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Original Article

Investigation of the prevalence of carbapenem resistance genes in faecal carriage of carbapenem resistant *Klebsiella spp.* isolates by multiplex real-time PCR method

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Abstract

Introduction: Increased carbapenem resistance in *Klebsiella spp.* strains causes high morbidity and mortality. The genes encoded for carbapenemaseare transferrable between different bacterial species. In the present study, we aimed to investigate carbapenem resistance genes in *Klebsiella spp. s*trains.

Methodology: Fifty *Klebsiella spp.* strains were isolated from rectal swabs of patients hospitalized in the neonatal intensive care unit (NICU). All strains were identified with API20E. The minimum inhibitory concentrations (MICs) of carbapenems were determined by the broth dilution method. The major five carbapenem genes (OXA-48, NDM, VIM, KPC, and IMP) were detected by the multiplex real-time PCR method.

Results: It was found that 49 (98%) of the strains were resistant to ertapenem (MIC ≥ 2μg/mL) and imipenem(MIC ≥ 4 μg/mL), and 47 (94%) of the strains were resistant to doripenem (MIC ≥ 4 μg/mL)and meropenem(MIC ≥ 4 μg/mL).NDM was detected in 42%, OXA-48 in 16%, and VIM in one (2%) isolate, and NDM + OXA-48 co-existed in 36% of the isolates. The KPC and IMP genes were not detected.

Conclusions: NDM and NDM co-existing with OXA-48 were prevalent in the NICU of Istanbul Medical Faculty Hospital. Paying attention to the hand hygiene of healthcare workers, screening of rectal swabs of hospitalized patients for the presence of carbapenem resistance strains, and isolation of infected patients can effectively control the spread of carbapenem-resistant strains.

Key words: Carbapenem resistance gene; *Klebsiella spp*.; neonatal; intensive care unit; rectal swab; multiplex real-time PCR.

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Introduction

Although carbapenems, which belong to the beta-lactam antibiotic group, are currently used as the last option in treating serious infections, various resistance pathways have emerged against carbapenems. Changes in penicillin-binding proteins (PBPs) in the bacterial cell, porin changes in the cell membrane, and synthesis of beta-lactamases encoded by various genes carried on the plasmids predispose to the spread of carbapenem resistance [1]. In recent years, carbapenem-resistant Gram-negative bacilli (CRGNB) have posed a great danger for hospitalized patients because the number of antibiotics to be used to treat CRGNB infections is gradually decreasing. Carbapenem resistance can be detected phenotypically by the disc diffusion test and by determining their minimum inhibitory concentrations (MICs) [2]. Genotypically, the polymerase chain reaction (PCR) method can determine which genes (IMP, VIM, NDM, KPC, and OXA-48) are responsible for this resistance. The presence and spread of these genes, called the 'major five,' differ between countries and regions [3,4]. OXA-48 was first identified in *Klebsiella pneumoniae* isolates in Turkey in 2001 [5]. Later, again in Turkey, OXA-48 was reported in *Escherichia coli*and *Citrobacter freundii* isolates [6]. OXA-48 has been identified only in Turkey for several years and later has spread to the Middle East and Europe [7]. In this study, we aimed to determine the MICs of four carbapenems and investigate the prevalence of the major five carbapenem genes in the *Klebsiella spp.* strains isolated from rectal swabs of patients hospitalized in the neonatal intensive care unit (NICU) of Istanbul Faculty of Medicine Hospital, Turkey.

Methodology

Istanbul University Faculty of Medicine Ethics Committee approval (No: 36909) was obtained on 18.12.2019.

In this study, rectal swabs of 206 patients hospitalized in the NICU were sent to the hospital infection laboratory in 2021 and evaluated for the

presence of carbapenem resistance Gram-negative bacilli strains. Swabs were cultured on Mac Conkey media (Becton Dickinson, USA) supplemented with meropenem (1 mg/L) [8] and incubated at 37 °C for 48 hours. Gram-negative Bacillus isolates were identified with API20E strips (Biomerieux France), and 50 *Klebsiella spp*. strains were stored at -20 °C for further tests.

Antibiotic Susceptibility Test (AST)

The disk diffusion method was used to determine AST of the strains. In brief, an overnight fresh culture of 50 *Klebsiella spp*. strains was suspended in cationic Mueller-Hinton Broth (CMHB) (Becton Dickinson, USA)and adjusted to 0.5 McFarland turbidity (10^8 CFU/mL). Then bacterial suspensions were swabbed on cation-adjusted Mueller-Hinton agar (CMHA) (Becton Dickinson, USA). Imipenem (10 µg IMP), meropenem (10 µg MEM), and ertapenem (10 µg ETP) disks were placed on the cultured CMHA plates and incubated at 37 °C for 18-24 hours. Growth inhibition zone diameters were evaluated according to the CLSI criteria [9]. *Klebsiella spp.* strains that demonstrated resistance to all tested carbapenem by the disk diffusion method were selected for further tests.

Determination of MICs for carbapenems

MICs of carbapenems were determined for *Klebsiella spp*. strains by the broth microdilution method. In brief, a two-fold dilution of each carbapenem (Glentham Life Science, United Kingdom) at concentrations ranging from 06–0.25 µg/mL, 0.008–0.06 µg/mL, 0.004–0.016 µg/mL, and 0.016–0.06 µg/mL for imipenem (IMP), meropenem (MEM), ertapenem (ETP), and doripenem (DOR), respectively, was prepared in CMHB(Becton Dickinson, USA). Bacterial suspensions were prepared as mentioned above but were diluted tenfold with sterile saline (0.85% NaCl). Then bacterial suspensions were added to each well, and the final bacterial count in each well

Table 1. The relationship between imipenem susceptibility and carbapenemases of isolates ($p > 0.05$).

Susceptibility of *Klebsiella spp.* strains to carbapenems (n)	**Type of carbapenem resistance gene (n)**				***Klebsiella spp.* strains not exist carbapenem resistance gene (n)**
	OXA-48	**NDM**	**OXA-48 + NDM**	**VIM**	
Resistance (n = 49)	8	21	18	1	1
Susceptible/intermediate resistance (n = 1)	0	0	0	0	1
Total (n = 50)	8	21	18	1	2

*$p = 0.06$.

Table 2. The relationship between meropenem susceptibility and carbapenemases of isolates ($p > 0.05$).

Susceptibility of *Klebsiella spp.* strains to carbapenems (n)	**Type of carbapenem resistance gene (n)**				***Klebsiella spp.* strains not exist carbapenem resistance gene (n)**
	OXA-48	**NDM**	**OXA-48 + NDM**	**VIM**	
Resistance (n = 47)	8	19	18	1	1
Susceptible/intermediate resistance (n = 3)	0	2	0	0	1
Total (n = 50)	8	21	18	1	2

*$p = 0.137$.

Table 3. The relationship between ertapenem susceptibility and carbapenemases of isolates ($p > 0.05$).

Susceptibility of *Klebsiella spp.* strains to carbapenems (n)	**Type of carbapenem resistance gene (n)**				***Klebsiella spp.* strains not exist carbapenem resistance gene (n)**
	OXA-48	**NDM**	**OXA-48 + NDM**	**VIM**	
Resistance (n = 49)	8	20	18	1	2
Susceptible/intermediate resistance (n = 1)	0	1	0	0	0
Total (n = 50)	8	21	18	1	2

*$p = 1.0$.

Table 4. The relationship between doripenem susceptibility and carbapenemases of isolates ($p > 0.05$).

Susceptibility of *Klebsiella spp.* strains to carbapenems (n)	**Type of carbapenem resistance gene (n)**				***Klebsiella spp.* strains not exist carbapenem resistance gene (n)**
	OXA-48	**NDM**	**OXA-48 + NDM**	**VIM**	
Resistance (n = 47)	8	19	18	1	1
Susceptible/intermediate resistance (n = 3)	0	2	0	0	1
Total (n = 50)	8	21	18	1	2

*$p = 0.137$.

was 10^4-10^5 CFU. Negative control wells contained only antibiotics, and positive control wells contained bacterial suspensions. The *Escherichia coli* ATCC 25922 strain was used to ensure the accuracy of MIC values of each carbapenem. MICs were evaluated after 24 h according to the CLSI criteria [9].

Detection of the 'major five' carbapenem resistance genes

The Xpert Carba-R (GeneXpert system, USA) system was used for the multiplex real-time PCR method. This system is a qualitative, on-demand real-time PCR test for detecting and differentiating the KPC, NDM, VIM, OXA-48, and IMP genes directly from rectal swabs in 48 minutes. We used 10 µL from bacterial suspensions adjusted to equal 0.5 McFarland turbidity (108 CFU/mL) according to the manufacturer's advice.

Statistical analysis

The IBM SPSS Statistics 20 program was used in the statistical analysis, and the relationship between the variables was examined by Fisher's exact test. A value of $p < 0.05$ was considered statistically significant.

Results

API20E revealed that 44 strains were *K. pneumoniae* and six strains were *Klebsiella oxytoca.* The disk diffusion method demonstrated that all 50 *Klebsiella spp.* strains were resistant to three tested carbapenems (IMP, MEM, and ETP).

MICs

According to MIC results, 94% (47/50) of the isolates were resistant to DOR and MEM, and 98% (49/50) were resistant to ETP and IMP. The MICs of one *K. pneumoniae* strain (strain no. 29) showed intermediate resistance to MEM and DOR (2 µg/mL). Furthermore, the MICs of one *K. oxytoca* strain (strain no. 37) showed intermediate resistance to IMP and MEM (2 µg/mL). The MICs of one *K. oxytoca* strain (strain no. 50) to DOR, MEM, and ETP were (0.125 µg/mL),(0.5 µg/mL), and (0.062 µg/mL), respectively, and in the susceptible range.

Carbapenem resistance genes

We detected that 48 (96%) out of 50 *Klebsiella spp.* strains contained three major carbapenem genes (OXA-48, NDM, and VIM), and two strains (4%) did not have any genes. The distribution of these genes was as follows; from 44 *K. pneumoniae* strains, 19 had NDM (43.2%), and eight had OXA-48 (18.2%), while six *K. oxytoca* strains carried only two (33.4%) NDM and one (16.7 %) VIM gene. NDM and OXA-48 co-existed in 16 (36.4%) *K. pneumoniae* and two (33.4%) *K. oxytoca* strains. The KPC and IMP genes were not detected in any tested *Klebsiella spp*. strains. No statistical significance was found between the susceptibility to four tested carbapenems and gene analyses of all strains ($p > 0.05$) (Tables 1-4). Table 5 shows carbapenem resistance profiles and gene analyses of all isolates.

Discussion

The recent increase in resistance to carbapenems, which are used as the last option in treating MDR-related infections, has become an important problem that complicates the treatment of hospital-acquired infections.In this study, we aimed to investigate carbapenem resistance genes in 50 *Klebsiella spp.* strains isolated from rectal swabs of patients hospitalized in the NICU. These isolates phenotypically displayed resistance to carbapenem by the disk diffusion method or had high MIC values determined by the broth microdilution method.The major five carbapenem genes (OXA-48, NDM, VIM, KPC, and IMP) were detected by multiplex real-time PCR. Accordingly, among 50 *Klebsiella spp.* strains, carbapenem resistance genes were detected in 48 (96%) of the strains:43 (97.8%) of 44 *K. pneumoniae* and 5 (83.3%) of 6 *K. oxytoca* strains. It was determined that one carbapenem resistance strain from each species did not carry any gene. A total of 66 carbapenem resistance genes were seen in all strains. These genes were detected as a single gene (21 NDM, 8 OXA-48, and 1 VIM) in 30 strains and a double gene (OXA-48 + NDM) in 18 strains. Of the 50 strains, 47 showed resistance to all carbapenems, and three strains (strain no. 29, strain no. 37, and strain no. 50) had different MIC values. *K. pneumoniae* strain no. 29, which contained NDM, showed intermediate resistance (2 µg/ml) to doripenem and meropenem.The MIC values of carbapenem in strains no.17, 37, and 50 were very interesting. Although *K. pneumoniae* strain no. 17 was negative for any tested gene, it was resistant to doripenem (4 µg/mL), meropenem (8 µg/mL), ertapenem (8 ≥ µg/mL), and imipenem (16 ≥ µg/mL). On the other hand, *K. oxytoca* strain no. 50 containing the NDM gene was susceptible to doripenem (MIC 0.125 µg/mL), meropenem (MIC 0.5 µg/mL), and ertapenem (MIC 0.062 µg/mL).

Conversely, *K. oxytoca* strain no. 37, which was tested as negative for the major five carbapenem resistance genes, exhibited intermediate resistance to doripenem (1 µg/mL), meropenem (2 µg/mL),

imipenem (2 μg/mL), and ertapenem (2 μg/mL).We think that strains no. 17 and 37 are more likely to have genes other than the major carbapenem genes that we sought in the present study. No statistically significant correlation was found between the carbapenem MIC values in the isolates and carbapenem resistance genes ($p > 0.05$). OXA-48 was first isolated from the *K. pneumoniae* strain in our hospital in 2003 [5], spread rapidly throughout the country, and caused epidemics in the central cities of the country [10]. A study conducted in Istanbul Medical Faculty between 2012 and 2016 found the OXA-48 carbapenemase gene in 86% (n = 43/50) of carbapenem-resistant *K. pneumoniae* strains and detected the NDM carbapenemase gene in 14% (n = 7/50) [11]. The *K. pneumoniae* strain producing NDM carbapenemase in Turkey was first identified from the patient's blood culture in 2011 [12]. It was the second most isolated carbapenemase gene type in Turkey after OXA-48 [13-16]. In our study, while the NDM gene was single in 21 strains and co-existed with OXA-48 in 18 strains in a total of 39 NDM (78%)-carrying strains, OXA-48

Table 5. Carbapenem resistance profiles and gene analyses of Klebsiella spp.strains.

No	Klebsiella spp.	Doripenem MIC (μg/mL)	Meropenem MIC (μg/mL)	Ertapenem MIC(μg/mL)	Imipenem MIC (μg/mL)	Type of gene
1	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
2	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
3	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
4	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
5	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
6	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
7	*K. oxytoca*	16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	VIM
8	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
9	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
10	*K. oxytoca*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
11	*K. oxytoca*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
12	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
13	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
14	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
15	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
16	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
17	*K. pneumoniae*	4 (R)	8(R)	≥ 8 (R)	≥ 16 (R)	Negative
18	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
19	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
20	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
21	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
22	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
23	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
24	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
25	*K. pneumoniae*	16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
26	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
27	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
28	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
29	*K. pneumoniae*	2 (I)	2 (I)	8 (R)	≥ 16 (R)	NDM
30	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
31	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
32	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
33	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
34	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
35	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
36	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
37	*K. oxytoca*	1 (I)	2 (I)	2 (R)	2 (I)	Negative
38	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
39	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
40	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
41	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
42	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
43	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
44	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
45	*K. oxytoca*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48 + NDM
46	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
47	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
48	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	OXA-48
49	*K. pneumoniae*	≥ 16 (R)	≥ 8 (R)	≥ 8 (R)	≥ 16 (R)	NDM
50	*K. oxytoca*	0.125 (S)	0.5 (S)	0.062 (S)	8 (R)	NDM

R: resistance; I: intermediate resistance; S: susceptible.

ranked the second and existed alone in 8 strains and co-existed with NDM in 18 strains, in a total of 26 (52%) strains.We found only the VIM gene in *K. oxytoca* strain no. 7 (2%).According to the European criterion for carbapenemase-producing *Enterobacteriaceae* (EuSCAPE), which was established in 2012 by the European Center for Disease Prevention and Control (ECDC), countries were divided into 5 phases in terms of the spread of cases: Phase 0 represents zero cases, phase 1 is sporadic, phase 2 is a hospital epidemic, phase 3 is regional spread, phase 4 is interregional spread, and finally, phase 5 is endemic. According to this research, Turkey is one of the phase 5 countries within the scope of OXA-48 producing *K. pneumoniae*. Concerning the NDM gene, while Turkey was among phase 1 countries in 2013, it was included in the class of phase 3 countries in 2015.

In our study, the presence of 66 carbapenems genes in 48 (96%) of the strains and NDM forming 39 (59.1%) of these genes indicate that the prevalence of NDM in our hospital exceeds that of OXA-48. It is possible to talk about NDM and NDM + OXA-48 epidemics in our hospital. NDM may spread rapidly and reach endemic levels in Turkey. A study conducted in 2015 emphasized that NDM-producing *Enterobacteriaceae* strains had increased especially in regions close to the Syrian border since 2013 [17]. We did not attribute the high NDM prevalence in our study to more than 450 thousand registered Syrian refugees living in Istanbul because this subject was out of our study scope. In another study conducted by Kilic and Baysallar in Turkey in 2015 [18] on 887 *Enterobacteriaceae* members, 5.52% of the isolates were resistant to at least one carbapenem, 48 isolates contained OXA-48, and one *K. pneumoniae* isolate was resistant to at least one carbapenem and carried OXA-48 together with NDM, which was considered the first *K. pneumoniae* strain containing co-existing OXA-48 and NDM genes in Turkey.The isolation of NDM-producing strains in patients of Syrian origin and the treatment of war-wounded patients in border cities such as Şanlurfa made the researchers conclude that Syria could be a source of NDM-producing strains in Turkey. In the world, *K. pneumoniae* co-producing NDM + OXA-48 was first detected in Morocco [19], secondly in Tunisia [20], and later in other countries [21].The number of cases reported in Turkey for KPC carbapenemase, which is the most frequently detected worldwide, is very low. VIM- and IMP-type carbapenemases from metallo-beta-lactamases have also been reported in small numbers. Our study supports this situation by the absence of the KPC and IMP genes in any of the 50 resistant strains and detecting the VIM gene in only one strain.The prevalence of carbapenemase resistance genes in other countries is also high. *K. pneumoniae* strains in the USA constitute 92% of all carbapenem-resistant *Enterobacteriaceae* [22]. A study conducted in Thailand revealed that 73% (n = 2888) of 3946 carbapenem-resistant *Enterobacteriaceae* strains collected within the scope of antimicrobial resistance surveillance between 2016-2018 were *K. pneumoniae* strains and 97% (n = 3844) of them had at least one carbapenemase-producing gene [23]. According to a study published in Saudi Arabia in 2018, OXA-48 was the most common carbapenemase (n = 48/71) among carbapenem-resistant *K. pneumoniae* strains, followed by NDM-1 (n = 9/71), and the number of strains containing both carbapenemases was six [24]. Although Greece is the epicenter for the spread of VIM-producing *Enterobacteriaceae* (especially *K. pneumoniae*) to other countries [25,26], *K. pneumoniae* strains producing KPC have also become endemic in this country [27]. In Iran, OXA-48 was detected in 72% of the 100 carbapenem-resistant *K. pneumoniae* strains and NDM in 31%. The KPC and VIM genes were not found in any isolate [28]. Although the high prevalence of OXA-48 has been reported in Turkey and the Middle East, our study indicates that NDM may become endemic in Turkey after OXA-48. Meanwhile, the importance of the presence of carbapenem resistance genes in *Klebsiella spp*. not solely restricted to carbapenems but these strains are candidates to harbour other antibiotic resistance genes.

To control and prevent spread of carbapenem resistance *Klebsiella spp.* in our hospital we highly recommended applying the Centers for Disease Control and Prevention (CDC) guidelines [29]. These can be summarized in three points; 1. Hand hygiene of healthcare personnel that may transmit pathogens after touching an infected or colonized body site on one patient or a contaminated inanimate object, 2. Instruments (for example, rectal temperature) that are inadequately cleaned between patients before disinfection or sterilization, and uncleaned laboratory coats, or isolation gowns used as personal protective equipment (PPE), may become contaminated with potential pathogens aftercare of a patient colonized or infected with an infectious agent.

Conclusions

The increased prevalence of NDM and NDM + OXA-48-producing *K. pneumoniae s*trains in our NICU is worrisome. Maintaining hand hygiene and isolation of infected or colonized patients can effectively prevent

the spread of carbapenem-resistant *Klebsiella spp.* isolates.

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Authors' contributions

MSc .Molecular biologist Hatice Kubra Demir (Demir HK) is student who performed the thesis work. Dr.Yasar Nakipoglu (Nakipoglu Y) is thesis supervisor, and writing the manuscript.

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