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Emergence of Carbapenem-Resistant *Enterobacteriaceae* in a Tertiary Care Hospital in Southern Thailand[†]

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Abstract

Carbapenem-resistant *Enterobacteriaceae* (CRE) is emerging as a major problem in healthcare settings globally, including Thailand, due to limited therapeutic options. We reported the detection, antimicrobial susceptibility profiles, and the presence of carbapenemase genes of CRE isolates obtained from Songklanagarind Hospital between July 2012 and June 2015. A total of 273 non-duplicated CRE isolates was recovered from 248 patients. The predominant organism was *Klebsiella pneumoniae* (183 [67.0 %]), followed by *Escherichia coli* (38 [13.9 %]). The susceptibility to 13 antibiotics was performed by disk diffusion assay. Most of the CRE isolates remained susceptible to amikacin. Minimum inhibitory concentrations (MIC) of carbapenems were determined by E-test. The MIC_{50} and MIC_{90} were varied among genera and species. Multiplex PCRs for the carbapenemase genes bla_{IMP}, bla_{VIM}, bla_{OXA-48}, bla_{NDM-1}, bla_{KPC}, and bla_{GES} were performed. One hundred and seventy-eight out of these 273 CRE isolates (65.2 %) harbored either single or multiple carbapenemase genes. One hundred and fifty nine isolates harbored the bla_{NDM-1} gene (113 *K. pneumoniae*, 25 *E. coli*, 17 *E. cloacae*, 2 *Citrobacter freundii*, 1 *Enterobacter aerogenes*, and 1 *Pantoea agglomerans*), 7 isolates carried bla_{IMP} (4 *K. pneumoniae*, 2 *C. freundii*, and 1 *E. cloacae*), 7 isolates possessed bla_{OXA-48} (1 *K. pneumoniae*, 5 *E. coli*, and 1 *E. aerogenes*), whereas 3 and 2 isolates harbored bla_{NDM-1} and bla_{IMP} (2 *K. pneumoniae* and 1 *E. cloacae*) and bla_{NDM-1} and bla_{OXA-48} (1 *E. coli* and 1 *E. cloacae*), respectively. In conclusion, this study revealed the detection of CRE, with the majority of *K. pneumoniae* harboring bla_{NDM-1} in this setting.

Keywords: Carbapenem resistance, *Enterobacteriaceae*, Carbapenemases, *K. pneumoniae*, NDM-1

Introduction

Carbapenems are antimicrobial agents from the β-lactam family which show the broadest spectrum of activity against Gram-negative bacteria [1]. Carbapenems are not inactivated by extended-spectrum β-lactamase (ESBL) or AmpC β-lactamase [2]. Hence, they are the drug of choice to treat infections caused by ESBL producers [3]. *Enterobacteriaceae*, which are resistant to antimicrobials of class carbapenem, are called carbapenem-resistant *Enterobacteriaceae* (CRE). Carbapenem resistance in *Enterobacteriaceae*

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has been reported globally, and it has emerged as a major public health threat with a worldwide impact [4,5]. CRE have been increasingly detected in Southeast Asia, including Thailand [6-8]; the danger is coupled with the fact that the mortality rate for CRE infections is higher, due to limited therapeutic options [9].

Carbapenem resistance in *Enterobacteriaceae* is mainly through the production of 3 classes of carbapenemase enzymes, as classified in the Ambler classification. The most common carbapenemases reported from different geographical regions are KPC, GES (class A serine β-lactamases), NDM, IMP, VIM (class B metallo-β-lactamases), and OXA (class D oxacillinase). The production of AmpC-type β-lactamase, or ESBL, with low permeability of outer membrane proteins and efflux pump expressions, is also involved in carbapenem resistance [5,10]. The localization of the carbapenemase genes is either on chromosomes or plasmids. The mobile genetic elements, plasmids and transposons, are related to bacterial gene transmission leading to infection outbreaks [10]. KPC-producing *Klebsiella pneumoniae* was first isolated from an intensive care unit in North Carolina in 1996 [11]. However, the wide spread of carbapenemase-producing *Enterobacteriaceae* was undetected until 2000, and the first outbreak of KPC-producing *K. pneumoniae* was reported in 2001 in the US. This KPC-producing strain spread throughout the US and appeared in different countries such as Greece, Italy, and Israel [12]. NDM was first isolated in India, and OXA-48 in Turkey. They have been frequently reported from Europe, Asia, and North America [5]. In Thailand, the first report of NDM-1 and IMP-14a in *Enterobacteriaceae* was from Khon Kaen province in 2012 [13], while the first case of *Enterobacteriaceae* harboring bla_{KPC-13} was reported from Bangkok in 2014. In 2015, the spread of carbapenem-resistant ST 340 *K. pneumoniae* was also reported, with concern shown as to limiting its spread [7,8].

The high prevalence of ESBL, along with the overuse of carbapenems, is widely considered to be the leading cause for the high incidence of CRE in Thailand. The use of carbapenems from 2010 to 2013 increased from approximately 2.1 to 3.1 million vials per year [14]. The rapid detection of CRE, along with the proper implementation of infection control measures, is important to prevent its spread. The aim of this present study was to investigate the prevalence of CRE over 3 years in Songklanagarind Hospital, the major tertiary care and referral center in Southern Thailand. We also studied the susceptibility profiles and characterized the main mechanisms of carbapenem resistance among these CRE isolates.

Materials and methods

This study was a retrospective cohort study conducted at Songklanagarind Hospital, an 863-bed tertiary level university hospital located in Southern Thailand, after obtaining approval from the Research Ethics Committee, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand (REC59-043-05-2).

Inclusion of CRE isolates

All antimicrobial-resistant isolates belonging to the family *Enterobacteriaceae* that have been stored in 30 % (V/V) glycerol broth at -80 °C as a part of the routine work in the Microbiology Unit, Songklanagarind hospital between July 2012 and June 2015 were investigated. Those isolates were previously identified for their species level at the time of isolation from routine cultures by standard laboratory methods. The isolates were collected from both clinical specimens (urine, sputum, pus/ discharge, tissue, blood, and various body fluids), and from surveillance screening (rectal swab). The isolates were defined as CRE on the basis of non-susceptibility to any tested carbapenems (ertapenem, imipenem, and meropenem) via susceptibility testing. The *Providencia*, *Proteus*, or *Morganella* genera that demonstrated an MIC of > 1 μg/mL for imipenem alone were excluded from the study. Duplicate CRE isolates (i.e., those of the same species from the same specimen type) from the same patient in the same year were excluded.

Antimicrobial susceptibility testing

The susceptibility testing of the *Enterobacteriaceae* isolates was carried out using the Kirby-Bauer disk diffusion method on Muller-Hinton agar plates, according to Clinical and Laboratory Standards Institute (CLSI) guidelines [15]. Commercial antibiotic discs from 2 manufacturers, Oxoid, Basingstoke, UK, and BD, Maryland, US, were used for the antimicrobial testing. The tested antibiotics were amikacin (30 µg), ceftazidime (30 µg), ceftriazone (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), ertapenem (10 µg), gentamicin (10 µg), imipenem (10 µg), meropenem (10 µg), sulperazone (30 µg), norfloxacin (10 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). The susceptible results were interpreted according to the CLSI guidelines [15]. *E. coli* ATCC®25922™ was used as the quality control for the antimicrobial susceptibility test. The MICs of ertapenem, imipenem, and meropenem were determined using commercial E-test strips (Liofilchem, Roseto degli Abruzzi, Italy).

Detection of carbapenemases genes

The bacterial DNA of CRE isolate was extracted by the boiling method. Briefly, an overnight-grown CRE isolate was centrifuged at 8,000 rpm for 3 min; then, the supernatant was discarded. The pellet was washed twice with normal saline. After washing, the pellet was mixed with 100 µl of deionized water and boiled for 15 min at 100 °C. The tube was immediately cooled on ice and centrifuged at 8,000 rpm for 8 min. The supernatant was collected as DNA templates for further investigation.

All CRE isolates were tested for carbapenemase genes by multiplex PCR. The most prevalent carbapenemase genes, bla_{IMP}, bla_{VIM}, bla_{OXA-48}, bla_{NDM-1}, bla_{KPC}, and bla_{GES}, were investigated by using the primers described earlier [16,17]. The PCR reaction was conducted under the following conditions: 94 °C for 10 min, followed by 36 cycles of 94 °C for 30 s, 52 °C for 40 s, and 72 °C for 50 s, with a final extension for 5 min at 72 °C. Amplification products were analyzed by 2 % agarose gel electrophoresis. The amplicons were sequenced at 1st BASE (Malaysia), and the sequence of each gene was confirmed using the NCBI website (https://www.ncbi.nlm.nih.gov).

Results and discussion

Distribution of CRE isolates

During the study period, a total of 19,495 *Enterobacteriaceae* were isolated and tested for antimicrobial susceptibility. Only 273 non-duplicated CRE, isolated from 248 patients admitted at various wards of the hospital, met the study inclusion criteria. In a 6-month interval period during the study, CRE were isolated from 5 patients in July-Dec 2012, and reached 107 patients in Jan-June 2015, indicating the increasing trend of CRE being circulated in this setting (**Figure 1**).

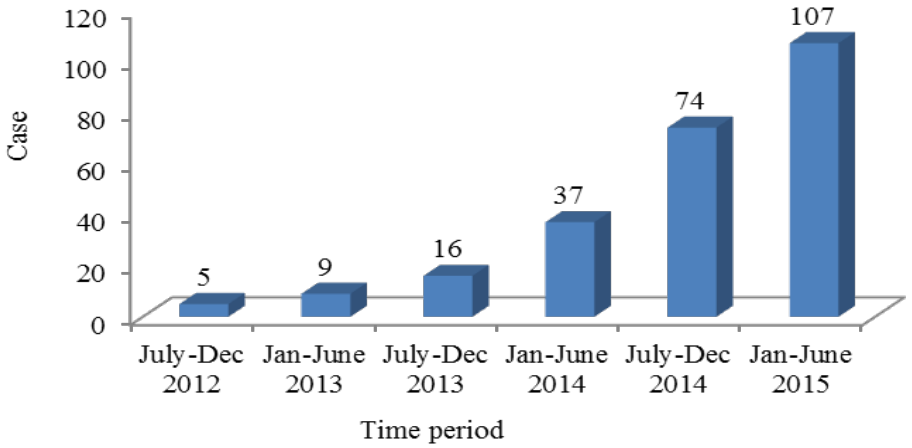

Figure 1 Number of CRE cases during July 2012 and June 2015.

The most common species were *K. pneumoniae* (183 isolates, 67.03 %), followed by *E. coli* (38 isolates, 13.92 %), *Enterobacter cloacae* (35 isolates, 12.82 %), *Citrobacter freundii* (8 isolates, 2.93 %), *Enterobacter aerogenes* (4 isolates, 1.47 %), *Pantoea agglomerans* (2 isolates, 0.73 %), *Klebsiella ozanae* (1 isolate, 0.37 %), *Proteus mirabilis* (1 isolate, 0.37 %), and *Serratia liquefaciens* (1 isolate, 0.37 %). Similar findings of CRE distribution were reported from Singapore and Malaysia, whereby the majority of the isolated CRE were *K. pneumoniae*, followed by *E. coli* and *E. cloacae* [18,19]. Nevertheless, the CRE species distributions were different from a previous study conducted in Thailand, in that *E. cloacae* (67.9 %) was found to be most predominant, whereas only 19.9 and 9.4 % of *K. pneumoniae* and *E. coli*, respectively, were CRE [14]. The difference in CRE species distribution may be due to variations in geography or the study site. However, further studies in CRE prevalence in Thailand's different healthcare setting would set a benchmark to trace CRE incidence and prevalence.

Overall, CRE isolates were obtained from 252 samples, and mainly isolated from rectal swabs (surveillance culture), 182/273 (85.7 %). The rectal swabs were collected from patients staying near newly identified CRE patients (index case) who were admitted at the same ward during the same interval time. The surveillance culture was performed to identify the unrecognized CRE colonization, as one of the recommended strategies by the Center for Disease Control and Prevention (CDC), in order to control CRE transmission in a ward [20]. For index cases, the majority of clinical samples were obtained from catheter urine, followed by sputum, pus, midstream urine, blood, and body fluid (**Table 1**). Few numbers were isolated from tissue. This finding was similar to another previous study that highlighted specimens from the urinary tract, followed by the respiratory tract, gastrointestinal tract, bloodstream, tissue, and wound, as the frequent isolation site of CRE [18].

Ward-wide distribution of all specimens is shown in **Table 1**. A high rate of infection and colonization of CRE isolates was observed in our setting, which led to major concern. Notably, fifty-three percent (n = 37) of the clinical specimens, in addition to 86 percent (n = 156) of rectal swabs, were from patients in 3 major wards: intensive care units, surgical wards, and medical wards. Patients in these wards usually have a longer hospital stay and have indwelling devices. Previous studies [18,21] suggested length of the stay, antimicrobial exposure, and indwelling devices (intravascular lines, urinary catheter, endotracheal tube, and feeding tube) were risk factors for both infection and colonization with CRE. Furthermore, colonization with CRE is considered as a risk factor for subsequent CRE infections [22].

Table 1 Ward-wise distribution of clinical specimens and surveillance screening (rectal swab).

Wards	Number of specimen							
	Sterile site (N=42)				Non-sterile site (N=30)			Rectal swab
	Blood	Body fluid	Catheter urine	Tissue	Sputum	Pus/ discharge	Midstream urine	
Intensive care units	3	2	1	-	5	1	3	41
Surgical	1	1	6	-	4	-	-	55
Medical	1	1	1	-	4	2	1	60
Orthopedics	2	-	2	-	-	1	1	8
Obstetrics and gynecology	1	-	3	-	-	-	1	3
Pediatrics	-	-	2	-	-	1	-	4
Emergency	-	-	5	-	1	1	1	1
Operation	-	-	2	2	-	1	-	2
Trauma	-	1	1	-	-	1	-	4
Others	-	1	3	-	1	-	-	4
Total	8	6	26	2	15	8	7	182

Table 2 CRE susceptibility test results by Kirby-Bauer method.

Antimicrobial class	Antimicrobial agent	Number of isolate (%), total=273		
		Susceptible	Intermediate	Resistant
Carbapenems	Imipenem	55 (20.1)	16 (5.9)	202 (74.0)
	Meropenem	22 (8.1)	12 (4.4)	239 (87.5)
	Ertapenem	1 (0.4)	4 (1.5)	268 (98.2)
Aminoglycosides	Amikacin	243 (89.0)	13 (4.8)	17 (6.2)
	Gentamicin	128 (46.9)	8 (2.9)	137 (50.2)
Cephalosporins	Cefoxitin	1 (0.4)	3 (1.1)	269 (98.5)
	Cefotaxime	0 (0.0)	1 (0.4)	272 (99.6)
	Ceftazidime	2 (0.7)	0 (0.0)	271 (99.3)
	Ceftriaxone	1 (0.4)	0 (0.0)	272 (99.6)
Other β-Lactam	Sulperazone	9 (3.3)	17 (6.2)	247 (90.5)
Fluoroquinolones	Ciprofloxacin	20 (7.3)	35 (12.8)	218 (79.9)
	Norfloxacin	98 (35.9)	24 (8.8)	151 (55.3)
Folate pathway inhiitors	Trimethoprim-sulfamethoxazole	54 (19.8)	9 (3.3)	210 (76.9)

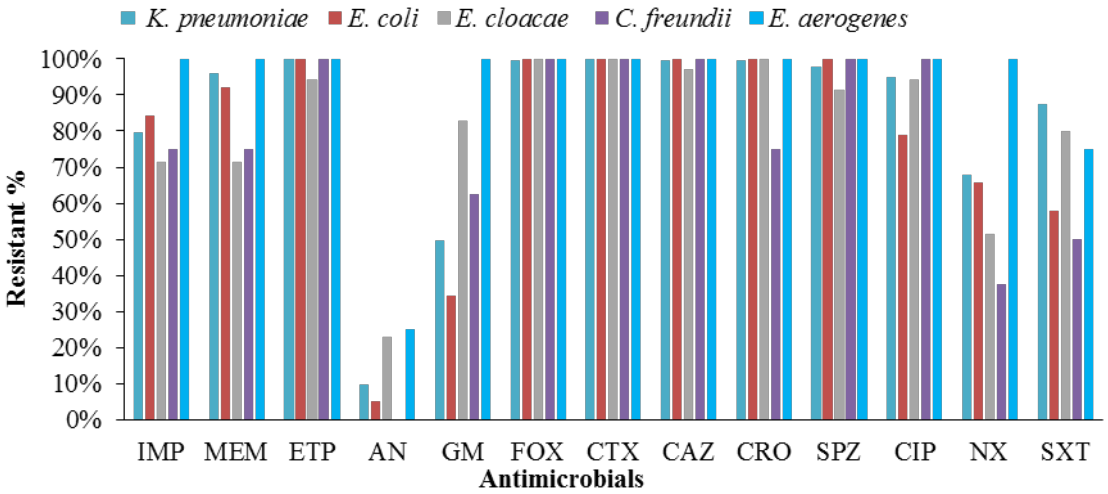

Figure 2 Antimicrobial resistant patterns of different *Enterobacteriaceae* species resistant to carbapenem IMP (imipenem); MEM (meropenem); ETP (ertapenem); AN (amikacin); GM (gentamicin); FOX (cefoxitin); CTX (cefotaxime); CAZ (ceftazidime); CRO (ceftriaxone); SPZ (sulperazone); CIP (ciprofloxacin); NX (norfloxacin); SXT (trimethoprim-sulfamethoxazole).

Antimicrobial susceptibilities

The susceptibility results are shown in **Table 2**. The susceptibility rates to meropenem and imipenem were 8.1 and 20.1 %, respectively. Of the 273 CRE isolates, only the single strain (0.4 %) was susceptible to ertapenem. From **Figure 2**, it is revealed that all of the CRE strains of *K. pneumoniae* (n = 183), *E. coli* (n = 38), *C. freundii* (n = 8), and *E. aerogenes* (n = 4) were resistant to ertapenem. Amikacin, an antimicrobial from the aminoglycosides group, displayed the most potent activity against

CRE, with an overall susceptibility rate of 89.01 % (243/273). A significant difference was observed between susceptibility to amikacin (89.0 %) and to gentamicin (46.9 %). However, there was a statistically significant difference between amikacin and gentamicin, as all the isolates (p-value < 0.05) that were susceptible to gentamicin were also susceptible to amikacin. Almost all of the CRE isolates were resistant to 2nd and 3rd generations of cephalosporins (susceptibility rates ranging from 0 - 3 %). Of the 273 CRE isolates, 2 isolates were found to be susceptible to ceftazidime, 1 isolate to each of cefoxitin and ceftriazone, and 9 isolates to sulperazone. None of the isolates were susceptible to cefotaxime. Only 20 isolates (7.3 %) were susceptible to ciprofloxacin, 98 isolates (35.9 %) to norfloxacin, and 54 (19.8 %) to trimethoprim-sulfamethoxazole.

K. pneumoniae isolates showed high levels of resistance to most of the antibiotics tested. The MIC_{50} for imipenem was 16 µg/mL, and for meropenem and ertapenem was ≥ 32 µg/mL. However, 90 and 50 % susceptibility were reported for amikacin and gentamicin, respectively. *E. coli* isolates were 94 and 65 % sensitive to amikacin and gentamicin, respectively, and none of the isolates were susceptible to cephalosporins. The MIC_{50} and MIC_{90} for all 3 tested carbapenems were ≥ 32 µg/mL for this species. Of 35 *E. cloacae* isolates, only 2 isolates were susceptible to ertapenem, and 10 were found susceptible to meropenem and imipenem. The MIC_{50} and MIC_{90} for ertapenem was ≥ 32 µg/mL. The MIC_{50} was 12 µg/mL, and the MIC_{90} was ≥ 32 µg/mL, for both imipenem and meropenem. All carbapenem-resistant *C. freundii* isolates developed 100 % resistance to 2nd and 3rd generations of cephalosporin, ertapenem, and norfloxacin. The MIC_{50} were, respectively, 1.5, 4, and 4 µg/mL for imipenem, meropenem and ertapenem. The MIC_{90} were all ≥ 32 µg/mL. Interestingly, all were found to be susceptible to amikacin. All *E. aerogenes* isolates showed resistance towards carbapenems, cephalosporins, quinolones, and gentamicin. The MIC_{50} and MIC_{90} of ≥ 32 µg/mL towards all tested carbapenems were reported. However, 3 *E. aerogenes* isolates showed susceptibility to amikacin.

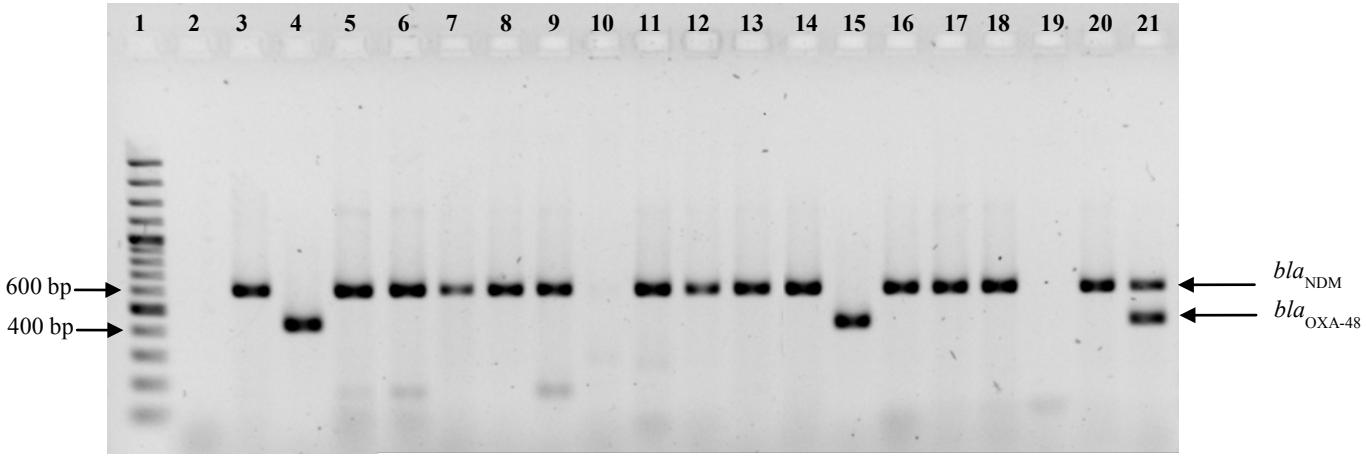

Figure 3 Example of agarose gel electrophoresis (2 %) of multiplex PCR products following amplification with specific primers for carbapenemase genes. Lanes: (1) the 100 bp Sharp Ladder (RBCBioscience, New Taipei City, Taiwan), (2) negative control, (3-20) clinical isolates and (21) positive control for bla_{NDM} (621 bp) and bla_{OXA-48} (438 bp).

Carbapenem resistance mechanisms and antimicrobial susceptibility

Carbapenem resistance in *Enterobacteriaceae* is mainly mediated by the enzymatic mechanism rather than the non-enzymatic mechanism [8]. In addition, carbapenemase is the most common, and major carbapenemase, KPC, GES, VIM, IMP, NDM-1, and OXA-48, are frequently detected in *Enterobacteriaceae* worldwide. Overall, out of 273 CRE, 173 (63.4 %) isolates were carrying a single carbapenemase gene, and 5 (1.8 %) isolates had double carbapenemase genes (**Table 3** and **Figure 3**). Out of 173 isolates, 159 (91.9 %) carried $bla_{\text{NDM-1}}$, and 7 (4.1 %) isolates harbored either the bla_{IMP} or bla_{OXA} gene alone. The remaining 5 isolates carried a dual mechanism for carbapenemase production, including $bla_{\text{NDM-1}}$ + bla_{IMP} (3/273, 1.1 %) and $bla_{\text{NDM-1}}$ + $bla_{\text{OXA-48}}$ (2/273, 0.7 %). Other types of carbapenemase genes, such as bla_{VIM}, bla_{KPC}, bla_{GES}, were not detected in this study. Antimicrobial susceptibility profiles of the *Enterobacteriaceae* harboring carbapenemase genes are summarized in **Table 4**. Ertapenem proved to be a reliable indicator of carbapenemase production, as all of the carbapenemase producing CRE isolates were 100 % non-susceptible to ertapenem. Nearly all CRE isolates were resistant to 2nd and 3rd generation cephalosporins tested in this study, with amikacin being the exception. The overall susceptibility of CRE (n = 273) to amikacin was 89.0 %. The susceptibilities to amikacin of $bla_{\text{NDM-1}}$-carrying *K. pneumoniae*, *E. coli*, and *E. cloacae* were 94.5, 92.0 and 88.2 %, respectively. Generally, most of the NDM-1 producers carry 16sRNA methylases genes and, as a result, show resistance to aminoglycosides [23]. The NDM-1-producing isolates remained susceptible to amikacin, which may be due to the loss of resistant genes which encode for 16sRNA methylase or $bla_{\text{CMY-4}}$ [24,25].

We reported on the high prevalence of NDM-1-producing *Enterobacteriaceae* (60.1 %) among 273 CRE isolates. Our finding was similar to the study by Tran and colleagues, wherein NDM prevalence was 68.1 % in all CRE [26]. A similar finding was observed in a collective survey from different countries, including the UK, India, Pakistan, and Bangladesh [23]. Indian subcontinent areas are supposed to be a reservoir for NDM-1 producing CRE isolates. The probable reason for CRE emergence is due to spread from hospitalized patients, who have a travel history in high risk areas [24,27]. In addition, the $bla_{\text{NDM-1}}$ gene is usually located on a plasmid, which can be easily transferred to other bacterial strains via horizontal gene transfer, resulting in drug-resistant phenotypes [23]. There is a concern of NDM-1 spreading from environment to community settings, as well as in hospitals settings. However, we did not perform environment sampling or clonal relatedness of CRE isolates; the origin or the source of NDM-1 spread in this setting remains unclear.

Table 3 Carbapenemase gene results for 273 CRE isolates.

Organism	No. tested	No. of positive carbapenemase gene results						No. tested negative
		bla_{KPC}	bla_{GES}	bla_{VIM}	bla_{IMP}	$bla_{\text{NDM-1}}$	$bla_{\text{OXA-48}}$	
K. pneumoniae[a]	183	0	0	0	4	115	1	63
E. coli[b]	38	0	0	0	0	26	5	7
E. cloacae[c]	35	0	0	0	1	19	0	15
C. freundii	8	0	0	0	2	2	0	4
E. aerogenes	4	0	0	0	0	1	1	2
P. agglomerans	2	0	0	0	0	1	0	1
K. ozanae	1	0	0	0	0	0	0	1
P. mirabilis	1	0	0	0	0	0	0	1
S. liquefaciens	1	0	0	0	0	0	0	1
Total	273	0	0	0	7	164	7	95

[a]2 *K. pneumoniae* isolates harbored $bla_{\text{NDM-1}}$ and bla_{IMP}
[b]1 *E. coli* isolate harbored $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$
[c]1 *E. cloacae* isolate harbored $bla_{\text{NDM-1}}$ and bla_{IMP} and 1 *E. cloacae* isolate harbored $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$

Table 4 Antimicrobial susceptibility of CRE strains harboring carbapenemase gene.

Antimicrobial agent	Susceptible strain [n, (%)]				
	bla_{NDM-1}			bla_{IMP}	bla_{OXA-48}
	***K. pneumoniae* (N=115)**	***E. coli* (N=26)**	***E. cloacae* (N=19)**	***K. pneumoniae* (N=4)**	***E. coli* (N=5)**
Imipenem	4, (3.5)	0, (0.0)	2, (11.8)	0, (0.0)	3, (60.0)
Meropenem	1, (0.9)	0, (0.0)	2, (11.8)	0, (0.0)	1, (20.0)
Ertapenem	0, (0.0)	0, (0.0)	0, (0.0)	0, (0.0)	0, (0.0)
Amikacin	107, (94.7)	23, (92.0)	15, (88.2)	2, (50.0)	5, (100.0)
Gentamicin	63, (55.8)	16, (64.0)	4, (23.5)	2, (50.0)	0, (0.0)
Cefoxitin	0, (0.0)	0, (0.0)	0, (0.0)	0, (0.0)	0, (0.0)
Cefotaxime	0, (0.0)	0, (0.0)	0, (0.0)	0, (0.0)	0, (0.0)
Ceftazidime	1, (0.9)	0, (0.0)	1, (5.9)	0, (0.0)	0, (0.0)
Ceftriaxone	1, (0.9)	0, (0.0)	0, (0.0)	0, (0.0)	0, (0.0)
Sulperazone	2, (1.8)	0, (0.0)	2, (11.8)	0, (0.0)	0, (0.0)
Ciprofloxacin	6, (5.3)	6, (24.0)	1, (5.9)	1, (25.0)	0, (0.0)
Norfloxacin	45, (39.8)	11, (44.0)	13, (76.5)	2, (50.0)	0, (0.0)
Trimethoprim-sulfamethoxazole	15, (13.3)	12, (48.0)	4, (23.5)	1, (25.0)	0, (0.0)

Conclusions

To our knowledge, this is the first study conducted in the southern region of Thailand that has reported the high prevalence of NDM-1 among CRE isolates. Most CRE isolates showed resistance to almost all antimicrobial agents, except amikacin. *K. pneumoniae* harboring bla_{NDM-1} was the most prevalent in this setting. Our study is the first to investigate and report on NDM-1-producing *E. cloacae* from Thailand. The prevalence of CRE was higher in certain specific wards; therefore, strict prevention and infection control measures should be taken to prevent the spread of CRE in hospitals, as well as in the community.

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