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RESEARCH ARTICLE



Prevalence of carbapenemases in Enterobacterales from urine specimens in an university hospital in Istanbul, Turkey

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ABSTRACT

We aimed to investigate the prevalence of carbapenemases in *Enterobacterales* strains isolated from urine specimens between July 2019 and July 2020.

CIM and modified CIM tests were applied as well as detection of *bla*_{OXA-48}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{KPC} and *bla*_{IMP} genes was performed by multiplex PCR.

One hundred fifty of 3,242 *Enterobacterales* strains were found to be carbapenem resistant and 46 were included in the study. Forty five (98%) of the 46 strains included in the study were *Klebsiella* spp. and one (2%) of them was *Escherichia coli*. Susceptibility to ceftazidime-avibactam, amikacin and gentamicin was 97%, 11% and 9%, respectively. Forty three (94%) isolates were found positive at 2 and 4 h with CIM test. Forty four (97%) strains were found positive at 4 h and 43 (94%) strains were found positive at 2 h with modified CIM test.

While *bla*_{OXA-48}, *bla*_{NDM} and *bla*_{OXA-48} with *bla*_{NDM} association were found in *Klebsiella* spp. isolates in 55%, 27% and 11%, respectively, *bla*_{VIM}, *bla*_{KPC}, *bla*_{IMP} were not found. Only *bla*_{OXA-48} and *bla*_{NDM-1} were detected in the *E. coli* strain.

None of the investigated genes were detected in three *Klebsiella* strains but with whole genome analysis the combination of *bla*_{OXA-534}, *bla*_{CMY-99} and *bla*_{KPC-3} was found in the first strain, *bla*_{OXA-370} in the second strain and no resistance gene was found in the third strain.

Ceftazidime-avibactam was found to be active against 97% of strains, and the most common resistance genes were *bla*_{OXA-48} and *bla*_{NDM-1}. Previously undetected resistance genes have been identified in our country.

KEYWORDS

urinary tract infection, *Enterobacterales*, carbapenem resistance, CIM test, PCR

INTRODUCTION

Urinary tract infections (UTIs) are common bacterial infections. UTIs are seen as different clinical diseases ranging from asymptomatic bacteriuria to urosepsis. They have an important role among community-acquired infections. Microorganisms that cause UTIs often originate from the person's own flora. Although *Escherichia coli* is most commonly isolated, members of the *Enterobacterales* order such as *Klebsiella* spp., *Proteus* spp. and *Enterobacter* spp. also appear to be causative agents. The *Enterobacterales* order is a large, heterogeneous

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community of Gram-negative rods with a large number of genera, species and subspecies. These bacteria can also cause bacteremia and sepsis, pneumonia, meningitis, osteomyelitis, typhoid fever, surgical site infections and gastrointestinal infections [1, 2].

Antibiotic resistance rates are increasing in bacteria that cause UTIs like in all other infections. In the last two decades, broad-spectrum antibiotics have been largely used to overcome the difficulty of treating urinary tract infections caused by Gram-negative rods [3, 4]. Carbapenems are β -lactam antibiotics that are preferred in the first place in multi-drug-resistant Gram-negative bacterial infections, as they have a broad spectrum of action, can pass through bacterial membranes quickly and are also resistant to enzymes such as AmpC and extended-spectrum beta-lactamases (ESBL). However, the frequent use of these antibiotics in empirical therapy has led to increased resistance to carbapenems [5].

Mechanisms of resistance to carbapenems are β -lactamase enzyme production, efflux pumps, loss of porin proteins and mutations that cause PBP modifications. The coexistence of some of these resistance mechanisms in bacteria can lead to high-level carbapenem resistance [6].

Carbapenemases identified prior to 1990 are β -lactamases that hydrolyze carbapenems to confer carbapenem resistance. They can be chromosomal or plasmid coded resistance determinants [7]. According to the Ambler classification system they are classified in class A carbapenemases (Guiana extended spectrum, GES; *Klebsiella pneumoniae* carbapenemase, KPC), class B carbapenemases or metallo-beta-lactamases (imipenemase, IMP; Verona integron metallo- β -lactamase, VIM; New Delhi metallo-beta-lactamase, NDM) and class D carbapenemase enzymes (oxacillinases) [8]. Carbapenemase production should be suspected in strains with an increase in carbapenem MIC values or a narrowing of the inhibition zone diameters. Then phenotypic methods such as Carbapenem Inactivation Method, Modified Hodge Test, inhibitor-based methods, biochemical methods and genotypic methods such as PCR, cloning and sequencing should be performed [9, 10].

METHODS

Enterobacterales species isolated from urine samples sent to our Medical Microbiology laboratory of Istanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine within a 12-month period between July 2019 and July 2020 were included in our study. Forty six isolates reported as intermediate-susceptible or resistant to a carbapenem (meropenem) antibiotic with the automated system BD Phoenix 100 (Becton Dickinson and Company, USA) or with the Kirby-Bauer disc diffusion method belonging to urine specimens with a colony count of $\geq 10^5$ cfu/mL of patients over 18 years of age were separated and included in the study. Before starting our study, the carbapenem susceptibility of the carbapenem intermediate susceptible/resistant isolates obtained as a result of both methods was reevaluated and confirmed by the disc diffusion

method. The susceptibility results obtained with this method were evaluated according to the EUCAST guidelines and the isolates that met the carbapenemase enzyme screening criteria were included in the study [11, 12]. The phenotypic tests CIM and modified CIM were used to determine carbapenemase synthesis.

In addition, carbapenemase genes encoding *bla*_{OXA-48}, *bla*_{KPC}, *bla*_{IMP}, *bla*_{NDM-1}, *bla*_{VIM} were investigated by high resolution melting curve analysis (HRM) and gel electrophoresis methods, following amplification with multiplex PCR.

Determination of carbapenemase production by phenotypic method

CIM test was applied to carbapenem-resistant (meropenem zone diameter <22 mm) isolates with the Kirby-Bauer disc diffusion method according to EUCAST recommendations. CIM and the modified CIM tests were considered as reference method. However, these methods can be applied in different ways. In our study, we applied CIM in four different ways.

1) The Carbapenem Inactivation Method

A ten μ l loop-filled with the bacterial strain to be tested was suspended in 400 μ l of distilled water in a sterile Eppendorf tube. A meropenem disc (10 μ g) was added into this prepared suspension and incubated at 35–37 °C for 2 h (second step) (Fig. 1). After incubation, a 0.5 McFarland suspension from *E. coli* ATCC 25922 strain in physiological saline was applied on to the surface of a 4 mm thick Mueller Hinton (MH) agar plate. The meropenem disc from the Eppendorf tube was removed and placed on the MH agar plate on which *E. coli* was applied. The plate was evaluated after 18–20 h incubation at 35 °C. The absence of an inhibition zone around the meropenem disc was considered positive for the presence of carbapenemase [13] (Fig. 2). In our study, as a different method from the classical method, instead of 2 h in the second step, meropenem disc (10 μ g) was incubated for 4 h at 35–37 °C and all the other steps were applied in the same way.

2) Modified Carbapenem Inactivation Method

The modified test has two differences from the classical CIM. From these, in the first step, tryptic soy broth is used to prepare the suspension instead of distilled water. The second is that the incubation period was applied as 4 h instead of 2 h in the inactivation step [14]. In addition, as a different method in our research, in this method, instead of 4 h in the second step, meropenem disc (10 μ g) was thrown into the bacterial suspension prepared with tryptic soy broth and incubated for 2 h at 35–37 °C, and other steps were applied in the same way.

Investigation of carbapenemase genes by molecular methods

Multiplex PCR method was used to detect *bla*_{OXA-48}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{KPC}, *bla*_{IMP} genes [15]. The PCR reaction



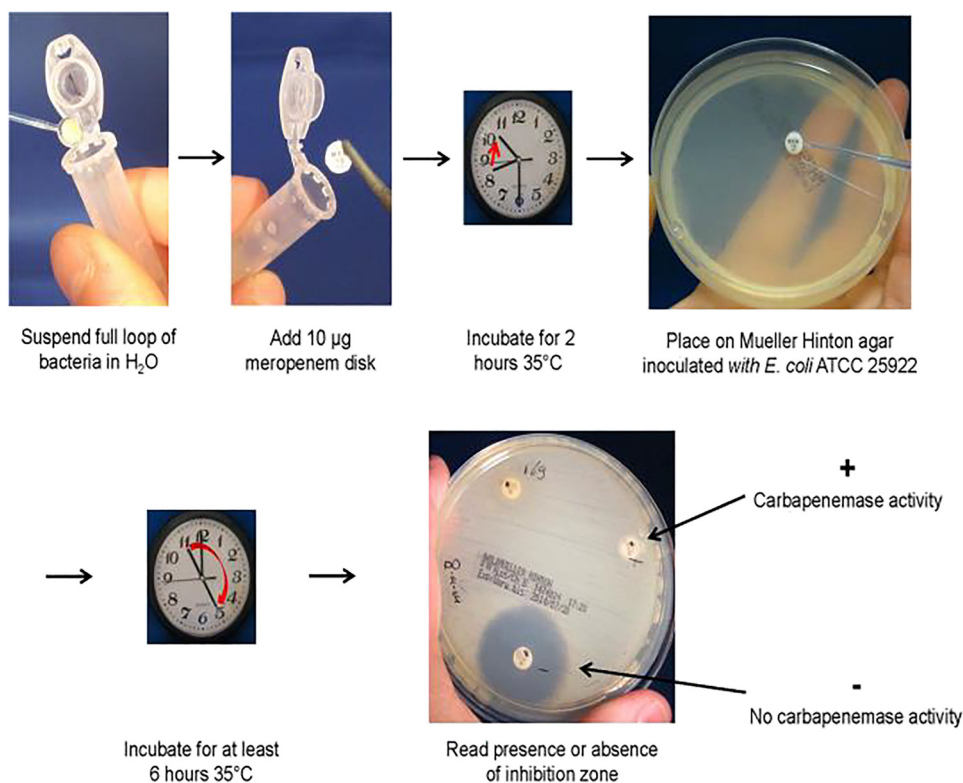


Fig. 1. The Carbapenem inactivation method [13]

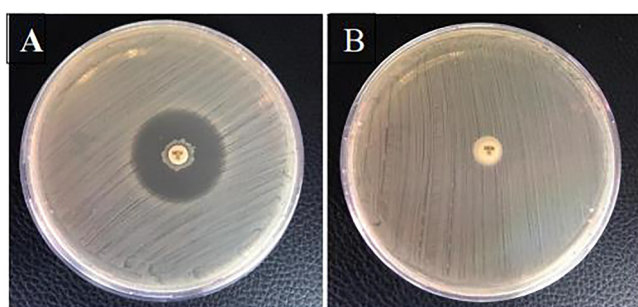


Fig. 2. The CIM test; A. non-carbapenemase-producing strain; CIM negative B. Carbapenemase-producing strain; CIM test positive

was performed on a BioRad CFX 96 (BioRad, Germany) instrument (Table 1).

After performing the polymerase chain reaction, the products were first evaluated using high resolution melting curve analysis (HRM) [16]. After that gel electrophoresis with agarose was performed to determine the band lengths.

In cases where carbapenem resistance could not be explained by the genes examined, the entire genome of the bacterium was extracted and a resistome analysis was performed from this data. Whole genome sequencing was performed by Illumina miniSeq instrument, after DNA extraction and fragmentation by sonication Nextera XT DNA Library Preparation Kit used for library preparation [17].

RESULTS

Forty five (98%) of the 46 strains included in the study were *Klebsiella* spp. and one (2%) of them is *E. coli*. Forty strains (87%) were resistant to meropenem and six (13%) were intermediate-susceptible to meropenem in all strains included in the study. Five strains (11%) were susceptible, 19 (41%) were intermediate-susceptible and 22 (48%) were resistant against imipenem. Forty six (100%) were found as resistant against ertapenem. All strains were found to be resistant against ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefotaxime, ceftazidime, ceftriaxone, cefepim, ciprofloxacin and trimethoprim-sulphamethoxazole. Forty five (97%) strains were susceptible to ceftazidime-avibactam, five (11%) to amikacin and four (9%) to gentamicin. Antibiotic susceptibility results are presented in Table 2.

From 46 carbapenem-resistant isolates included in our study, forty three (94%) isolates were detected as positive when the inactivation time of 2 and 4 h was applied with the CIM test. Forty four (97%) isolates were found to be positive when a 4-h inactivation time was applied and 43 isolates (94%) were found to be positive when a 2-h inactivation period was applied with the modified CIM test.

Comparative data about the phenotypic tests and molecular method for the determination of *Enterobacterales* strains producing carbapenemase enzyme is presented in Table 3.

Table 1. Primers used for beta-lactamase detection [15]

Resistance gene	Primer abbreviation	Primer sequence	T _m * (°C)	Gene bank	Position	Product (bp)	Reference
<i>bla</i> _{OXA-48} **	OXA-48_Fw	GCGTGTATTAGCCTTATCGGC	52	JN626286	5,518–5,537	722	[15]
	OXA-48_Rev	RGGCATATCCATATTCATCGC			6,240–6,220		
<i>bla</i> _{IMP} **	Pan_IMP_Fw	GGAATAGAGTGGCTTAAYTCTC	50	GU207399	372–393	188	[15]
	Pan_IMP_Rev	ARCCAAACYACTASGTTATC			560–543		
<i>bla</i> _{VIM} **	Pan_VIM_Fw	TTCTCGCGGAGATTGARAAGC	54	JN819277	219–239	264	[15]
	Pan_VIM_Rev	TTGTCTGGYGAATGCGCAGC			483–464		
<i>bla</i> _{NDM-1} **	NDM_Fw	GGGCAGTCGCTTCCAACGGT	55	JQ734687	212–231	475	[15]
	NDM_Rev	GTAGTGCTCAGTGTCGGCAT			687–668		
<i>bla</i> _{KPC} **	KPC_Fw	GCTGTCTTGTCTCTCATGGCC	55	JQ867396	394–414	836	[15]
	KPC_Rev	AATCCCTCGAGCGCGAGTCTA			1,230–1,210		
<i>bla</i> _{CTX-M} **	CTXM_Fw	ATCTGACGCTGGGTAAAGC	50	JQ686201	695–713	162	[15]
	CTXM_Fw	ATATCGTTGGTGGTGCCATA			857–838		

*T_m, Temperature.

**OXA-48, Oxacillinase-48; IMP, Imipenemase; VIM, Verona integron-encoded metallo-beta-lactamase; NDM-1, New Delhi metallo-beta-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; CTX-M, Cefotaxime-hydrolyzing beta-lactamase.

Table 2. Antibiotic susceptibility results

Antibiotic	Susceptible n (%)	Intermediate susceptible n (%)	Resistant n (%)
Ertapenem	-	-	46 (%100)
Meropenem	-	6 (%13)	40 (%87)
Imipenem	5 (%11)	19 (%41)	22 (%48)
Ampicillin	-	-	46 (%100)
Amoxicillin-Clavulanic Acid	-	-	46 (%100)
Piperacillin-Tazobactam	-	-	46 (%100)
Cefuroxime	-	-	46 (%100)
Ceftazidime	-	-	46 (%100)
Cefotaxime	-	-	46 (%100)
Ceftriaxone	-	-	46 (%100)
Cefepim	-	-	46 (%100)
Ciprofloxacin	-	-	46 (%100)
Trimethoprim-Sulphamethoxazole	-	-	46 (%100)
Amikacin	5 (%11)	1 (%2)	40 (%87)
Gentamicin	4 (%9)	2 (%4)	40 (%87)
Ceftazidime-Avibactam	45 (%97)	-	1 (%3)

When CIM results were evaluated, 43 of 43 isolates found positive for carbapenemase gene by PCR had positive results with CIM (2 and 4 h) and/or modified CIM (2 and 4 h).

While *bla*_{OXA-48}, *bla*_{NDM} and *bla*_{OXA-48} with *bla*_{NDM} association were found in *Klebsiella* spp. isolates in 55%, 27% and 11%, respectively, *bla*_{VIM}, *bla*_{KPC}, *bla*_{IMP} were not found. Only *bla*_{OXA-48} and *bla*_{NDM-1} were detected in the *E. coli* strain.

None of the investigated genes were detected in three *Klebsiella* strains however, whole genome analysis detected the combination of *bla*_{OXA-534}, *bla*_{CMY-99} and *bla*_{KPC-3} in the first strain, *bla*_{OXA-370} in the second strain and no resistance

Table 3. Comparison of phenotypic tests and molecular method for the determination of *Enterobacterales* strains producing carbapenemase enzymes

	Phenotypic test				Molecular test
	CIM test		Modified CIM test		
	2 h	4 h	2 h	4 h	PCR test
Positive	43	43	43	44	43
Negative	3	3	3	2	3
Total	46	46	46	46	46

gene was found in the third strain. Resistance genes of carbapenemase-producing *Enterobacterales* strains are listed in Table 4.

DISCUSSION

In the last two decades, broad-spectrum antibiotics have been used to overcome the difficulty in the treatment of urinary tract infections caused by Gram-negative rods. However, these microorganisms have developed multiple antimicrobial resistance mechanisms, such as changes in drug target, increased drug efflux and plasmid-mediated β-lactamases [3, 4]. Carbapenem group antibiotics are often preferred as the first choice antibiotics that are effective against ESBL-producing bacteria. However, with the increase over the years in infections caused by ESBL producing *Enterobacterales* strains, an increase in carbapenem resistance has been observed with the increase in the frequency of use of carbapenem group antibiotics [18–20].

According to Centers for Disease Control and Prevention (CDC) and National Healthcare Safety Network (NHSN) data, the rate of carbapenem-resistant *Enterobacterales* increased from 1.2% to 4.2%, the rate of



Table 4. Resistance genes of carbapenemase-producing *Enterobacterales* strains

No	Bacteria	Sequence	IMP	VIM	NDM-1	KPC	OXA-48	CTX-M
1	<i>Klebsiella</i> spp.		—	—	—	—	+	—
2	<i>Klebsiella</i> spp.	*	—	—	—	—	—	—
3	<i>Klebsiella</i> spp.		—	—	—	—	+	+
4	<i>Klebsiella</i> spp.	**	—	—	—	—	—	—
5	<i>Klebsiella</i> spp.		—	—	—	—	+	+
6	<i>Klebsiella</i> spp.		—	—	+	—	—	+
7	<i>Klebsiella</i> spp.		—	—	+	—	—	+
8	<i>Klebsiella</i> spp.		—	—	+	—	—	—
9	<i>Klebsiella</i> spp.		—	—	—	—	+	—
10	<i>Klebsiella</i> spp.		—	—	+	—	—	+
11	<i>Klebsiella</i> spp.		—	—	—	—	+	+
12	<i>E. coli</i>		—	—	+	—	+	+
13	<i>Klebsiella</i> spp.		—	—	+	—	+	+
14	<i>Klebsiella</i> spp.		—	—	—	—	+	—
15	<i>Klebsiella</i> spp.		—	—	+	—	—	—
16	<i>Klebsiella</i> spp.		—	—	—	—	+	+
17	<i>Klebsiella</i> spp.	***	—	—	—	—	—	+
18	<i>Klebsiella</i> spp.		—	—	+	—	+	+
19	<i>Klebsiella</i> spp.		—	—	—	—	+	+
20	<i>Klebsiella</i> spp.		—	—	—	—	+	+
21	<i>Klebsiella</i> spp.		—	—	—	—	+	—
22	<i>Klebsiella</i> spp.		—	—	—	—	+	—
23	<i>Klebsiella</i> spp.		—	—	+	—	+	+
24	<i>Klebsiella</i> spp.		—	—	—	—	+	+
25	<i>Klebsiella</i> spp.		—	—	—	—	+	+
26	<i>Klebsiella</i> spp.		—	—	+	—	—	+
27	<i>Klebsiella</i> spp.		—	—	—	—	+	+
28	<i>Klebsiella</i> spp.		—	—	—	—	+	+
29	<i>Klebsiella</i> spp.		—	—	—	—	+	+
30	<i>Klebsiella</i> spp.		—	—	+	—	—	+
31	<i>Klebsiella</i> spp.		—	—	—	—	+	+
32	<i>Klebsiella</i> spp.		—	—	—	—	+	+
33	<i>Klebsiella</i> spp.		—	—	—	—	+	+
34	<i>Klebsiella</i> spp.		—	—	—	—	+	+
35	<i>Klebsiella</i> spp.		—	—	—	—	+	+
36	<i>Klebsiella</i> spp.		—	—	+	—	+	+
37	<i>Klebsiella</i> spp.		—	—	+	—	—	+
38	<i>Klebsiella</i> spp.		—	—	+	—	+	+
39	<i>Klebsiella</i> spp.		—	—	—	—	+	—
40	<i>Klebsiella</i> spp.		—	—	+	—	—	+
41	<i>Klebsiella</i> spp.		—	—	+	—	—	+
42	<i>Klebsiella</i> spp.		—	—	—	—	+	+
43	<i>Klebsiella</i> spp.		—	—	—	—	+	—
44	<i>Klebsiella</i> spp.		—	—	—	—	+	+
45	<i>Klebsiella</i> spp.		—	—	+	—	—	+
46	<i>Klebsiella</i> spp.		—	—	+	—	—	+

*: *bla*_{OXA-534}, *bla*_{CMY-99}, *bla*_{KPC-3} resistance genes found.**: *bla*_{OXA-370} resistance gene found.

***: No carbapenem resistance gene found.

K. pneumoniae increased from 1.6% to 10.4%, and *Enterobacter* spp. rate increased from 1.4% to 3.6% [21].

According to the European Center for Disease Prevention and Control 2015 data, it has been stated that carbapenem-resistant *Enterobacterales* have become widespread over time. Whereas the prevalence (endemic) or inter-regional spread of these strains was detected in 6 countries in total according to 2013 data, this endemic situation was

detected in thirteen countries in 2015. While NDM enzyme was reported in individual hospital outbreaks in England and Italy in 2013, 51 interregional spreads were detected in seven countries in 2015. Similarly, the OXA-48 enzyme has spread quite rapidly. While it was detected endemic in only one country in 2013, it was shown that it was endemic in two countries with an interregional spread in four countries in 2015 [22].



In the study conducted by Eser et al. [23] between 2005 and 2009, the rate of carbapenem resistance was determined as 11% (23) in 210 *Enterobacterales* strains (153 *E. coli*, 10 *K. oxytoca*, 47 *K. pneumoniae*) obtained from blood culture samples. They also reported resistance against meropenem, imipenem and ertapenem as 5.7% (12), 1.9% (4) and 2.4% (5) respectively. In a study by Aytar et al. [24], in 199 *Enterobacterales* strains (*K. pneumoniae* 79 strains, *E. coli* 120 strains) obtained from blood culture samples, carbapenem resistance was determined only in *K. pneumoniae* with a rate of 6% (11 strains), while in *E. coli* strains, carbapenem resistance was not found. As a result of our study, we determined the rate of carbapenem resistance to be 5% (150) among 3,242 *Enterobacterales* strains obtained urine samples. We included in our study forty six isolates. Eighty seven percent (40 strains) of the 46 isolates were resistant and 13% (6) were intermediate-susceptible to meropenem; 11% (5) were susceptible, 41% (19) were intermediate-susceptible and 48% (22) of them were determined as resistant to imipenem, while 100% (46) of them were resistant to ertapenem. The data we obtained as a result of the study show compatibility with the studies conducted in our country in recent years. In our study, CIM was used for the phenotypic detection of carbapenem-resistant *Enterobacterales* and this method was first described by Van der Zwaluw et al. [13]. As a result of the research, the specificity and sensitivity of the test was determined as 100%. The CIM test was applied with various modifications by different researchers in the periods after the work of Van der Zwaluw et al. and different results were obtained. In the study of Tijet et al. [25], the specificity of the CIM test was reported as 100% and the sensitivity as 98–100%. In the study of Aguirre Quiñonero et al. [26], the specificity of the test was reported as 95.7% and the sensitivity as 85.7%. In a study by Yamada et al. [27], the specificity of the test was reported as 95.7% and the sensitivity as 97%.

In various studies conducted in Turkey, the CIM test was preferred to determine carbapenem resistance and different results were obtained. In a study by Aktaş et al. [28], the specificity and the sensitivity was 100%. In a study by Bayramoğlu et al. [29], the specificity and the sensitivity of the test was also 100%. In a study by Yıldız et al. [30], the specificity of the test was 100% and the sensitivity was 97.59%. In our study, we preferred to leave all isolates for 2 and 4 h for disc incubation. Forty six carbapenem-resistant isolates were evaluated with the CIM test and 43 isolates (94%) were found to be positive when the inactivation time of 2 and 4 h was applied. When evaluated with the modified CIM test, 44 isolates (97%) were found to be positive when a 4-h inactivation time was applied, and 43 isolates (94%) were found to be positive when a 2-h inactivation period was applied. All forty tree isolates that were found to be positive for the carbapenemase gene by PCR gave positive results with CIM (2 and 4 h) and/or modified CIM (2 and 4 h). Our modified CIM (4-h inactivation period) study yielded higher positive results than the CIM (2 and 4-h) and modified CIM (2-h) studies. The results of the CIM (2 and 4-h) and modified CIM (2-h) studies were also equivalent.

Many molecular studies have been carried out in our country to determine carbapenemase resistance genes. In a study by Genç et al. [31], *bla*_{OXA-48} was reported in 92.14% (129), *bla*_{NDM-1} in 7.14% (10) and *bla*_{VIM} in 0.71% (1) of 140 carbapenem-resistant *Enterobacterales* strains. Bayramoğlu et al. [32] reported in 65 carbapenem-resistant *Enterobacterales* strains, *bla*_{OXA-48}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM-1}, *bla*_{KPC-2} and *bla*_{OXA-48} + *bla*_{VIM} in 66.2% (43), 15.4% (10), 13.9% (9), 1.51% (1), 1.5% (1) and 1.5% (1), respectively. In a study by Baran et al. [8], *bla*_{OXA-48} was reported as 47.51% (86), *bla*_{NDM-1} as 3.31% (6) and *bla*_{VIM} as 0.55% (1) in 181 carbapenem-resistant *Enterobacterales* strains. In a study by Poirel et al. [33], *bla*_{NDM-1} was found in 54.5% (12), *bla*_{KPC-2} in 9.09% (2) and *bla*_{OXA-48} in 36.36% (8), among 22 carbapenem-resistant *Enterobacterales* strains. In our study, carbapenemase resistance genes such as *bla*_{IMP}, *bla*_{KPC} and *bla*_{VIM}, which are very rare in our country, were not detected. While *bla*_{OXA-48} was detected per total of strains in 54% (25), *bla*_{NDM} in 26% (12), *bla*_{OXA-48} and *bla*_{NDM} association was detected in 13% (6), no investigated gene region was detected in 7% (3). The rate of *bla*_{OXA-48} of 54% (25) that we found in our study and the rates reported in these studies from our country confirm that *bla*_{OXA-48} resistance is endemic in our country [10]. In the whole genome analysis of three *Klebsiella* strains, for which no investigated gene region was detected, *bla*_{OXA-534}, *bla*_{CMY-99} and *bla*_{KPC-3} were found in the first strain, *bla*_{OXA-370} was found in the second strain, and in the third strain no carbapenem resistance gene was found. Previously undetected resistance genes in our country like *bla*_{KPC-3} and *bla*_{CMY-99}, were detected for the first time in our study.

As in every study, there are some limitations in our research. The first of these limitations is that since only one-year-old strains were included in the study, the presence and frequency of carbapenem resistance could not be evaluated by years. Secondly, the absence of a control group due to the fact that *Enterobacterales* strains without carbapenem resistance were not included in the study constituted a limitation in the evaluation of the specificity and sensitivity of the phenotypic method we applied.

In conclusion, the phenotypic and genotypic diversity in enzymes responsible for carbapenem resistance poses some difficulties in the identification of enzymes. The tests to be preferred in routine applications should be easy, cheap and accessible. Although phenotypic tests have this feature, their results may not be 100% reliable. Determination of carbapenem resistance by phenotypic methods creates a need for a working algorithm that starts with phenotypic methods and progresses to genotypic methods. As more information about resistance mechanisms is acquired, more up-to-date phenotypic methods suitable for these mechanisms, with high sensitivity and specificity, and which can be used in routine applications by many laboratories, are being developed. Although these methods are standardized and preferred in routine applications, genotypic methods still maintain their importance as they are the gold standard methods for detecting resistance. We also applied an algorithm covering phenotype and genotypic methods in our



study, and as a result, we detected the presence of *bla*_{OXA-48} type and *bla*_{NDM} carbapenemase genes as the most frequently in carbapenemase producing *Enterobacterales* strains and as an important resistance problem in our hospital. KPC carbapenemase genes have been reported recently in our country, and as a result of our study, *bla*_{KPC-3} carbapenemase type was detected for the first time in a *Enterobacterales* strain. We detected also for the first time in our country *bla*_{OXA-534} and *bla*_{CMY-99}.

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