Antibiotic Resistance Genes Among Carbapenem-resistant Enterobacterales (CRE) Isolates of Prapokklao Hospital, Chanthaburi Province, Thailand

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Background: The global spread of carbapenem-resistant Enterobacterales (CRE) inflicts a severe threat to human health. The CRE infections have resulted in an increased mortality rate in hospitals and other health-care settings worldwide. In this study, the antibiotic-resistance pattern and prevalence of carbapenemase-encoding genes among CRE isolated from patients of one hospital in Thailand were investigated.

Methods: By using conventional biochemical tests, we identified and isolated all species of Enterobacterales from the clinical samples kept at Prapokklao Hospital, Chanthaburi, Thailand, which were collected during 2016–2017. Multidrug-resistant (MDR) bacteria were determined by disc diffusion method and minimum inhibitory concentration (MIC) test strips. Carbenapenem genes were detected by PCR and confirmed by Sanger sequencing.

Results: Klebsiella pneumoniae complex, Escherichia coli, and Enterobacter spp. were isolated from the specimens. Of 9,564 isolated Enterobacterales, 282 were multidrug-resistant (MDR). The MIC test strips revealed that the MDR CRE were resistant to ertapenem (92.9%) and meropenem (81.3%). All these isolates carried carbapenemase-coding genes, including blaNDM (90%) and blaIMP (71%), the two most commonly found genes among CRE strains. There were 39.2% of the isolates that carried a combination of blaNDM-blaIMP and 22.6% carried combined blaNDM-blaIMP-blaOXA-48-like genes.

Conclusion: This study demonstrates a significantly high prevalence of CRE isolates with the MDR phenotypes. A minority of the isolates carried a single carbapenem-resistant gene, while the majority harbored multiple genes in combination. Regular monitoring of MDR CRE and characterization of their drug resistance are important for guiding treatment, intervention and control of the CRE spread and outbreak in a health-care setting.

Keywords: carbapenemase, CRE, drug resistant, Enterobacterales, nosocomial infections

Introduction

Carbapenem-resistant Enterobacterales (CRE) are gram-negative bacteria that have become pathogens of a major public health concern globally. These bacteria infect hospitalized patients who are under long-term medical care from severe entities, such as those with major surgery/injury or urinary/intravascular/respiratory catheters.1-3 Normally, CRE inhabit the digestive tract of healthy individuals or animals and are nonpathogenic.4 They become pathogens when they are misplaced into other anatomical sites, e.g., the blood stream, urinary tract, or respiratory tract, causing hospital-acquired...
diseases that encompass pneumonia, sepsis, urinary tract infection, wound infection, meningitis, etc. The infections are difficult to treat as these bacteria have propensity to resist multiple antimicrobial drugs, particularly carbapenem, which is considered the last antibiotic resort for treatment of multidrug-resistant bacterial infections.\(^5\)\(^6\) Over the past decades, increased CRE infections have been reported worldwide.\(^7\)\(^-\)\(^16\) The Centers for Disease Control and Prevention (CDC)’s Detect and Protect Against Antibiotic Resistance Initiative (known as the AR Initiative) and the World Health Organization (WHO) have specifically recommended the detection and tracking of CRE as a critical priority of public health.\(^17\)\(^,\)\(^18\)\(^,\)\(^19\) The most common species of CRE causing infections in the clinical settings are *Klebsiella pneumoniae* and *Escherichia coli*.\(^20\)\(^,\)\(^21\) The carbapenemase resistance is usually mediated by mobile genetic elements harboring beta-lactamase encoding genes, which also confer resistance to most penicillin-derivatives and cephalosporins.\(^5\) Meropenem, doripenem, ertapenem, and imipenem are the most commonly used carbapenems in the health-care settings.\(^22\) Carbapenemase gene products, such as *K. pneumoniae* carbapenemase (KPC), metallo-\(\beta\)-lactamases, and oxacillinase (OXA-48), are often found in carbapenemase-producing Enterobacteriales.\(^23\)\(^-\)\(^28\) Among them, the *bla*KPC is the most common gene reported; however, prevalence of other genes, such as *bla*OXA, *bla*VIM, *bla*NDM, and *bla*IMP are gradually increasing worldwide.\(^29\) Emergence of drug resistant bacteria have led to more severe drug resistance problems.\(^30\) In this study, the antibiotic resistance patterns and carbapenem-resistant genes among CRE isolated from the clinical samples kept in a tertiary health-care setting in Thailand were investigated as this kind of information is needed for emergency preparedness and response to outbreaks of MDR organisms in a locality.

**Materials and Methods**

**Study Design, Sample Collection, and Ethical Approval**

The stored clinical samples were from a cross-sectional study conducted during January 2016 to December 2017 by the Department of Microbiology, Prapokklao Hospital, Chanthaburi Province, located about 226 km Southeast of Bangkok, Thailand. Of the 9,564 Enterobacteriales isolates recovered from the specimens, 282 isolates (2.95%) were CRE. They were from different types of the samples from different patients, i.e., 18 blood samples, 8 body fluids, 120 urine samples, 108 sputum samples and 28 pus samples. Unfortunately, the demographic data of the patients were untraceable. This study was approved by the Ethics Committee of Prapokklao Hospital, Chanthaburi Province, Thailand (approval number: CTIREC 041).

**Bacterial Isolation and Identification**

The stock samples were streaked on McConkey agar and incubated at 37°C for 24 hours. Isolated bacterial colonies on the plates were subjected to conventional biochemical tests\(^31\) including oxidase, triple sugar iron utilization, ornithine decarboxylase, indole production, motility, and citrate utilization tests, as well as antimicrobial susceptibility tests.\(^32\)

**Antimicrobial Susceptibility Testing**

The antimicrobial susceptible testing was performed based on the Clinical and Laboratory Standards Institute (CLSI) guidelines 2017.\(^33\) The Kirby–Bauer disc diffusion method and 16 antimicrobial drugs/drug combinations were used, including ampicillin (10 μg), amoxicillin/clavulanate (20/10 μg), ampicillin/sulbactam (10/10 μg), piperacillin/tazobactam (100/10 μg), cefazolin (30 μg), cefazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), ertapenem (10 μg), meropenem (10 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), and fosfomycin (200 μg) (Oxoid, Thermo Fisher Scientific, Basingstoke Hampshire, UK). Multidrug-resistance criteria were defined as bacteria resistant to carbapenem and at least three out of five drug groups—cephalosporins, ampicillin, \(\beta\)-lactam/\(\beta\)-lactamase inhibitor combination, fluoroquinolones, folate pathway, and fosfomycin. Extended-spectrum \(\beta\)-lactamase (ESBL) production of all isolates were tested by using a combination disc test of ceftazidime with and without clavulanate, and cefotaxime with and without clavulanate (BD BBL, Identification System, New York, USA). A positive test result was defined as ≥5 mm difference in the clear zone diameter between the discs with and without clavulanate. Criteria of CLSI 2017 were used for interpretation of the antimicrobial-susceptibility results. Carbapenem resistance was determined using Liofilchem\(^36\) MIC Test Strips (Liofilchem, Roseto degli Abruzzi (TE), Italy). CRE was defined as Enterobacteriales organisms showing decreased susceptibility to carbapenems (MIC for imipenem ≥2 μg/mL, meropenem ≥2 μg/mL, or ertapenem ≥1 μg/mL) and resistance to all third-generation cephalosporins, regardless of carbapenemase production.\(^34\) All antibiotic
susceptibility tests were independently performed in duplicative experiments.

**PCR and DNA Sequencing for Detection and Identification of Carbapenemase Genes**

The genomic DNA of all CRE isolates was extracted and amplified for *bla* genes, including *bla*KPC, *bla*NDM, *bla*VIM, *bla*IMP and *bla*OXA-48-like, using specific primers. Briefly, the bacterial isolates were grown on tryptic soy agar plates at 37°C overnight. The single bacterial colony was re-suspended in sterile distilled water and boiled at 100°C for 10 minutes. The lysates were centrifuged at 12,000 × g for 10 minutes and 1 μL of supernatant was used as DNA template for PCR. The amplification reaction (25 μL) contained 10× *Taq* buffer, 25 mM of MgCl₂, 10 mM of dNTPs, 10 μM each of of forward and reverse primers, >50 ng/μL of genomic DNA and 1.25 units/μL of *Taq* DNA Polymerase (Thermo Scientific, Waltham, MA, USA). The thermal cycles were 94°C for 5 minutes, followed by 35-cycles of denaturing at 94°C, 30 seconds; annealing at a temperature specific for each primer, 30 seconds; and extension at 72°C, 30 seconds, with a final extension at 72°C for 7 minutes. PCR products were analyzed using 1.5% (w/v) agarose gel and ethidium bromide staining. Individual genes in representative CRE isolates were verified by DNA sequencing. The nucleotide sequences were analyzed using the BLASTn program, software available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

**Results**

**Bacterial Identification and CRE Isolates**

From a total of 9,567 Enterobacterales isolates, 282 (2.95%) were carbapenem resistant Enterobacterales (CRE). They were 187 (66%) *K. pneumoniae* complex, 81 (29%) *E. coli*, and 14 (5%) *Enterobacter* spp., 11 (4%) *E. cloacae* and 3 (1%) *E. aerogenes* (Figure 1).

**Antimicrobial Susceptibility Testing**

Antimicrobial pattern of carbapenem-resistant Enterobacterales isolates are shown in Table 1. The 2016 CRE isolates showed high resistance to third-generation cephalosporin (100%); carbapenem: ertapenem 99%, meropenem 94.6%, imipenem 81.9%; β-lactam/β-lactamase inhibition combination: amoxicillin/clavulanate 100%, ampicillin/sulbactam 99%, piperacillin/tazobactam 88.3%; and ampicillin 99%. Resistance to other antibiotics was observed—ciprofloxacin 82.9%, trimethoprim-sulfamethoxazole 90%, amikacin 61.3%, gentamicin 21.6%, and fosfomycin 18%. The results of the MIC test strips showed resistance to ertapenem and meropenem at 85.6 and 78.4%, respectively. The MIC ranges of CRE isolates, i.e., *E. coli*, *K. pneumoniae* complex, and *Enterobacter* spp., for ertapenem were 0.5–32, 0.064–32, and 1–32 µg/mL, respectively, and for meropenem were 0.032–32, 0.047–32 and 2–32 µg/mL, respectively. The CRE isolates were positive for extended-spectrum β-lactamase (ESBL); the values for ceftazidime and cefotaxime were 85.5 and 71.4%, respectively (Figure 2).

The 2017 CRE isolates were highly resistant to third-generation cephalosporin (cefazolin 100%, ceftazidime...
Table 1: Antimicrobial Pattern of Carbapenem-Resistant Enterobacterales Isolates

| Antimicrobial Susceptibility Tests | Microorganisms | Total Number of Bacterial Isolates |
|-----------------------------------|----------------|-----------------------------------|
|                                   | E. coli        | K. pneumoniae Complex             | Enterobacter spp. | Total |
|                                   | 2016 (n=111)   | E. coli                          | 2017 (n=171)      |       |
|                                   | n=50 (%)       | n=56 (%)                         | n=31 (%)          | n=171 |
|                                   |                | 2016 (n=111)                     | 2017 (n=171)      |       |
| Disc diffusion                    |                | E. coli                          | K. pneumoniae     | Enterobacter spp. | Total |
| Ertapenem                         | 50 (100)       | 55 (98.2)                        | 30 (96.8)         | 279 (98.9) |
| Meropenem                         | 47 (94)        | 53 (94.6)                        | 28 (90.3)         | 265 (93.9) |
| Imipenem                          | 44 (88)        | 44 (78.6)                        | 27 (87.1)         | 239 (84.7) |
| Ampicillin                        | 50 (100)       | 56 (100)                         | 31 (100)          | 280 (90.2) |
| Ampicillin/ Sulbactam             | 50 (100)       | 55 (98.2)                        | 31 (100)          | 273 (96.8) |
| Amoxicillin/ Clavulanate          | 48 (96)        | 47 (83.9)                        | 31 (100)          | 253 (89.7) |
| Piperacillin/ Tazobactam          | 50 (100)       | 56 (100)                         | 31 (100)          | 171 (100) |
| Cefazolin                         | 45 (90)        | 47 (83.9)                        | 26 (91.4)         | 170 (96.4) |
| Cefotaxime                        | 39 (78)        | 45 (80.4)                        | 22 (77.4)         | 157 (85.7) |
| Ceftriaxone                       | 50 (100)       | 56 (100)                         | 27 (77.4)         | 139 (81.5) |
| Amikacin                          | 35 (70)        | 30 (53.6)                        | 12 (62.8)         | 141 (82.9) |
| Gentamicin                        | 2 (4)          | 22 (39.3)                        | 6 (31.6)          | 105 (60.1) |
| Ciprofloxacin                     | 49 (98)        | 42 (73.9)                        | 11 (64.7)         | 158 (89.2) |
| Trimethoprim/ sulfamethoxazole    | 50 (100)       | 47 (83.9)                        | 26 (74.2)         | 132 (77.4) |
| Fosfomycin                        | 1 (2)          | 19 (33.9)                        | 45 (84.5)         | 174 (97.1) |
| MIC test                          |                | 20 (18)                          | 3 (33.3)          | 70 (24.8) |
| Ertapenem                         | 45 (90)        | 47 (83.9)                        | 95 (85.6)         | 167 (97.7) |
| Meropenem                         | 39 (78)        | 45 (80.4)                        | 87 (78.4)         | 142 (83.1) |
| Cefazidime                        | 40 (80)        | 54 (96.4)                        | 97 (87.4)         | 144 (84.2) |
| Cefotaxime                        | 36 (72)        | 40 (71.4)                        | 79 (71.2)         | 133 (77.8) |

Abbreviations: n, number of isolates; MIC, minimum inhibitory concentration; ESBL, extended-spectrum β-lactamase.
100%, cefotaxime 99.4%, ceftriaxone 99.4%); carbapenem (ertapenem 98.8%, meropenem 93.6%, imipenem 86.5%); β-lactam/β-lactamase inhibition combination (amoxicillin/clavulanate 100%, ampicillin/sulbactam 96.8%, piperacillin/tazobactam 89.7%); and ampicillin (99.2%). Resistance to other antibiotics were—ciprofloxacin (87.1%), trimethoprim-sulfamethoxazole (76.1%), amikacin (67.8%), gentamicin (40.4%), and fosfomycin (29.2%). Resistance to other antibiotics were—ciprofloxacin (87.1%), trimetho-
prim-sulfamethoxazole (76.1%), amikacin (67.8%), genta-
icin (40.4%), and fosfomycin (29.2%). The MIC test strips that demonstrated resistance to ertapenem and meropenem were 97.7 and 83.1%, respectively. These CRE isolates were positive for ESBL; the values for cefazidime and cefotax-
ime were 84.2 and 77.8%, respectively.

**Carbapenemase Coding Genes Among the Carbapenem-resistant Enterobacterales**

The PCR amplicon sizes of the resistant genes, ie, bla\textsubscript{KPC}, bla\textsubscript{VIM}, bla\textsubscript{IMP}, bla\textsubscript{NDM}, and bla\textsubscript{OXA-48-like} were 489, 382, 188, 490, 218 and 743 bp, respectively (Supplementary Data 1).

Of the 282 CRE isolates, 256 (90%) carried bla\textsubscript{NDM}; 203 (72%) and 134 (48%) isolates were positive for bla\textsubscript{IMP} and bla\textsubscript{OXA-48-like}, respectively. There were five (1.8%) isolates carried bla\textsubscript{VIM} and one (0.4%) isolate with bla\textsubscript{KPC}.

Distribution of the carbapenemase genes was also analyzed. Among 282 isolates, 31 were singly positive for bla\textsubscript{NDM} (23 isolates, 7.8%) and bla\textsubscript{IMP} (9 isolates, 3.2%). The others 251 isolates (89%) had combination of different genes. The predominant combination was bla\textsubscript{NDM} and bla\textsubscript{IMP} (111 isolates, 39.2%), followed by combined bla\textsubscript{NDM}, bla\textsubscript{IMP} and bla\textsubscript{OXA-48-like} (64 isolates, 22.6%) and combined bla\textsubscript{NDM} and bla\textsubscript{OXA-48-like} (55 isolates, 19.4%). Other combination types were the bla\textsubscript{IMP} and bla\textsubscript{OXA-48-like} (15 isolates, 5.3%), bla\textsubscript{NDM}, bla\textsubscript{IMP} and bla-

**Abbreviations:** CAZ/CLA, ceftazidime + clavulanate; CAZ, ceftazidime + cefotaxime; CTX, cefotaxime; CTX/CLA, cefotaxime + clavulanate; ETP, ertapenem; MEM, meropenem.
Table 2 Distribution of Carbapenemase-encoding Genes Among Carbapenemase-producing Carbapenem-resistant Enterobacterales Isolates by PCR

| Carbapenemase Genes | E. coli n=81 (%) | K. pneumoniae complex n=187 (%) | Enterobacter spp. n=14 (%) | Total Number of Bacterial Isolates n=282 (%) |
|---------------------|------------------|-------------------------------|--------------------------|--------------------------------------------|
| blaKPC             | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaVIM             | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaIMP             | 3 (3.7)          | 6 (3.21)                      | 0 (0)                    | 9 (3.2)                                    |
| blaNDM             | 11 (13.58)       | 9 (4.81)                      | 2 (1.428)                | 22 (7.8)                                   |
| blaOXA-48-like     | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaVIM     | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaIMP     | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaNDM     | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaOXA-48-like | 0 (0)        | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaOXA-48-like     | 2 (2.46)         | 0 (0)                         | 0 (0)                    | 2 (0.7)                                    |
| blaKPC, blaOXA-48-like | 0 (0)           | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaNDM, blaOXA-48-like | 19 (23.45) | 88 (47.05)                    | 4 (28.57)                | 111 (39.4)                                 |
| blaIMP, blaNDM     | 0 (0)            | 14 (7.48)                     | 1 (7.14)                 | 15 (5.3)                                   |
| blaNDM, blaOXA-48-like | 22 (27.16)     | 27 (14.44)                    | 6 (42.85)                | 55 (19.5)                                  |
| blaKPC             | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaVIM     | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaNDM     | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaOXA-48-like | 0 (0)           | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaIMP, blaNDM | 0 (0)           | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaOXA-48-like | 2 (2.46)        | 1 (0.53)                      | 0 (0)                    | 1 (0.35)                                   |
| blaOXA-48-like     | 0 (0)            | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaNDM, blaOXA-48-like | 22 (27.16) | 41 (21.93)                    | 1 (7.14)                 | 64 (22.7)                                  |
| blaKPC, blaVIM, blaNDM | 0 (0)           | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaVIM, blaOXA-48-like | 0 (0)        | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaNDM, blaOXA-48-like | 0 (0)        | 0 (0)                         | 0 (0)                    | 0 (0)                                      |
| blaKPC, blaVIM, blaRD, blaNDM | 0 (0)       | 0 (0)                         | 0 (0)                    | 0 (0)                                      |

Abbreviation: n, number of isolates.

resistant gene was *blaIMP* gene, which was found in 151 (53.5%) of *K. pneumoniae* complex and in 46 (29.1%) of *E. coli* isolates. The *blaOXA-48-like* gene was found in 82 isolates (29.1%), and 44 isolates (15.6%); the *blaVIM* gene was found in 4 (1.4%) *E. coli* isolates and 1 (0.4%) *K. pneumoniae* complex isolate; and the *blaKPC* gene was found in 1 (0.4%) *K. pneumoniae* complex isolate. For *Enterobacter* spp., the predominant resistant gene was *blaNDM* (13 isolates, 4.6%; 11 *E. cloacae* isolates and 2 *E. aerogenes* isolates). Others genes were *blaOXA-48-like* (8 isolates, 3%; 5 *E. cloacae* isolates and 3 *E. aerogenes* isolates) and *blaIMP* (6 isolates, 2%; 5 *E. cloacae* isolates and 1 *E. aerogenes* isolate) (Table 2 and Figure 3).

Discussion

Carbapenem-resistant Enterobacterales (CRE) are considered nowadays as a public health threat worldwide because they are one of the major causes of death among patients with hospital-acquired infections. Regular monitoring of CRE in a particular health-care setting is rational to guide effective treatment of the infection. This study assessed the antibiotic-resistance pattern and the prevalence of the carbapenem-resistant genes among CRE isolated from clinical specimens of patients kept at Prapokklao Hospital which is one of the tertiary care hospitals in Southeastern Thailand. Our study demonstrates a high prevalence of CRE that carried a variety of carbapenem-resistance coding genes. Three species of Enterobacterales, i.e.,
K. pneumoniae complex, E. coli, and Enterobacter spp. were predominant among the CRE isolates (282 of 9,567 Enterobacterales isolates). Five families of carbapenemase genes including \textit{bla}_{KPC}, \textit{bla}_{NDM}, \textit{bla}_{IMP}, \textit{bla}_{VIM}, and \textit{bla}_{OXA-48-like} were identified by PCR and DNA sequencing.

In this study, the prevalence of CRE isolated during 2016–2017 showed marked increase compared to those of 2012–2013 study in the same hospital, Prapokklao Hospital. The National Antimicrobial Resistance Surveillance Center, Thailand (NARST), reported that between January and December 2017, 13% of \textit{K. pneumoniae} complex isolates and 1% of \textit{E. coli} isolates were resistant to carbapenem. In this study, 66% of the \textit{K. pneumoniae} complex accounted for the largest percentage of CRE isolates (50% in 2016 and 77% in 2017), 29% of the \textit{E. coli} and 5% of the \textit{Enterobacter} spp., respectively.

Most resistance to carbapenem agents is caused by carbapenemase and the presence of other resistance mechanisms, such as extended-spectrum beta-lactamases (ESBLs), porin mutations, and/or the presence of efflux pumps. While our antimicrobial susceptibility testing results showed that carbapenem-resistant Enterobacterales strains exhibited resistance to many antimicrobial agents including third generation cephalosporin, carbapenem, the \beta-lactam/\beta-lactamase inhibition combination, ampicillin, ciprofloxacin, trimethoprim sulfamethoxazole, and amikacin, most CRE strains are still sensitive to gentamicin and fosfomycin which conformed to the results of previous studies. The results of this study showed that CRE isolates were highly resistant to carbapenem agents. The isolates were resistant to ertapenem (93%) which was more than meropenem (81%). The prevalence of ESBL-producing isolates (75–85%) in this study was higher than that observed in a previous study among Thai community volunteers (32.0–66.5%).

Among the CRE isolates of this study, the prevalence of three carbapenemase genes was high—\textit{bla}_{NDM}, followed by \textit{bla}_{IMP} and \textit{bla}_{OXA-48-like}. The \textit{bla}_{VIM} and \textit{bla}_{KPC} genes were found in smaller numbers in this study, even though this gene has been reported to spread rapidly worldwide. In Thailand, \textit{bla}_{NDM}, \textit{bla}_{OXA-48-like}, and \textit{bla}_{IMP-14} were frequently detected in clinical Enterobacterales isolates, while the prevalence of \textit{bla}_{KPC} in the country remained low; the latest study reported 0.02% of \textit{bla}_{KPC-13}-carrying isolates among Enterobacterales and 1.7% among CRE isolates. The prevalence of CRE in Asia and South East Asia showed an increasing trend and is mainly caused by carbapenem-hydrolyzing \beta-lactamases, including NDM and IMP-type enzymes. In this study, the \textit{bla}_{NDM} gene was found to be the most common carbapenemase gene, i.e., carried by about 90% of the tested isolates. The other most common carbapenemase genes were \textit{bla}_{IMP}, carried by 203 isolates.
(71%) and frequently found in K. pneumoniae complex (53%); the blaOXA-48-like gene, was found in 134 isolates (48%) and 29% among the K. pneumoniae complex. There are limited data on blaIMP and blaOXA-48-like in relation to CRE prevalence in Thailand. However, Rimrang et al\textsuperscript{43} reported in 2012 that two isolates of K. pneumoniae complex out of 4,818 from clinical Enterobacteriales isolates were blaIMP. In addition, our results also demonstrated that 89% of the CRE contained combinations of genes. The most commonly found were the bla\textsubscript{NDM} and bla\textsubscript{IMP} gene combination (39.2%), followed by the bla\textsubscript{NDM} and bla\textsubscript{OXA-48-like} combination, and the bla\textsubscript{IMP} and bla\textsubscript{OXA-48-like} combination. In addition, we observed the triple combination of bla\textsubscript{NDM}, bla\textsubscript{IMP} and bla\textsubscript{OXA-48-like} (22.6%), and 11% of the CRE isolates with single gene, i.e., bla\textsubscript{NDM} (7.8%) and bla\textsubscript{IMP} (3.2%).

The high prevalence of CRE harboring the carbapenem-coding genes along with multiple drug-resistant phenotypes signals a decreasing therapeutic efficacy of the currently available antimicrobial agents. Thus, an effective alternative option to combat these drug resistant bacteria is urgently needed. Natural products with antibacterial activities against various gram-positive and gram-negative bacterial pathogens have been used with some success, such as essential oils (Melaleuca alternifolia, Thymus vulgaris, Mentha piperita, and Rosmarinus officinalis), a commercial ophthalmic solution containing povidone-iodine 0.6% (IODIM), hexamidine disethionate 0.05% (Keratosept).\textsuperscript{45–48} These products should be studied further along with other novel synthetic drugs toward clinical use against the MDR CRE.

**Conclusion**

We have demonstrated a significantly high prevalence of carbapenem-resistant Enterobacteriales (CRE) isolates with the multidrug-resistant (MDR) phenotypes isolated from clinical samples of patients of one of the tertiary care hospitals in Thailand. The MDR CRE isolates carried carbapenem-resistant genes either singly (minority) or multiple genes in combination (majority). Regular monitoring of the MDR CRE is needed regularly for guiding treatment, intervention and control of the spread and outbreak of these superbugs.

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**Disclosure**

The authors report no conflicts of interest in this work.

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