dis Health. 2019;24:124-33.
6. Beware, centers for disease control and prevention report (cdc). department of health and human services. 2015;02.04.21
7. Rodríguez-Baño J, Gutiérrez-Gutiérrez B, Machuca I, et al. Treatment of infections caused by extended spectrum beta lactamase, AmpC, and carbapenemase producing *Enterobacterales*. Clin Microbiol Reviews. 2018;31:2.
8. Suay-García B, Pérez-Gracia MT. Present and Future of Carbapenem-Resistant *Enterobacterales* (CRE) Infections. Antibiotics. 2019;8:122.
9. Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. 2009;53:5046-54.
10. Karabay O, Altindis M, Koroglu M, et al. The carbapenem-resistant *Enterobacterales* threat is growing: NDM-1 epidemic at a training hospital in Turkey. Ann Clin Microbiol Antimicrob. 2016;15:6.
11. Berçot B, Poirel L, Dortet L, et al. In vitro evaluation of antibiotic synergy for NDM-1-producing *Enterobacterales*. J Antimicrob Chemother. 2011;66:2295-7.
12. Jin Y, Shao C, Li J, et al. Outbreak of multidrug resistant NDM-1-producing *Klebsiella pneumoniae* from a neonatal unit in Shandong Province, China. PloS One. 2015;10:0119571.
13. Binod GC, Nim RS, Binod R, et al. Detection of blaNDM-1gene among the carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from a children's hospital in Nepal. Novel Res Microbiol J. 2018;2:65-74.
14. Yoon EJ, Kang DY, Yang JW, et al. New Delhi Metallo-Beta-Lactamase-Producing *Enterobacterales* in South Korea Between 2010 and 2015. Front. Microbiol. 2018;9:571.
15. Xiang T, Chen C, Wen J, et al. Resistance of *Klebsiella pneumoniae* Strains Carrying blaNDM-1Gene and the Genetic Environment of blaNDM-1. Front Microbiol. 2020;11:700.
16. Poirel L, Ozdamar M, Ocampo-Sosa AA, et al. NDM-1-producing *Klebsiella pneumoniae* now in Turkey. Antimicrob Agents Chemother. 2012;56:2784-5.
17. Alp E, Perçin D, Çolakoğlu S, et al. Molecular characterization of carbapenem-resistant *Klebsiella pneumoniae* in a tertiary university hospital in Turkey. J Hospital Infec. 2013;84:178-80.
18. Davarci I, Senbayrak S, Aksaray S, et al. Molecular Epidemiology of Carbapenem resistant *Klebsiella pneumoniae* Isolates. Anatolian Clinic J Med Sci. 2019;24:1-7.
19. Beware: European Committee on Antimicrobial Susceptibility Testing (EUCAST). Eur Society Clin Microbiol Infect Dis. 2015;02:04.21
20. Kaase M, Szabados F, Wassill L, et al. Detection of carbapenemases in *Enterobacterales* by a Commercial Multiplex PCR. J Clin Microbiol. 2012;50:3315-9.
21. Wang JT, Wu UI, Lauderdale TLY, et al. Carbapenem nonsusceptible *Enterobacterales* in Taiwan. PLoS ONE. 2015;10:e0121668.
22. Zaidah AR, Mohammad NI, Suraiya S, et al. High burden of Carbapenem-resistant *Enterobacterales* (CRE) fecal carriage at a teaching hospital: cost-effectiveness of screening in low-resource setting. Antimicrob Resist Infect Control. 2017;6:42.
23. Pollett S, Miller S, Hindler J, et al. Phenotypic and molecular characteristics of carbapenem-resistant *Enterobacterales* in a health care system in Los Angeles, California, from 2011 to 2013. J Clin Microbiol. 2014;52:4003-9.
24. Hammoudi D, Moubareck CA, Aires J, et al. Countrywide spread of OXA-48 carbapenemase in Lebanon: surveillance and genetic characterization of carbapenem-non- susceptible *Enterobacterales* in 10 hospitals over a one-year period. Int Infec Dis. 2014;29:39-44
25. Baran I, Aksu N. Phenotypic and genotypic characteristics of carbapenem-resistant *Enterobacterales* in a tertiary-level reference hospital in Turkey. Ann Clin Microbiol Antimicrob. 2016;15:20.
26. Sahin K, Tekin A, Ozdas S, et al. Evaluation of carbapenem resistance using phenotypic and genotypic techniques in *Enterobacterales* isolates. Ann Clin Microbiol Antimicrob. 2015;14:44.
27. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10:597-602.
28. Bushnell G, Mitrani Gold F, Mundy LM. Emergence of New Delhi metallo-β-lactamase type 1-producing *Enterobacterales* and non-*Enterobacterales*: global case detection and bacterial surveillance. Int J Infec Dis. 2013;17:325-33.
29. Yanık K, Emir D, Eroğlu C, et al. Investigation of the Presence of New Delhi Metallo-Beta-Lactamase-1 (NDM-1) by PCR in Carbapenem-Resistant Gram-Negative Isolates. Mikrobiyol Bul. 2013;47:382-4.
30. Doyle D, Peirano G, Lascols C, et al. Laboratory detection of *Enterobacterales* that produce carbapenemases. J Clin Microbiol. 2012;50:3877-80.
31. Rasheed JK, Kitchel B, Zhu W, et al. New Delhi metallo-β-lactamase-producing *Enterobacterales*, United States. Emerging Infec Dis. 2013;19:870-8.
32. Göttig S, Riedel-Christ S, Saleh A, et al. Impact of bla_{NDM-1} on fitness and pathogenicity of *Escherichia coli* and *Klebsiella pneumoniae* . Int Journal of Antimicrobial Agents. 2016;47:430-5.
33. Fazeli H, Norouzi Barough M, Ahadi AM, et al. Detection of New Delhi Metallo-Beta-Lactamase-1 (NDM-1) in carbapenem-resistant *Klebsiella pneumoniae* isolated from a university hospital in Iran. Hippokratia. 2015;19:205-9.
34. Hamzan NI, Yean CY, Rahman RA, et al. Detection of blaIMP4 and blaNDM1 harboring *Klebsiella pneumoniae* isolates in a university hospital in Malaysia. Emerg Health Threats J. 2015;8:26011.
35. Sidjabat H, Nimmo G.R, Walsh TR, et al. Carbapenem Resistance in *Klebsiella pneumoniae* Due to the New Delhi Metallo-β-lactamase. Clin Infec Dis. 2011;52:481-4.
36. Shahcheraghi F, Nobari S, Ghezelgeh FR, et al. First Report of New Delhi Metallo-Beta-Lactamase-1-Producing *Klebsiella pneumoniae* in Iran. Microbial Drug Resis. 2013;19:30-6.