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ORIGINAL ARTICLE

The First *Enterobacter cloacae* Co-Producing NDM and OXA-48 Carbapenemases and Interhospital Spread of OXA-48 and NDM-Producing *Klebsiella pneumoniae* in Turkey

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SUMMARY

Background: **The aim of this study was to investigate the occurrence of carbapenemase-producing Enterobacteriaceae.**
Methods: **A total of 54 carbapenem nonsusceptible Enterobacteriaceae (CRE) isolates were recovered from clinical samples sent to the Dr. Lutfi Kirdar Kartal Training and Research Hospital from the period 2011 through 2014. Forty-four isolates were *Klebsiella pneumoniae* (CRKP) and the other 10 were *Enterobacter cloacae* (CREC).The isolate identifications and antibiotic sensitivity tests were performed using a Vitek2 automatic system. The clonality of isolates was determined using rep-PCR Diversilab. Presence of blaOXA-48, blaNDM, blaVIM, blaIMP, and blaKPC genes were screened using polymerase chain reaction (PCR) with specific primers.**
Results: **CRKP were isolated from blood, urine, wounds, catheter tips, and tracheal aspirate samples; a total 44 isolates were evaluated. All isolates were nonsusceptible to ertapenem/imipenem or meropenem. Eighteen percent of the isolates were resistant to colistin. CREC were isolated from blood, urine, cerebrospinal fluid and sputum; a total of 10 isolates were evaluated. They were resistant to all carbapenems and 90% were resistant to cefoperazone/sulbactam and trimethoprim/sulfamethoxazole, and 50 - 70% isolates were resistant to gentamicin, amikacin, and ciprofloxacin. Thirty-three (75%) OXA-48 producing CRKP were identified. Thirteen (29.5%) were positive and two (4.5%) NDM-producing *K. pneumoniae* were co-producing OXA-48. Of the ten CREC strains tested, eight were positive for blaNDM, one isolate was positive for blaVIM and another for blaIMP genes. rep-PCR typing revealed the presence of a clonal dissemination in CRKP and CREC in the hospital.**
Conclusions: **To our knowledge, this is the first identification of blaNDM in *E.* cloacae isolates in Turkey. These findings describe an interhospital spread of CRKP-producing OXA-48 and NDM carbapenemases that started in 2011. Continuous monitoring is necessary to better understand their dissemination in the hospital, which probably occurred as a result of transmission from an environmental reservoir. These findings emphasize the need for intensive surveillance and precautions.**
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INTRODUCTION

Antibiotic resistance has become an important worldwide health problem with a gradually growing prevalence. In particular, resistance to carbapenems in the Enterobacteriaceae family, which leads to infections in humans with the highest frequency being reported from all countries with larger populations. Three main carbapenemase classes have been defined in carbapenem resistance: Ambler class A beta-lactamase (KPC), class B (metallo-enzymes), and, class D (OXA-48 type). KPC was first reported from the United States of America and later observed in Greece and Italy. NDM-1 was first reported in India and has also been observed in the other countries with an increasing prevalence. OXA-48 has been mostly reported from Mediterranean countries and Southern Europe [1]. Resistance to many penicillin and cephalosporin derivatives may also be observed because carbapenem resistance genes are transmitted by beta lactamase enzymes. In addition, resistance to other non-beta lactam antibiotics may also be observed, because resistance is transferred by the same mobile genetic element [2,3]. Mortality and morbidity rates are substantially high in the presence of severe infections including pneumonia, urinary infections, intraabdominal infection, and sepsis caused by these bacteria, which limit antibiotic options to a great extent [4-6]. The diagnosis should be made rapidly and strict infection control precautions should be taken to prevent the spread of infection, because treatment options are very limited [1]. In this study, we aimed to determine the susceptibility, genotype, and clonal relationship of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) and *Enterobacter cloacae* (CREC) isolates that we isolated from various clinical samples over a four-year period in our hospital.

MATERIALS AND METHODS

Strains

Forty-four *Klebsiella pneumoniae* and 10 *Enterobacter cloacae* isolates that were nonsusceptible to carbapenems found in various clinical samples, which had been sent to Dr. Lütfi Kirdar Education and Research Hospital, Clinical Microbiology Laboratory between 2011 and 2014 were included in the study.

The isolates and antibiotic resistance tests were defined using an automated VITEK2 (bioMérieux, France) system, and susceptibilities were determined according to the Clinical & Laboratory Standards Institute's (CLSI) recommendations [7]. The limiting values recommended by the Food and Drug Administration (FDA) for *Enterobacteriaceae* were used for tigecycline susceptibility; a minimum inhibitory concentration (MIC) value of ≤ 2 µg/mL was considered susceptible, an MIC value of 4 µg/mL was considered moderately susceptible, and an MIC value of ≥ 8 µ/mL was considered resistant [8]. Susceptibilities were decided by repeating susceptibility tests of strains that were found moderately susceptible and resistant to carbapenems on the automated system using the antimicrobial gradient test (Etest, BioMérieux, France) [7].

Strains that were resistant to carbapenems were kept at -70°C in microbanks until molecular tests were performed. Molecular analysis was performed with pure colonies obtained after they were defrosted and passaged twice.

PCR method

DNA templates were prepared by boiling a bacterial suspension for 10 minutes. Isolates were examined using PCR with the following sets of primers; KPC FW (5-ATGTCACTGTATCGCCGTC-3'), RW (5'-TTACT GCCCGTTAACGCC-3') (785 bp) [9], OXA48-A (5'-TTGGTGGCATCGATTATCGG-3'), OXA48-B (5'-GAGCACTTCTTTTGTGATGGC-3') (743 bp) [10], NDM FW (5'-GGGCAGTCGCTTCCAACGGT-3'), RW (5'-GAGCCCGTTTTATGGACCC-3') in a 50 µL volume containing 10 x PCR buffer, 2 mM deoxynucleoside triphosphates, 3.4 pmol of each primer, 2.5 mM MgCl2, and 1 U Taq DNA polymerase, and 1 µL of genomic DNA. PCR conditions were initial denaturation at 95°C for 5 minutes, denaturation at 94°C for 60 seconds, annealing at 56°C for 45 seconds, and extension at 72°C for 60 seconds, which was repeated for 35 cycles, and final extension at 72°C for 7 minutes on a thermal cycler. Amplification products were separated in 1% agarose gels stained with ethidium bromide and visualized under UV light. X174 Hae III fragments were used to assess PCR product size (MBI Fermentas, St. Leon-Rot, Germany) [10].

The clonal relationship between the isolates was investigated using DiversiLab typing

The DiversiLab Microbial Typing System (BioMérieux, Marcy l'Etoile, France) is based on repetitive sequence-based PCR (rep-PCR), which amplifies the regions between the noncoding repetitive sequences in bacterial genomes. DNA was isolated by the DiversiLab Mo Bio UltraClean microbial DNA isolation kit, as recommended by the manufacturer (MO-BIO, USA). The DNA concentration was measured and set between 25 ng/L and 30 ng/L. Subsequently, the DNA was amplified using the DiversiLab fingerprinting kit for *Klebsiella* spp. and *Enterobacter* spp, in accordance with the manufacturer's instructions. PCR was performed using the following parameters: initial denaturation (94°C) for 2 minutes, and then 35 cycles of 30 seconds of denaturation (94°C), 30 seconds of annealing (50 - 55°C depending on the species), and 90 seconds of extension (70°C), followed by 3 minutes of final extension (70°C), and ending at 4°C. The amplification products were separated using an Agilent B2100 bioanalyzer. Five microliters of DNA standard markers (used for normalization of sample runs) and 1 µL of the DNA product were used. The detection of rep-PCR products was made using kits for the Diversilab DNA LabChip (BioMeriéux, France). All data were entered and ana-

lyzed in the DiversiLab software system (version 3.4), which uses Pearson's correlation coefficient and the unweighted-pair group method with arithmetic averages to determine distance matrices, and to create dendograms with a similarity matrix and gel image of the fingerprint for DNA. The reports were generated automatically [11, 12].

The isolates were classified as indistinguishable (similarity > 97% and no band difference), similar (similarity between 95% and 97%, 1 - 2 different bands), and different (similarity < 95% ad > 2 different bands). Strains that showed more than 95% similarity with each other were considered main clones, and clones that showed more than 97% similarity among the main clones were considered subclones. Clones with a similarity below 95% were considered different clones [11,13].

RESULTS

Forty-four *K. pneumoniae* from blood, urine, wounds, catheter ends, and tracheal aspirate samples and 10 *E. cloacae* from blood, urine, CSF, and sputum were evaluated.

When we evaluated the number of *K. pneumoniae* isolates by years, we found that we isolated 4 in 2011, 5 in 2012, 4 in 2013, and 31 in 2014 (8 in the month of August) (Figure 1). All *K. pneumoniae* isolates were resistant to pipercillin-tazobactam, seftazidim, sefepim, and ertapenem. It was found that 42 (95.5%) isolates were resistant to imipenem, 42 (95.5%) were resistant to meropenem, 31 (70.5%) were resistant to amikacin, 38 (86.4%) were resistant to gentamycin, 40 (91%) were resistant to ciprofloxacin, 1 (2.3%) was resistant to tigecycline, and 30 (68.2%) isolates were resistant to trimethoprim-sulfametoxazole. Colistin resistance was found in 8 (18%) strains (Table 1). The MIC (mg/L) range of the isolates was found as 0.25 - > 32 for imipenem, 0.25 - > 32 for meropenem, and 2 - > 32 for ertapenem. The MIC_{50} and MIC_{90} values were found as > 32 for all carbapenems.

All *E. cloacae* strains were resistant to carbapenems, piperacillin/tazobactam, ceftazidim, and cefepim. Nine were found resistant to trimetoprim/sulfametoxazol (90%), 7 were resistant to gentamycin (70%), 6 were resistant to ciprofloxacin (60%), and 5 were resistant to amikacin (50%). All *E. cloacae* strains were susceptible to tigecycline and colistin (Table 2). The MIC (mg/L) range of the isolates was found as 2 - > 32 for imipenem, 2 - > 32 for meropenem, and 1 - > 32 for ertapenem. The MIC_{50} and MIC_{90} values of all carbapenems were found as > 32 mg/L.

Antimicrobial susceptibility test results

Table 1, Table 2.

Molecular characterization of carbapenemase genes

It was found that 11 of the 44 isolated CRKP carried NDM-1 (KL1, 3, 7, 17, 18, 26, 32, 40, 44, 52, 60) genotype, 2 carried NDM-1/OXA-48 (KL10, 22) genotype, and 31 carried OXA-48 genotype.

When the clonal relationship of *K. pneumoniae* isolates was examined with rep-PCR, it was observed that all strains had a similarity rate of 96.8% and established 28 different subclones (P1-P28) (Figure 2). It was observed that 6 strains in the P1 subclone (KL5,14, 18, 21, 24, 28), 5 strains in the P2 subclone (KL3, 7, 17, 26, 37), 3 strains in the P3 subclone (KL27, 35, 45), 2 strains in the P4 subclone (KL50, 53), 2 strains in the P5 subclone (KL1, 11), 2 strains in the P6 subclone (KL31, 41), 2 strains in the P7 subclone (KL 32,36), and 2 strains in the P8 subclone (KL22, 48) were indistinguishable. Each of the remaining strains were considered different subclones (P9-28) because they were different from each other. It was observed that 1 strain in the P1 subclone possessed the NDM-1 (KL18) gene and the remaining 5 strains possessed OXA-48 (KL5, 14, 21, 24, 28), and one strain in the P2 subclone possessed OXA-48 (KL37) and the remaining strains possessed the NDM-1 (KL3, 7, 17, 26) gene. It was found that the strains in P3 (KL27, 35, 45), P4 (KL50, 53), P5 (KL1, 11), P6 (KL31, 41) subclones carried OXA-48. It was found that one strain in P7 subclone carried the (KL32) NDM-1 gene and the other strain carried the (KL36) OXA-48 gene, and one strain in the P8 subclone carried the (KL48) OXA-48 gene and the other strain carried the (KL22) NDM-1+OXA-48 gene. It was observed that 4 of the strains in the remaining different subclones (P9-28) carried NDM-1 the (KL40, 44, 52, 60) gene, 15 strains carried the OXA-48 (KL2, 8, 19, 23, 29, 30, 34, 42, 46, 47, 55, 56, 57, 58, 59) gene, and one strain carried the (KL10) NDM-1+OXA-48 gene (Figure 2 and Figure 3).

It was observed that 6 of the 10 CRECs isolated had a genotype of NDM-1 (EC5, 7, 15, 20, 51, 54), 2 had a genotype of NDM-1+ OXA-48 (EC1, 10), one had a genotype of VIM-1(EC2), and the remaining one had a genotype of IMP-1 (EC3).

When the clonal relationship of *E. cloacae* isolates was examined with rep-PCR, it was observed that the similarity rate of all strains was 95%, and 6 subclones that were different from each other were established (P1-P6). It was observed that 4 strains in the P1 subclone (EC1, 5, 15, 20) and 2 strains in the P2 subclone (EC51, 54) were indistinguishable. Each remaining strain was considered a different subclone (P3-6) because they were different from each other. It was found that three of the strains in the P1 subclone carried the NDM-1 gene (EC5, 15, 20), and one strain carried the NDM-1+ OXA-48 (EC1) gene; both strains in the P2 subclone carried the NDM-1gene (EC51, 54); one strain in the P3 subclone carried VIM-1(EC2) gene; one strain in the P4 subclone carried the IMP-1(EC 3) gene; one strain in the P5 subclone carried the NDM-1+ OXA-48 (EC10) gene, and one strain in the P6 subclone carried the NDM-1(EC7) gene (Figure 4).

Two isolates that were susceptible to imipenem were found to carry the OXA-48 (KL23, 55) gene, one of the

Table 1. Antibiotic susceptibility of *K. pneumoniae* (n = 44).

	Susceptible	Nonsusceptible
	n (%)	n (%)
Colistin	36 (82)	8 (18)
Pipercillin-tazobactam	0 (0)	44 (100)
Ceftazidime	0 (0)	44 (100)
Cefepime	0 (0)	44 (100)
Imipenem	2 (4.5)	42 (95.5)
Meropenem	2 (4.5)	42 (95.5)
Ertapenem	0 (0)	44 (100)
Amikacin	13 (29.5)	31 (70.5)
Gentamycin	6 (13.6)	38 (86.4)
Ciprofloxacin	4 (9)	40 (91)
Tigecycline	43 (97.7)	1 (2.3)
Trimethoprim-sulfamethoxazole	14 (31.8)	30 (68.2)

Table 2. Antibiotic susceptibility of *E. cloacae* (n = 10).

	Susceptible	Nonsusceptible
	n (%)	n (%)
Colistin	10 (100)	0 (0)
Pipercillin-Tazobactam	0 (0)	10 (100)
Ceftazidim	0 (0)	10 (100)
Cefepime	0 (0)	10 (100)
Imipenem	0 (0)	10 (100)
Meropenem	0 (0)	10 (100)
Ertapenem	0 (0)	10 (100)
Amikacin	5 (50)	5 (50)
Gentamycin	3 (30)	7 (70)
Ciprofloxacin	4 (40)	6 (60)
Tigecycline	10 (100)	0 (0)
Trimethoprim-sulfamethoxazole	1 (10)	9 (90)

two isolates that were susceptible to meropenem carried the NDM-1(KL60) gene, and the other carried the OXA-48 (KL23) gene.

DISCUSSION

Carbapenem-resistant *K. pneumoniae* has been reported from many parts of the world since the time it was defined initially. Carbapenemases, including KPC, NDM, IMP, VIM, and OXA-48, are the main mechanisms of carbapenem resistance in CRE [14].

NDM-1 metallo beta lactamase was isolated initially in 2008 from a patient who travelled from India to Sweden for treatment, and was also reported from England as cases spread via patients who originated from India and Pakistan [15,16]. Although it is also observed in other countries in Europe, a high rate has been reported especially in Greece [16-18]. In our country, NDM-1 producing *K. pneumoniae* was reported for the first time in 2011 in Istanbul in a patient who came from Iraq. In 2012, it was reported that NDM-1 and OXA-48 produc-

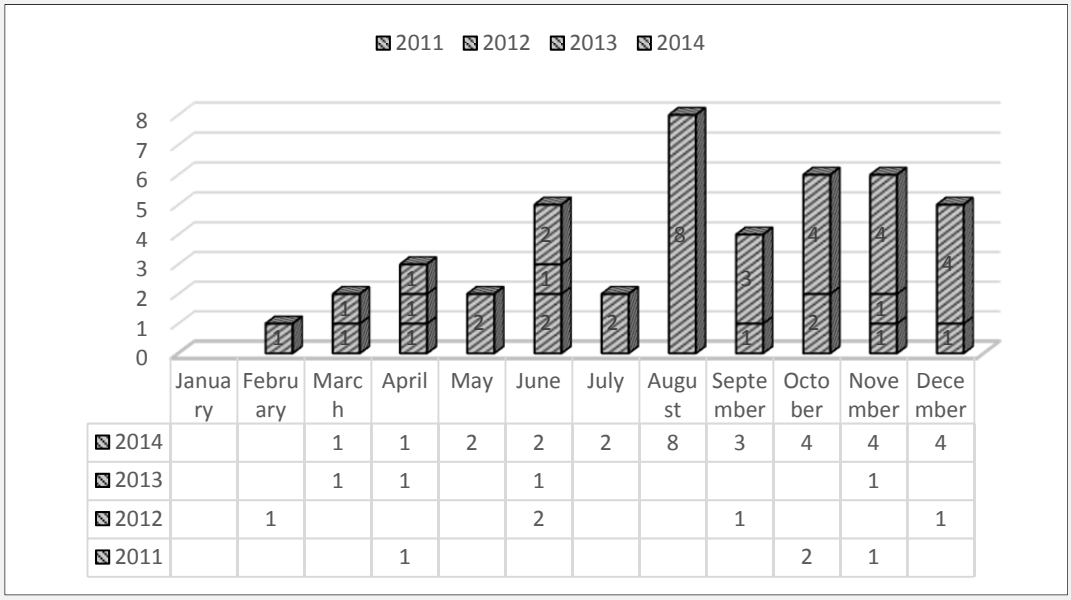

Figure 1. Distribution of *K. pneumoniae* isolates (n = 44) by months and years.

ing CRKP isolate was obtained from rectal swab cultures [19].

In our country, OXA-48 carbapenemase-producing *K. pneuomoniae* was reported in 2001 from Istanbul for the first time [20]. The first epidemic with OXA-48 was reported in 2008, again from Istanbul [21]. Later, epidemics from many other provinces were also reported [22-24].

In our hospital, we isolated the first CRKP isolate in 2011 in the hematology ward from a blood culture obtained on the 45th day of hospitalization from a patient with acute myeloid leukemia (KL60) who was being followed up. We learned that the patient had no relation with foreign countries, but was hospitalized in different hospitals in Istanbul for treatment. We found that this first strain in our hospital had a NDM-1 genotype and was not similar to the other isolates found in 2011 (KL17, 57, 58). We found that the five CRKP isolates (KL10, 19, 52, 55 59) that we found in 2012 were in different subclones (P26, P18, P28, P9, P18, respectively), three isolates carried the OXA-48 genotype (KL 19, 55, 59), one isolate carried the NDM-1 genotype (KL52), and one isolate carried the NDM-1/OXA-48 (KL 10) genotype. The 4 CRKP isolates that we isolated in 2013 were in different subclones (P25, P16, P2, P23), 2 isolates carried a genotype of OXA-48 (KL8 in P25 and KL42 in P16), and 2 isolates had a genotype of NDM-1 (KL3 in P2 and KL40 in P23). In 2014, an increase in carbapenem-resistant isolates occurred and we obtained 31 isolates; 8 isolates were obtained in August 2014. When the clonal relationship of these 8 isolates was examined, we observed that the isolate found first in this month and 2 that were subsequently isolated were in the same subclone (KL27, 35, 45 in P3 subclone) and belonged to patients who were hospitalized in the same intensive care unit; these strains had a genotype of OXA-48. Although two of the other isolates in this group (KL7, 37) were in the same subclone (P2), the patients had been hospitalized in different units and had different genotypes (OXA-48 and NDM-1). The other isolates that were isolated in August (KL30, 22, 44) were in different subclones (P22, P8, P10, respectively) and carried OXA-48 (KL30), NDM-1+OXA-48 (KL22), and NDM-1 (KL44) genotypes.

Five of the six *K. pneuomoniae* isolates in the P1 subclone carried the NDM-1 gene and one carried the OXA-48 gene; all except one were patients hospitalized in the intensive care unit. Four of the strains in the P1 subclone that possessed the highest number of isolates in 2014 were isolated from patients who were hospitalized in the same intensive care unit (KL21, 18, 24, 5), the remaining two belonged to patients who were hospitalized in different wards (KL28 and 14), one of these six strains carried the NDM-1 gene (KL18) and the others had a genotype of OXA-48. This suggested that there might be a spread between wards. At the end of August 2014, when an increase in strains was observed, 33 environmental cultures including the patients' bedsides, monitors, shelves and desks, the nurses' medication desks and treatment trays, and the computer key-

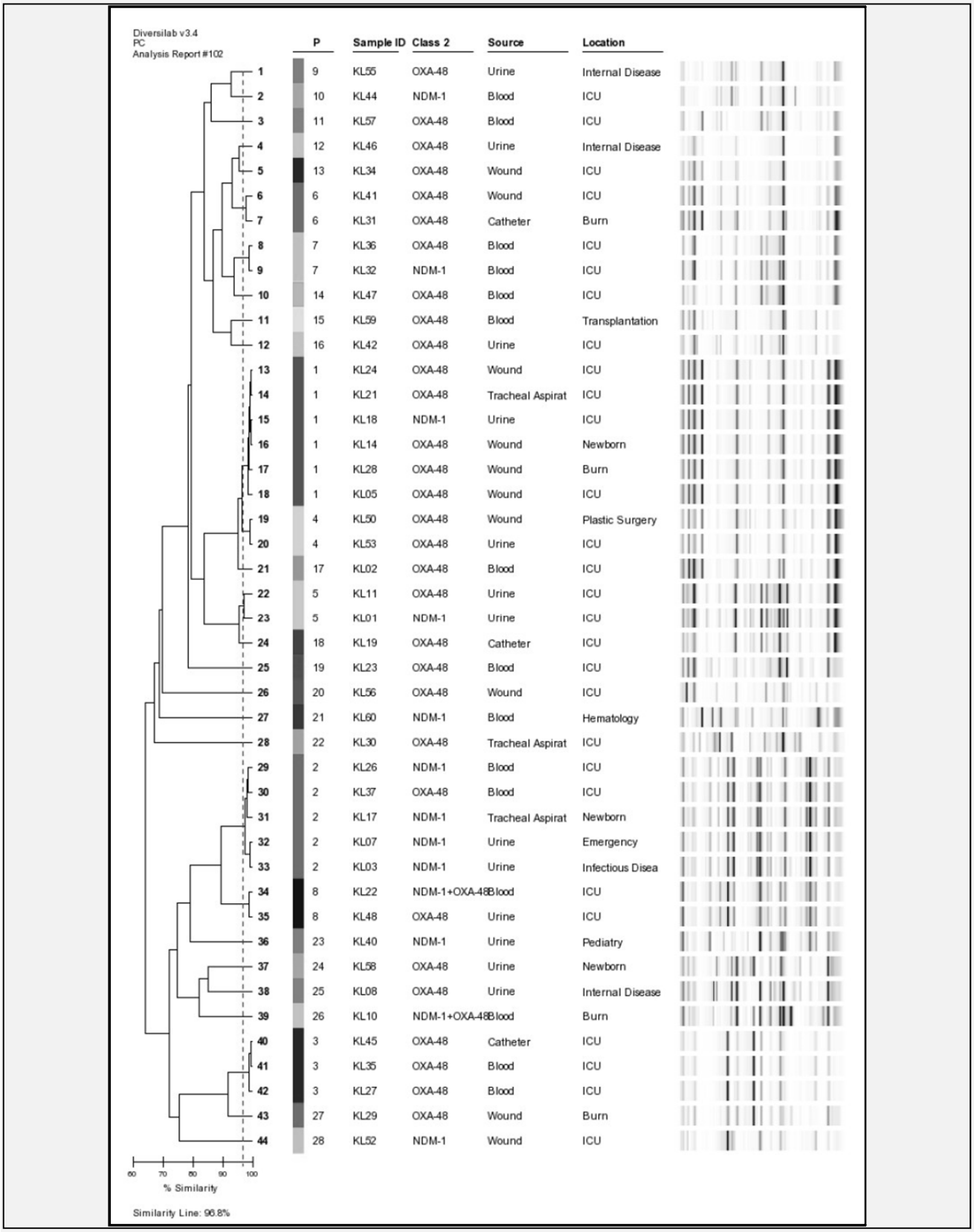

Figure 2. Results of Rep-PCR analysis for *K. pneumoniae* isolates (n = 44).

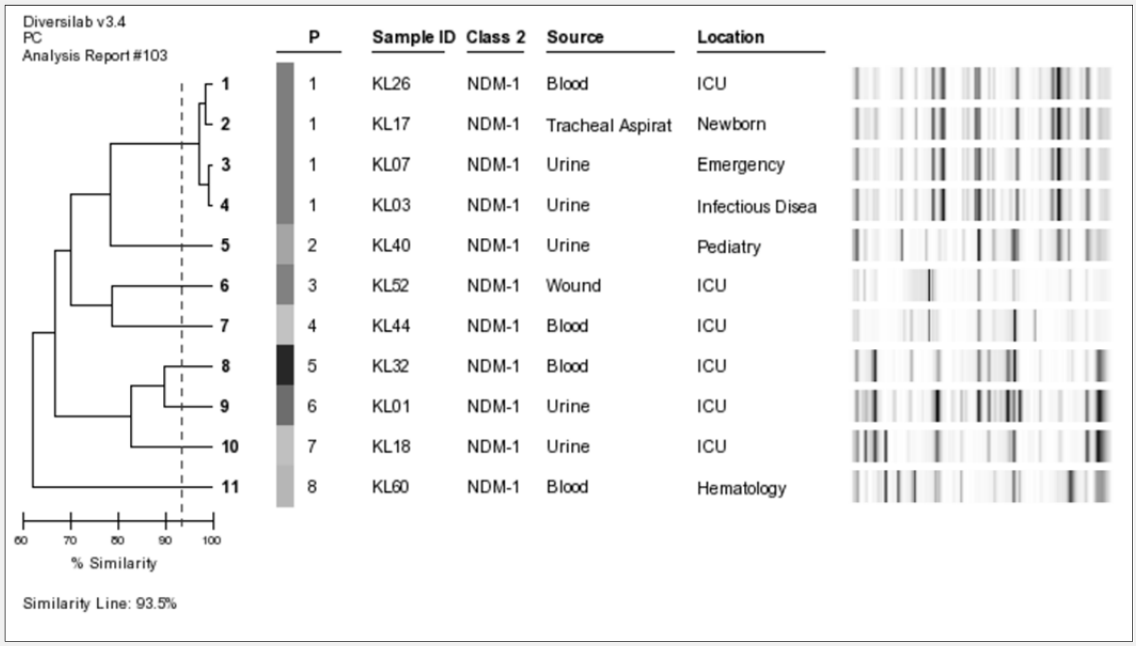

Figure 3. Results of Rep-PCR analysis for NDM producing *K. pneumoniae* isolates (n = 11).

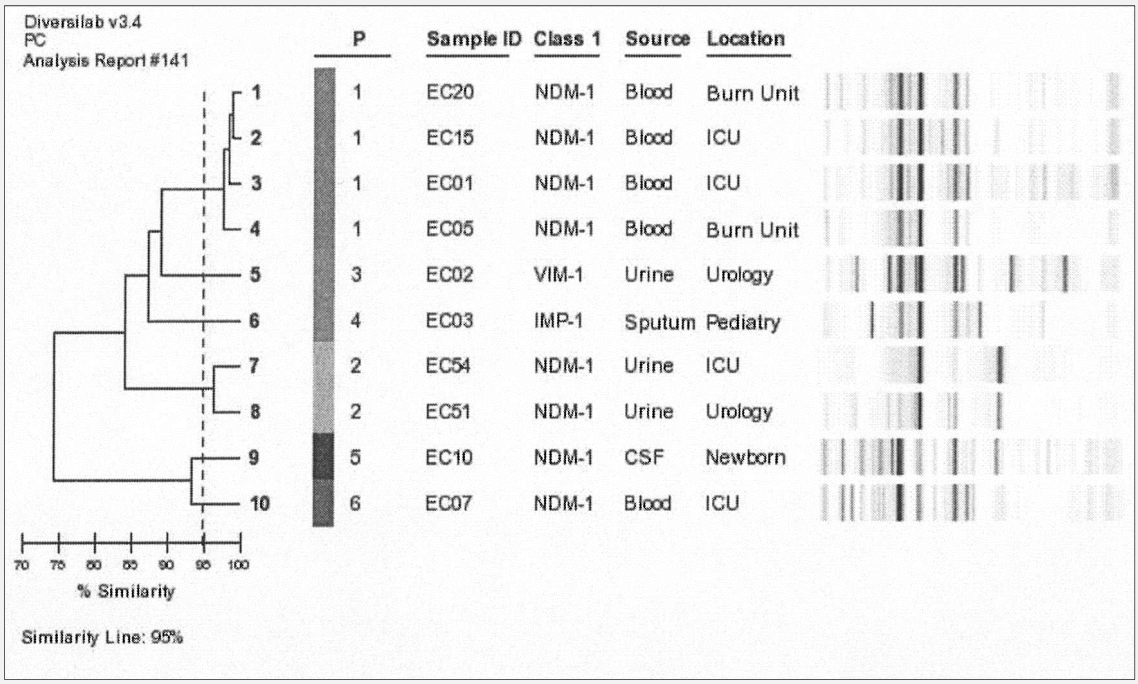

Figure 4. Results of Rep-PCR analysis for *E. cloacae* isolates (n = 10).

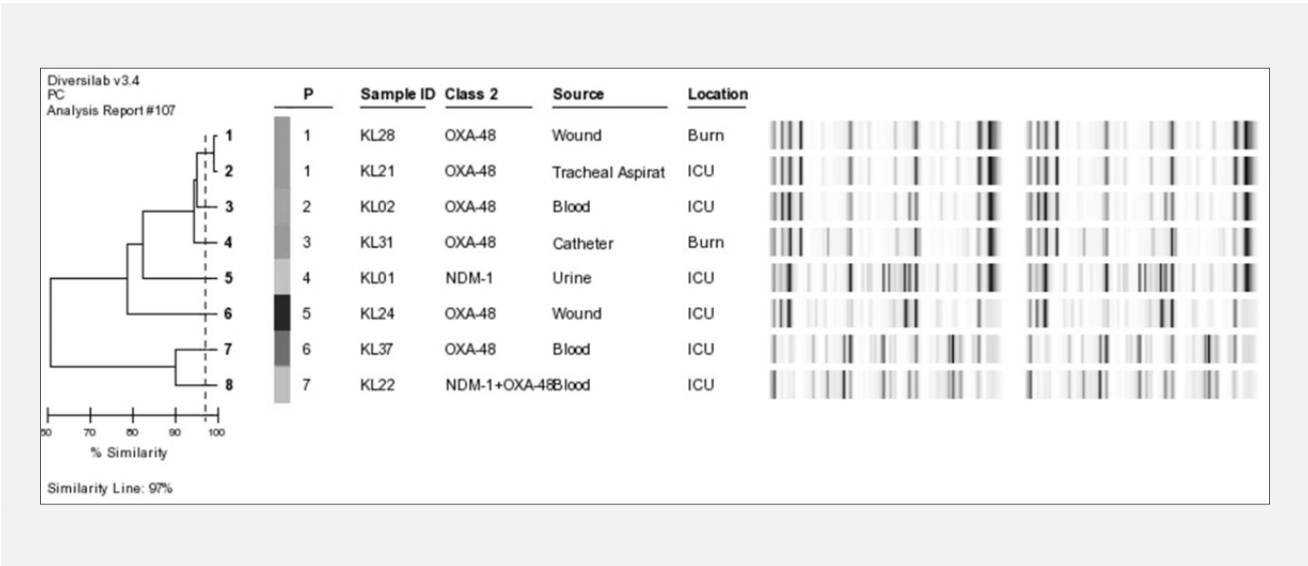

Figure 5. Results of Rep-PCR analysis for Colistin-resistant *K. pneumoniae* isolates (n = 8).

boards used, and hand cultures from 18 healthcare workers who cared for the patients were obtained, but no carbapenem-resistant isolate could be isolated. The fact that we could not grow CRKP from the environmental cultures obtained at the end of the month might be related with strict infection precautions as soon as we noted the increase in these strains and with repetition of training including cleaning and disinfection rules. Based on our previous experiences, we know that an increase in multiple-drug resistant microorganisms occurs in periods when infection control surveillance is not strictly performed or when the intensive care team changes. When the colistin-resistant CRKP strains were examined, it was found that 3 strains (KL21, 24, 28) were in the same subclone (P1), carried a genotype of OXA-48, and belonged to two patients including one patient who was hospitalized in a different intensive care unit (KL24) 4 months after the first patient (KL21), who was hospitalized in the intensive care unit in which the colistin-resistant strain was grown, and one patient who was hospitalized in a ward near this second intensive care unit (KL28). The other colistin-resistant strains were in different subclones. In addition, three of these strains were found to cary the OXA-48 genotype (KL2, 31, 37), one carried the NDM-1 genotype (KL1), and the remaining one carried the NDM-1/OXA-48 genotype (KL22). When we evaluated this situation, we understood that one strain (KL21) could occasionally spread to the intensive care unit and other wards (Figure 5).

Bacteriae that carry carbapenemase enzymes are usually susceptible to a limited number of antibiotics. Most are susceptible to colistin, tigecycline, and one or more aminoglycosides, and are resistant to the remaining antibiotics. This increases mortality in severe infections including sepsis [20]. Susceptibility may show variance for different carbapenem groups. It has been reported that the most sensitive agent to determine carbapenem resistance is ertapenem [25]. All our strains were resistant to ertapenem, but two were susceptible to imipenem (KL23,55) and two were susceptible to meropenem (KL23,60).

In this study, two imipenem-susceptible strains and one meropenem-susceptible strain (KL23) were identified among 31 *K. pneuomoniae* strains that carried a genotype of OXA-48. The remaining strains were found to be resistant to imipenem and meropenem. All 31 strains were resistant to ertapenem. Among these, all 6 isolates that were resistant to colistin were found susceptible to tigecycline, 4 were susceptible to SXT, and 1 was susceptible to amikacin.

All *K. pneuomoniae* strains that carried NDM-1 were found resistant to pipercillin-tazobactam, ceftazidim, cefepim, imipenem, and ertapenem, 7 were resistant to amikacin, 9 were resistant to gentamycin, 8 were resistant to ciprofloxacin and trimethoprim-sulfamethoxazole, and 1 was resistant to tigecycline. One of these isolates carried the NDM-1 genotype and it was found reistant to all antibiotics except tigecycline (KL1). Only one strain was susceptible to meropenem (KL60). One of the strains that carried a genotype of NDM-1 + OXA-48 was susceptible to colistin, amikacin, and tigecycline, and the other was resistant to colistin and susceptible to tigecycline and amikacin. These data show that the most susceptible antibiotics for our isolates were colistin and tigecycline.

Nosocomial infections caused by *E. cloacae*, which is another member of Enterobacteriaceae and possesses natural resistance to ampicillin and narrow-spectrum cefalosporins, are gradually gaining more importance.

Development of resistance in these strains is related with porin loss or excessive production of chromosomal cefalosporinase [25]. *E. cloacae* isolates, which possess resistance genes related with enzymes hydrolizing carbapenems have been reported from different countries and from our country [13,26-29].

In this study, we found that all *E. cloacae s*trains were resistant to carbapenems. We observed that *E.cloacae* isolates formed 6 different subclones (P1-P6). Six of the strains carried the NDM-1 gene, one carried the VIM-1 gene, one carried the IMP-1 gene, and two carried the NDM-1/OXA-48 gene. As far as we know, an *E. cloacae* isolate carrying NDM-1 and OXA-48 genes in association are presented for the first time in Turkey in this study.

When the genetic relationship of *E. cloacae* strains was examined, it was observed that 4 strains in the P1 subclone belonged to patients who were hospitalized in the same ward and its associated intensive care unit at different times. The first strain, which was isolated in 2012, was in this subclone and was found resistant for the first time; it belonged to a patient who originated from Iraq and was hospitalized in the ward. The agent was grown from a blood culture obtained at the time of hospitalization and the patient was transferred to another hospital after 2 days of hospitalization. This strain carried a genotype of NDM-1. The second strain in the P1 subclone (EC1) was isolated on the 14th day of hospitalization of another foreign patient in the same ward, 5 months after the patient in whom the first strain was isolated, and the patient was lost on the day the positive culture was sent. It was observed that this strain carried NDM-1 and OXA-48 in association. These two strains were considered indistinguishable. One of the other strains in the P1 subclone isolated in 2013 (EC5) and another isolated in 2014 (EC15) had a genotype of NDM-1. One of the patients in whom the two strains were considered indistinguishable in the P2 subclone was hospitalized in one ward (EC51), and the other (EC54) had no relation with that ward and was hospitalized in the intensive care unit; these patients were separated by an interval of 22 days. These two strains had a genotype of NDM-1. Based on these findings, we can state that CREC carrying a genotype of NDM-1 was transferred and spread to our hospital via a patient who originated from abroad. The fact that there were 4 years between the strains makes it difficult to explain how they transferred from patient to patient. In this aspect, we can speculate that the strain was also transmitted to other patients, did not lead to a clinical picture or no sample was sent even though a clinical picture was present, because these patients were already using antibiotics or we could not isolate this agent, even though a sample was sent.

CONCLUSION

Our findings show that carbapenem-resistant isolates remained persistent for a long time and spread in our hospital among patients hospitalized in the intensive care unit and other wards. This means that they may cause epidemics at any time period when infection control measures are not implemented. It is clear that we will have difficulties in the event of larger epidemics, because antibiotics with the highest susceptibility to our carbapenem-resistant isolates were tigecycline and colistin, and strict infection control measures should be implemented.

Declaration of Interest:
None declared.

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