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Original Article

Investigation of NDM, VIM, KPC and OXA-48 genes, blue-carba and CIM in carbapenem resistant *Enterobacterales* isolates

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Abstract

Introduction: Carbapenem resistance is an emerging problem in *Enterobactarales*. We aimed to investigate the presence of carbapenemase genes bla_{NDM}, bla_{KPC}, bla_{VIM} and bla_{OXA-48} and evaluate the phenotypic blue-carba method and carbapenem inactivation method (CIM) in *Enterobacterales* isolates.

Methodology: Total of 153 *Enterobacterales* isolates were tested in the study. Presence of bla_{NDM}, bla_{KPC}, bla_{VIM} and bla_{OXA-48} genes was investigated by polymerase chain reaction (PCR) method. Carbapenemase production of the isolates was also tested by blue-carba method and CIM.

Results: The presence of bla_{OXA-48} gene was detected in 110 (71.4%) and bla_{NDM} gene was detected in 2 (1.3%) of the *Enterobacterales* isolates by PCR method. None of the isolates were positive for bla_{KPC} and bla_{VIM} genes. The 121 (78.54%) of the isolates were found to be positive by blue-carba method and CIM. And 105 (68.18%) of the isolates were determined as positive by both PCR, blue-carba and CIM.

Conclusions: In our study, 112 (72.7%) of the *Enterobacterales* isolates were found to be positive for carbapenemase genes (bla_{oxa-48} and bla_{NDM}), and 121 (78.57%) of different isolates were found to be positive for blue-carba and CIM. However, 105 (68.18%) of the carbapenem resistance isolates found to be positive for all three methods.

Key words: Carbapenem resistance; *Enterobacterales*; blue-carba; CIM.

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Introduction

Enterobacterales are inhabitants of the intestinal flora and are among the most common human pathogens, causing infections such as cystitis, pyelonephritis, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections [1]. The emergence and transmission of carbapenem-resistant *Enterobacterales* (CRE) over the past two decades has attracted worldwide attention, for its indication that most currently available broad-spectrum antibiotics may no longer be a therapeutic option for some patients [2]. It is therefore mandatory to maintain the clinical efficacy of carbapenems (imipenem, ertapenem, meropenem, doripenem), which have become antimicrobial drugs of last resort [1].

Multidrug-resistant organisms are a major public health concern worldwide; of particular concern has been the emergence of resistance to carbapenem antimicrobial drugs among *Enterobacterales* [3]. Therefore, preventing transmission of carbapenemase-producing, carbapenem-resistant *Enterobacterales* is a public health priority [4].

Carbapenem resistance in *Enterobacterales* is often associated with presence of carbapenemases located on plasmids or other mobile genetic structures [5]. Carbapenem resistance due to the production of acquired carbapenemase genes is increasingly reported among members of *Enterobacterales*, these mechanisms are still less commonly observed in *Enterobacter* spp. than in *Klebsiella* spp. or *Escherichia coli* [6]. The other carbapenem resistance mechanisms for *Enterobacterales* species are combination of presence of extended-spectrum β-lactamases (ESBLs), increased efflux, porin alteration, and a strongly expressed (derepressed) endogenous AmpC enzyme [7].

Carbapenemases have been slow to emerge in the *Enterobacterales*, but now their prevelance is increasing. They are notable for their diversity, including enzymes belonging to molecular classes such as A [KPC (*Klebsiella pneumoniae* carbapenemase), SME and IMI types)], B [IMP (imipenemase), NDM (New Delhi metallo-β-lactamase) and VIM (Verona integron–encoded metallo-β-lactamase)] and D [OXA-48 (oxacillinase)] [2].

Many phenotypic methods for detection of carbapenemase-producers have been described for the *Enterobacterales* [8]. Phenotypic methods defined by the Clinical and Laboratory Standards Institute (CLSI) that clinical microbiology laboratories can apply to detect carbapenemase producers include the Modified Hodge Test (to be removed from the M100 in 2018; CLSI, January 2017 meeting minutes), the Carba NP and most recently the modified Carbapenem Inactivation Method (mCIM) [9]. The blue-carba, has the same principle as the CarbaNP but uses bromothymol blue as a pH indicator and does not need the enzymatic extraction step [10]. The CIM utilizes readily available reagents not requiring reagent preparation and results are more objective in nature as a zone diameter reading is used for interpretation of results [9]. The CIM concept has the potential to also be applied to assess enzymatic hydrolysis of other antibiotics, e.g. allowing detection of ESBL activity. Preliminary experiments in laboratory have shown this maybe feasible [11].

In our study, we aimed to investigate the presence of bla_{NDM}, bla_{VIM}, bla_{KPC} and bla_{OXA-48} genes, which are responsible for the development of resistance to carbapenem antibiotics in *Enterobacterales* isolates, and to evaluate the phenotypic carbapenemase detection methods blue-carba and CIM.

Methodology

Carbapenem resistant 153 *Enterobacterales* isolates that were isolated from various clinical samples sent to the Medical Microbiology Laboratory of Ondokuz Mayis University Faculty of Medicine were included in the study (Table 1). Identification of the isolates was done using conventional methods and Vitek-MS (Biomeirux, France) automated system. The antibiotic susceptibility was tested with the Vitek2 Compact (Biomeirux, France) automated system. Susceptibility of the isolates were evaluated according to the EUCAST criteria. For quality control, reference strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used. Isolates that were resistant to one of the carbapenems (imipenem, meropenem or ertapenem) were considered as carbapenem resistance and enrolled in the study. *Enterobacterales* isolates were stored at -20°C until the molecular study. The existence of bla_{NDM}, bla_{VIM}, bla_{KPC} and bla_{OXA-48} genes were studied by polymerase chain reaction (PCR) using specific primers (Table 2) [12,13]. DNA extraction were made by the method of boiling. Positive strains for bla_{NDM}, bla_{VIM}, bla_{KPC} and bla_{OXA-48} were as positive control and *E. coli* ATCC 25922 was used as negative control in the PCR assays. Positive strains for bla_{NDM}, bla_{VIM}, bla_{KPC} and bla_{OXA-48} were obtained as a part of national quality control program. bla_{NDM} and selected bla_{OXA-48} positive isolates were sequenced in Sentegen (Turkey), they confirmed positive for bla_{NDM} and bla_{OXA-48}.

Table 1. Distribution of *Enterobacterales spp* in our study.

Microorganisms	No (%)
Klebsiella pneumoniae	120
Escherichia coli	14
Klebsiella oxytoca	10
Enterobacter cloacae	4
Enterobacter aerogenes	2
Proteus mirabilis	2
Proteus vulgaris	1
Providencia rettgeri	1

Bromothymol blue was selected as the indicator in the blue-carba test. A commercially available imipenem (Tienam500; Merck Sharp & Dohme, France) was used as the substrate for carbapenemases. The test solution consisted of an aqueous solution of bromothymol blue at 0.04% (Merck Millipore, Germany) adjusted to pH 6.0, 0.1 mmol/liter $ZnSO_4$, and 3 mg/mL of imipenem, with a final pH of 7.0. A negative-control solution (0.04% bromothymol blue solution, pH 7.0) was prepared. A loop (approximately 5 µl) of bacteria was directly suspended in 100 µl of both test and negative-control solutions in a plate and incubated at 37°C for 2 hours. Carbapenemase activity was revealed when the test and negative-control solutions, respectively, were (i) yellow versus blue, (ii) yellow versus green, or (iii)

Table 2. Sequences of the primers.

Gene	Primer	Sequence	Expected amplicon size (bp)	Reference
NDM	NDM-F	GCA GCT TGT CGG CCA TGC GGG C	782	[13]
	NDM-R	GGT CGC GAA GCT GAG CAC CGC AT		
VIM	VIM-F	GTT TGG TCG CAT ATC GCA AC	389	[13]
	VIM-R	AAT GCG CAG CAC CAG GAT AG		
KPC	KPC-F	TGTCACTGTATCGCCGTC	900	[13]
	KPC-R	CTCAGTGCTCTACAGAAAACC		
OXA-48	OXA-48-F	TTG GTG GCA TCG ATT ATC GG	438	[14]
	OXA-48-R	GAG CAC TTC TTT TGT GAT GGC		

green versus blue. Non-carbapenemase producers remained blue or green on both solutions. The test was performed in duplicate for all isolates [10].

In carbapenem inactivation method (CIM), a suspension was made by suspending a full 10 µL inoculation loop of culture, taken from blood agar plate in 400 µL water. Subsequently, a susceptibility-testing disk containing 10 µg meropenem was immersed in the suspension and incubated for a minimum of two hours at 35°C. After incubation, the disk was removed from the suspension, placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* ATCC 29522 and subsequently incubated at 35°C. The inhibition zone around the disc was evaluated after 6 or 24 hours. If the tested bacterial isolate produced carbapenemase, the meropenem in the susceptibility disk was inactivated allowing uninhibited growth of the susceptible E. coli ATCC 29522. Disks incubated in suspensions that do not contain carbapenemases yielded a clear inhibition zone. [11].

Results

Total of 153 carbepenem resistant isolates were tested in the study. These isolates were isolated from various clinical specimens. Majority of the clinical specimens were urine specimens (n = 75, 49%), and followed by blood specimen (n = 35, 22.9%), tracheal aspirate (n = 16, 10.4%), wound specimen (n = 16, 10.4%), sterile body fluid (n = 6, 3.9%), sputum (n = 5, 3.4%). Ertapenem resistance were determined at 150 of the isolates. And 103 of the isolates and 87 of the isolates were found to be resistant and/or intermediate to imipenem and meropenem, respectively. MIC distrubution of the imipenem resistant isolates were 0.25 ≥ 32 µg/mL and 1 ≥ 32µg/mL.

Figure 1. PCR gel image with NDM and OXA-48 positive strains (1-2; NDM positive strain, 3; OXA-48 positive strain, M; marker (100bp plus)).

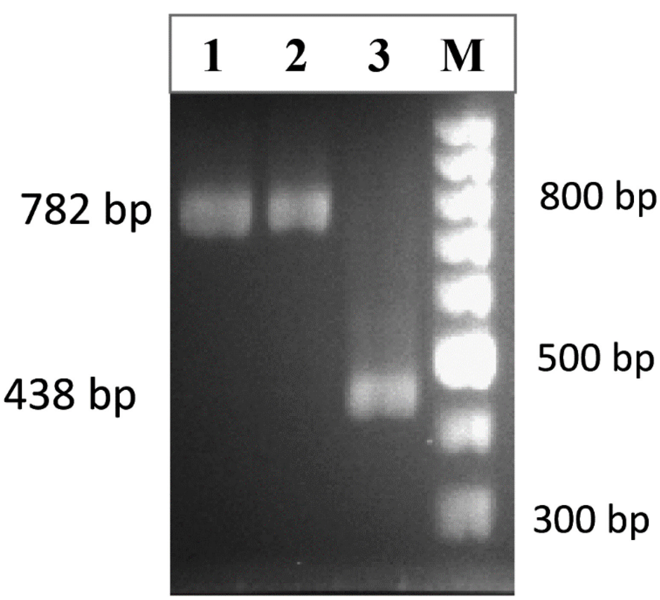

Table 3. Clinical ward distribution of OXA-48 and NDM positive Enterobacteriaceae isolates.

Carbapenemase gene	Clinical ward
OXA-48 (n = 110)	Internal medicine: 95 (61.68%) Intensive care unit: 16 (10.38%) Surgery: 11 (7.14%)
NDM (n = 2)	Intensive care unit Internal medicine (nephrology)

In this study, 110 (71.9%) isolates were determined positive for *bla*$_{OXA-48}$ and 2 (1.3%) isolates were positive for *bla*$_{NDM}$. *Bla*$_{VIM}$ and KPC genes were not detected (Figure 1). Most of the isolates were sent from internal medicine clinics (77.92%). And majority of the *bla*$_{OXA-48}$ positive isolates were isolated from the specimens that sent form internal medicine clinics (61.68%) *bla*$_{NDM}$ positive isolates were isolated the clinical specimens that sent from intensive care unit and internal medicine clinic (Table 3). The sequenced *bla*$_{NDM}$ and selected *bla*$_{OXA-48}$ positive isolates were confirmed positive for *bla*$_{NDM}$ and *bla*$_{OXA-48}$.

For blue-carba test and CIM, 120 isolates were determined positive (78.43%). In our study 99 (64.7%) of the tested isolates were found positive by three methods (PCR, blue-carba and CIM). And 19 (12.4%) isolates were found to be negative by three methods. For the *bla*$_{OXA-48}$ and *bla*$_{NDM}$ positive isolates 101 (90.2%) of them were found to be positive by blue-carba and 111(99.1%) of them were found to be positive by CIM.

But 12 of the PCR positive isolates were determined negative by blue-carba test, and two of the PCR positive isolates were found negative by CIM. Nineteen and nine of the PCR negative isolates were detected positive by blue-carba and CIM, respectively (Table 4). The sensitivity and specitivity of the blue-carba was determined as 89.4% and 52.5%, respectively. The sensitivity and specitivity of CIM was determined as 98.2% and 77.5%.

Discussion

The *Enterobacterales* account for up to 25% of healthcare associated infections (HAIs) reported to the U.S. National Healthcare Safety Network [14]. Carbapenemases increasingly have been reported in *Enterobacterales* in the past 10 years [1]. The emergence and spread of carbapenemase-producing *Enterobacterales* (CPE) is a serious global threat that considerably limits therapeutic options available for life threatening Gram-negative infections [15]. Detection of infected patients and carriers with carbapenemase producers is necessary for prevention of their spread, also may help prevent development of nosocomial outbreaks caused by carbapenemase producers, particularly *K. pneumoniae* [1].

Carbapenem-resistant *Enterobacterales* have been reported worldwide as a result of various kind of carbapenemase genes [16]. The first carbapenemase producer in *Enterobacterales* (NmcA) was identified in 1993 [17]. *Bla*$_{KPC}$ have been reported from worldwide with higher endemicity in USA and Greece. *bla*$_{OXA-48}$ type have been reported mostly from European and Mediterranean countries and India [1].

NDM-1 firstly isolated in 2008 and now it is the focus of worldwide attention [18,19]. Deshpande *et al.* (2010) reported that of the 24 carbapenem resistant *Enterobacterales*, 22 were *bla*$_{NDM}$ producers while 2 were *bla*$_{NDM}$ non-producers. Amongst the 22 *bla*$_{NDM}$ producing organisms 10 were *Klebsiella* spp, 9 were *Escherichia coli*, 2 were *Enterobacter* spp and 1 was *Morganella morganii* [20]. Since mid-August 2010, *bla*$_{NDM}$ producers have been identified on all continents except in Central and South America with, in most of the cases, a direct link with the Indian subcontinent, also recent findings suggest that the Balkan states and the Middle East may act as secondary reservoirs of *bla*$_{NDM}$ producers [19]. Yanık *et al*. (2013) investigated the presence of *bla*$_{NDM}$ by PCR method in 210 carbapenem-resistant Gram-negative isolates (132 *Acinetobacter baumannii*, 54 *Pseudomonas aeruginosa*, 5 *Pseudomonas putida*, 8 *Enterobacter cloacae*, 3 *Enterobacter aerogenes*, 3 *Klebsiella pneumoniae*, 2 *Providencia rettgeri*, 2 *Escherichia coli* and 1 *Citrobacter freundii*) recovered from clinical specimens in Samsun, Turkey. But *bla*$_{NDM}$ gene was not detected in any of the clinical isolates [21]. Poirel *et al*. (2012) report a 16-year-old male patient who was admitted to the hematology unit of a hospital situated near Istanbul, Turkey, in October 2011. That leukemic patient had been transferred from a hospital in Baghdad, Iraq, and received allogeneic hematopoietic stem cell transplantation the day after his admission. Blood cultures grew two types of multidrug-resistant *Enterobacterales* isolates, *K. pneumoniae* and *E. coli*. PCR, sequencing revealed that *K. pneumoniae bla*$_{NDM}$ carbapenemase. This study has also constituted to the very first report of an *bla*$_{NDM}$ positive isolate in Turkey [22].

The first acquired MBL, *bla*$_{IMP}$, was reported in *Serratia marcescens* in Japan in 1991 [23]. IMP types and, more recently, of the New Delhi metallo-β-lactamase-1 (*bla*$_{NDM}$) type. Endemicity of VIM- and IMP-type enzymes has been reported in Greece, Taiwan, and Japan [24,25]. Death rates, associated with MBL producers, range from 18% to 67% [26].

The first *bla*$_{KPC}$ producer (KPC-2 in *K. pneumoniae*) was identified in 1996 in the eastern United States [27]. KPC-mediated carbapenem resistance in members of the *Enterobacterales* has emerged recently in Israel, as observed in clinical strains of *Escherichia coli* [28], *Enterobacter cloacae* [29], and *Klebsiella pneumoniae* [30]. *Bla*$_{KPC}$ producers have been reported, mostly from nosocomial *K. pneumoniae* isolates and to a much lesser extent from *E. coli* (especially in Israel) and from other *Enterobacterales* species [2]. Institutional outbreaks of KPC-producing *Enterobacterales* due to the spread of a single strain have also been reported for other species, including *Enterobacter spp.* and *Serratia marcescens* [29,31].

The first identified *bla*$_{OXA-48}$ producer was from a *K. pneumoniae* strain isolated in Turkey in 2001 [32]. More than 250 class D β-lactamases (OXAs) have been described in recent years, with variations in hydrolytic activity for β-lactams [33]. Although *bla*$_{OXA-48}$ β-lactamase and its variants typically have low-level hydrolytic activity against many carbapenems, they can contribute to high-level carbapenem resistance in combination with other mechanisms [34]. Since then, *bla*$_{OXA-48}$ producers have been extensively reported from Turkey as a source of nosocomial outbreaks [35,36]. Their worldwide distribution now includes countries in Europe, in the southern and eastern part of the Mediterranean Sea, and Africa [32,36]. Several *bla*$_{OXA-48}$ producing clones have been identified, and dissemination of this resistance trait is associated with a 62.5-kb plasmid (previously identified as a plasmid of ≈ 70 kb) [35]. Although reported in various enterobacterial species, *bla*$_{OXA-48}$ producers are mostly identified in *K. pneumoniae* and *E. coli*, and the level of

Table 4. PCR, blue-carba and CIM test results in *Enterobacterales* isolates.

	Blue-carba positive (n = 120)	**Blue-carba negative (n = 33)**	**CIM positive (n = 120)**	**CIM negative (n = 33)**
PCR positive (n = 112)	101	12	111	2
PCR negative(n = 41)	19	21	9	31

resistance to carbapenems is usually higher when ESBL and permeability defects are associated [35,37]. Out of 296 carbapenem-nonsusceptible isolates, *bla*$_{OXA-48}$ gene was detected in 12 *K. pneumoniae* isolates in Croatia [38].

Perçin *et al*. (2012) investigated the presence of *bla*$_{IMP}$, *bla*$_{OXA}$ OXA, *bla*$_{NDM}$, *bla*$_{KPC}$ genes in carbapenem-resistant *K. pneumoniae* from rectal swabs. Carbapenem susceptibility was evaluated by using E-test method, the presence of beta-lactamases was determined by using modified Hodge test (MHT). Of the 801 isolates 33 were determined as carbapenem resistant. No resistance gene were identified by PCR in 13 of 33 isolates, whereas *bla*$_{OXA-48}$ was detected in 19 and *bla*$_{IMP}$ in 1 of 20 positive isolates [39].

Eser *et al.* (2014) investigated the presence of carbapenem resistance in 210 *Enterobacterales* isolates (*E. coli* (n = 153), *K. pneumoniae* (n = 47) and *Klebsiella oxytoca* (n = 10)) recovered from invasive infections, in Hacettepe University Hospital, Ankara, Turkey, between 2005-2009, by PCR (AmpC, CTX-M, KPC, NDM, OXA, IMP and VIM). One *K. pneumoniae* isolate was found to inhabit *bla*$_{OXA-48}$ gene. Five isolates were positive for OXA-1 and one for OXA-10. Two isolates were positive for *bla*$_{CTX-M}$, however *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{KPC}$ and *bla*$_{NDM-1}$ genes were not detected among the isolates [40].

Helvacı (2014) investigated the *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{KPC,}$ *bla*$_{OXA}$ and *bla*$_{NDM-1}$ genes in carbapenem resistant *Enterobacterales* isolates from blood cultures by PCR. PCR was performed on 11 *E. coli* strains and 36 *Klebsiella spp.* strains. They reported that 7 of *E. coli* strains (63.6%) and 28 of *Klebsiella spp.* strains (77%) were carbapenemase positive. *Klebsiella spp.* subgroup analysis showed that 22 strains were OXA, 3 were VIM, 2 were IMP and one strain was OXA+VIM positive. For *E.coli* strains; 5 were OXA, 2 were OXA+VIM pozitive. NDM and KPC subtypes weren't detected [41].

A total of 312 isolates were included in Chea *et al.* (2015) study; a carbapenemase gene was identified in 94 (30%). Seventy-two (65%) *Klebsiella spp.* isolates had a carbapenemase gene, of which 67 (93%) were *bla*$_{KPC}$ and 5 (7%) were *bla*$_{NDM}$. Of all *Enterobacter spp.* and *E. coli* isolates, 14 (14%) and 8 (8%), respectively, had a carbapenemase gene, and all were *bla*$_{KPC}$. The percentage of carbapenemase-producing CRE at the various sites was 73% in Maryland (40 [93%] *bla*$_{KPC}$, 3 [7%] *bla*$_{NDM}$); 30% in Minnesota (31 [94%] *bla*$_{KPC}$, 2 [6%] *bla*$_{NDM}$); 20% in Tennessee (13 [100%] *bla*$_{KPC}$); 6% in New York (3 [100%] *bla*$_{KPC}$); 7% in New Mexico (1 [100%] *bla*$_{KPC}$); and 0 in Colorado [4].

Studies have shown that locally class D OXA-48-like enzymes and NDM are the most common carbapenemases in *Enterobacterales* with sporadic occurrence of KPC and VIM type enzymes [42,43]. Turkey is known to be an area of endemicity for OXA-48-producing *Enterobacterales* [22], and in this study most of the carbapenem resistant isolates were positive OXA-48 and NDM were determined in two isoates.

In García-Fernández *et al.* (2015) study, the 159 CPE comprised the following variants: *bla*$_{OXA-48}$ (n = 53), *bla*$_{KPC-2}$ (n = 45), *bla*$_{KPC-3}$ (n = 36), *bla*$_{VIM-1}$ (n = 24), and *bla*$_{NDM-1}$ (n = 1). In 8 and 6 isolates of the 159 CPE, ambiguous or false negative results were obtained with blue-Carba test and Carba NP test, respectively. With the blue-carba test, 156 out of 159 isolates were correctly detected, thus sensitivity and negative predictive result were slightly lower, 98% and 96%, respectively. Blue-carba detected 100% of KPC, VIM and NDM and 94% of OXA-48 enzymes. To note that the three OXA-48-producing isolates (2 *K. pneumoniae*, 1 *E. aerogenes*) with negative result with the Blue-Carba test had a hypermucoid phenotype [12].

In Erdem *et al.* (2017) study, carbapenem-resistant *Enterobacterales* (CRE) isolates were used to evaluate modified Blue-Carba test for the rapid detection of OXA-48 carbapenemase in comparison with polymerase chain reaction (PCR) amplification. These CREs of various enterobacterial species were isolated from various clinical samples including OXA-48 (47), NDM-1 (6), KPC-1 (1), IMP-1 (1), VIM-2-4 (2), IMP-2 (1), OXA-51 (1), and OXA-23 (1) producers. The Blue-Carba test detected carbapenemase producers with 93% sensitivity and 100% specificity [13].

In Tamma *et al*. (2017) study, two collections of carbapenem-resistant *Enterobacterales* (CRE) isolates were evaluated including 191 retrospective isolates (122 CP-CRE and 69 non-CP isolates) as well as 45 prospective clinical isolates (15 CP-CRE and 30 non-CP-CRE) obtained over a 3-month period. Among the retrospective cohort, sensitivities ranged from 72% for the boronic acid synergy test for the detection of KPC-producers to ≥ 98% for the Modified Carba NP, the RAPIDEC® CARBA NP, the manual Blue-Carba, and the modified carbapenem inactivation method for the detection of any CPE. All assays had excellent specificity exceeding 95% with the exception of the boronic acid synergy test (88%) and modified Hodge test (91%). Prospectively, 45 CRE isolates were encountered over a three-month period including 15 CPE (33%) and 30 non-CP-CRE (67%) [44].

A new phenotypic test called the carbapenem inactivation method (CIM) was developed to detect carbapenemase activity in Gram-negative bacilli within six hours. This method has been found to be highly compatible with the results obtained with PCR to detect genes encoding bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP} and bla_{OXA-23} carbapenemases. Comparing the results of PCR and CIM determined by genes producing several carbapenemase in *Enterobacterales*, *P. aeruginosa* and *A. baumannii* isolates; CIM (92.1%) has been shown to be a phenotypic screening method that can reliably detect carbapenemase activity. It was observed that, 67 isolates (16.3%) whose carbapenemase gene determined by PCR method of 411 isolates tested in the study were positive and according to CIM result, 65 (97.0%) of 67 isolates were positive. It was seen that bla_{KPC} gene was not detected in the *Pseudomonas* isolates used in the study [11].

In Laolerd *et al*. (2018) study, A total of 287 *Enterobacterales* isolates, which were at least resistant to one of the carbapenems, were identified for carbapenemase genes by multiplex PCR (bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}), mCIM and Carba NP. Species of carbapenem-resistant isolates mainly *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae* were detected. Of these isolates, three families of carbapenemase genes [223 (77.70%)], including bla_{NDM} [160 (46.64%)], bla_{OXA-48} [56 (25.11%)] and bla_{IMP-14} [7 (3.14%)] were found. In addition, 25.11% (56/223) of the carbapenemase-producing isolates harbored a combination of NDM and OXA-48-like. Phenotypic detection methods, mCIM and Carba NP, showed 100% sensitivity and specificity to NDM, IMP-14, and OXA-48, while the mCIM was positive in all OXA-181 and OXA-232 isolates, only 12.5% (1/8) and 28.95% (11/38), respectively, were detected by the Carba NP test. This result reflected the greater efficacies of mCIM over the Carba NP test on detection of OXA-181 and OXA-232.This study revealed of carbapenemase genes in Bangkok, Thailand, where NDM-1 and OXA-232 were predominant [45].

A total of 125 isolates were included in Bayraktar *et al.* (2018) study. The strain collection of CPEs included 89 OXA-48 (80 *K. pneumoniae*, six *E. coli*, and three *K. oxytoca*), two KPC (two *K. pneumoniae*), five NDM (four *K. pneumoniae* and one *E. cloacae*), two VIM (one *K. pneumoniae* and one *E. coli*), one IMP (*K. pneumoniae*), 10 OXA-48+NDM (eight *K. pneumoniae*, one *E. coli*, and one *E. cloacae*), and one OXA-48+VIM (*K. pneumoniae*) producer. Of the 110 CPE isolates (including the positive control isolate), 100 were found to be positive by all three tests and 10 isolates showed discordant results. Carba NP-direct, CIM, and b-CARBA tests detected 109 (99.0%), 102 (92.7%) and 108 (93.6%) isolates as positive, respectively [46]. Tijet *et al*. (2015) reported that the sensitivity (98.8%) of the CIM test were higher than those of the Carba NP test (90.1% and 88.2%, respectively) [47]. In our study sensitivity of the CIM test was higher than blue-carba test. The sensitivity of the CIM and blue-carba tests found to be similar to the other studies. However specitivity of the blue-carba test was found to be 52.5%.

Conclusions

Carbapenem resistance is an emerging problem in world wide. Determination of carbapenem resistance determinants is important for prevention of health-care associated infection.

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