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ORIGINAL RESEARCH

Association Between Types of Carbapenemase and Clinical Outcomes of Infection Due to Carbapenem Resistance Enterobacterales

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Purpose: Compared with non-carbapenemase producing carbapenem-resistant Enterobacterales (non-CP-CRE), carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) are associated with considerable mortality. However, given that the patients are treated with various therapeutic options, it remains unclear whether differences in types of carbapenemase genes yield different mortality rates. Therefore, this study aims to identify carbapenemase genes and identify whether clinical outcomes differ according to the prevalence of genotype and phenotype of carbapenemase among Enterobacterales clinical isolated.

Patients and Methods: A retrospective cohort study was performed to determine whether types of carbapenemase genes have an impact on clinical outcomes. Carbapenem-resistant clinical isolates were collected at a tertiary care university hospital in Songkhla, Thailand, between June 2018 and February 2020. Demographic and microbiological data such as antimicrobial susceptibility, carbapenemase genes, and overall mortality were evaluated.

Results: A total of 121 Enterobacterales clinical isolated were evaluated. The $bla_{\text{NDM-1}}$ gene was detected in 44% of the isolates, followed by $bla_{\text{OXA-48}}$ (28%) and $bla_{\text{NDM-1/OXA-48}}$ (28%). NDM-1- or NDM-1/OXA-48- producing isolates were more likely to require meropenem MICs of ≥16 mg/L, while OXA-48-producing isolates were more likely to require meropenem MICs of <16 mg/L. The patients with NDM-1 or NDM-1/OXA-48 had a higher 14 days mortality rate than those with OXA-48 after treating with carbapenem-containing regimens (*P*-value 0.001) or colistin-containing regimens (*P*-value < 0.001).

Conclusion: Our findings suggest that the mortality for CP-CRE infection in patients with NDM-1 or NDM-1/OXA-48 was higher than the mortality in those with OXA-48, which It seems that the type of carbapenemase gene may affect meropenem MIC levels. Hence, in treatment decisions involving the use of either carbapenem-containing regiment or colistin-containing regiment in patients with CP-CRE infection, especially those in the NDM-1 and NDM-1/OXA-48 groups, the patient symptoms should be closely monitored.

Keywords: carbapenemase, carbapenem resistance Enterobacterales, NDM-1, OXA-48, NDM-1/OXA-48

Introduction

The emergence of carbapenem-resistant Enterobacterales (CRE) has become a major public health crisis worldwide over the last decade, because of their rapid spread and the lack of development of new antimicrobial drugs.[1–3] When found in clinical culture, CRE can represent an infection or colonization. Colonization means that the organism can be found in or on the body but it is not causing any symptoms or disease. Colonizing CRE strains can go on to cause infections or spread to other patients.[4]

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A variety of molecular mechanisms are thought to mediate carbapenem resistance, including the carbapenemase-production, the production of extended-spectrum beta-lactamase (ESBLs) and/or AmpC cephalosporinase (AmpC) combined with altered membrane permeability caused by the loss of outer membrane porin or active drug efflux (non-CP-CRE).[5,6]

There are 3 classes of carbapenemase enzymes, as classified in the Ambler classification. The most common carbapenemases reported from different geographical regions are class A serine beta-lactamases (KPC and GES), class B metallo-beta-lactamases or known as MBLs (NDM, IMP, and VIM), and class D oxacillinase (OXA).[1,7,8] The global spread of CRE has occurred involving different epidemic strains across the region.[1,8] CRE have been increasingly detected in Southeast Asia, including Thailand.[9–11] In Thailand, the main carbapenemase enzymes were MBLs (bla_{NDM} and $bla_{\text{IMP-14}}$) and OXA types.[11–13] A recent report from Thailand revealed the two most common genotypes among CRE isolates were bla_{NDM}, of which 95.63% were the $bla_{\text{NDM-1,}}$ and bla_{OXA} ($bla_{\text{OXA-48,-181,-232}}$) of 50.22%.[13] Furthermore, Paveenkittiporn et al showed data of all the CRE isolates carried *mcr* genes were 0.3% in 2016–2019.[14] The *mcr* gene has been shown to encode a phosphoethanolamine transferase that alters lipid A in the lipopolysaccharide of the bacterial outer membrane by adding a phosphoethanolamine.[15] This reduces the attachment of colistin to the bacterial outer membrane and, therefore, prevents cell lysis. The production of carbapenemase is commonly associated with infection control and increased mortality compared with carbapenem-susceptible strain.[16–19] NDM-producers are of particular concern as they also harbor multiple chromosomally and plasmid-encoded resistance genes resulting in a multi-drug-resistant.[20,21] NDM can impair the efficacy of almost all beta-lactams (except aztreonam), and the therapeutic options for infection are mostly limited to polymyxin, tigecycline, fosfomycin and cefiderocol,[22,23] whereas the minimal inhibitory concentrations (MICs) of carbapenems against OXA-48-type producers range between 0.5 and ≥64 mg/L for ertapenem, 1 and ≥64 mg/L for Imipenem, and 1 and ≥64 mg/L for meropenem.[24] The OXA-48-type producers with low MICs, categorized as susceptible to carbapenems by the EUCAST and CLSI guidelines.[24–26]

In 2017, the World Health Organization (WHO) pointed out that the attributable mortality rate of CRE infection had reached more than 26%.[27] Invasive CRE infections have been associated with mortality rates of 40% to 50%.[28] Moreover, the mortality was 14% higher in the non-susceptibility to other carbapenems (imipenem, meropenem, or doripenem) Enterobacteriaceae (NSOCE) group compared to the nonsusceptibility to ertapenem alone Enterobacteriaceae (NSEE) group at a tertiary care hospital in Thailand.[29]

Although many reports demonstrated different kinds of treatment options for CRE infection,[1,30–32] the mortality of CRE bloodstream infection was 38.5% in patients receiving appropriate treatment in a retrospective international cohort study conducted in ten countries.[33]

Since the prevalence of infections due to CRE is expected to increase, screening for carbapenemase-production and the specific type of carbapenemase produced is important to guide treatment decisions. Therefore, we aimed to identify carbapenemase gene in CRE and correlate it with clinical outcomes by the prevalence of genotype and phenotype of carbapenemase among clinical Enterobacterales isolated in Thailand.

Materials and Methods

Study Design and Patients

This retrospective cohort study was performed in patients with CRE infection who were hospitalized at Songklanagarind Hospital in Southern Thailand between June 2018-February 2020. Patient data were collected via chart review and include the following: demographics, preexisting medical conditions, source of infections, microbiological data, antibiotic therapy, and clinical outcome data. Consistent with the current Centers for Disease Control and Prevention (CDC) definition, CRE was defined as Enterobacterales isolates demonstrating resistance to any carbapenem (ertapenem, meropenem, imipenem, and/or doripenem). Patients were excluded if they were discharged or expired within 3 days of infection onset, which was before the results of antimicrobial susceptibility testing (AST) were available for the treatment of patients.

This retrospective study was approved by the Institutional Review Board of the Faculty of Medicine, Prince of Songkla University with EC: 63-021-14-1 for clinical data from medical record review and microbiological data

extraction. The researchers were granted permission to extract the data from the database with waiver of consent. All data were fully anonymized before the researcher accessed and analyzed them. Medical records of patients admitted between 1 June 2018 and 29 February 2020 were used in the study. The author also confirmed that this current study was this study was conducted in accordance with the Declaration of Helsinki.

Inclusion Criteria for the CRE Isolates

CRE isolates were obtained from the clinical Microbiology Laboratory (CML), Songklanagarind Hospital between June 2018 and February 2020. These isolates were collected from different clinical specimens. Duplicate CRE isolates (ie, those of the same species from the same specimen type) from the same patient in the same year were excluded. We studied susceptibility using automated systems or disk diffusion and interpreted it using the 2020 Clinical and Laboratory Standards Institute (CLSI) breakpoints for defined CRE isolates.[25] 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* spp., *Proteus* spp., or *Morganella morganii* that demonstrated an MIC of >1 μg/mL for imipenem alone were determined by meropenem and ertapenem.

Bacterial Identification and Detection of Carbapenemase Production

The species of 736 CRE isolates were confirmed using Matrix-Assisted Laser-Desorption Ionization Time-of-Flight mass spectrometry (MALDI-ToF MS). The production of carbapenemases in all CRE isolates was determined using a modified carbapenem inactivation method (mCIM) according to CLSI guidelines.[25]

Antimicrobial Susceptibility Testing

The MICs of amikacin, amoxicillin/clavulanic, aztreonam, cefotaxime, ceftazidime, ceftazidime/avibactam, ciprofloxacin, colistin, gentamicin, ertapenem (concentration range 0.12–2), Imipenem (concentration range 0.5–16), meropenem (concentration rage 0.12–16), piperacillin-tazobactam, tigecycline and trimethoprim-sulfamethoxazole were evaluated in the CRE isolates using the automated microbroth dilution testing systems (Sensititre™ Vizion™ system; ThermoFisher Scientific, Waltham, MA, USA). However, the susceptibilities to ampicillin, amoxicillin, cefepime, cefoxitin, ceftriaxone and trimethoprim-sulfamethoxazole were tested using the disk diffusion method, fosfomycin susceptibility was checked using the agar dilution method. All MIC results were interpreted according to the CLSI guidelines.[25] and *E. coli* ATCC 25922™ was used for quality.

Detection of Carbapenemase and Mobilized Colistin Resistance (*mcr*) Genes

Genomic DNA of all CRE isolates was extracted using the DNeasy Blood & Tissue Kit (QIAGEN, Inc., Valencia, California). The most prevalent carbapenemase genes (eg, bla_{IMP}, bla_{VIM}, bla_{OXA-48}, bla_{NDM-1}, and bla_{KPC}) and the *mcr-1* gene were investigated by multiplex PCR using previously reported primers.[34,35]

Endpoints

The primary outcome was 14-day mortality, with day 1 as the day the first positive culture was collected. Fourteen-day mortality was selected as the primary endpoint, as it was thought to be most reflective of death attributable to CRE infection.

Statistical Methods

Descriptive statistics for patient variables were calculated using mean (standard deviation) or frequency count (percentage), as appropriate. The Pearson x^2 test and Fisher's exact test were used for cells with a frequency of 5 or fewer, for categorical variables. The relationship between variables and outcomes was evaluated using univariable logistic regression, as summarized by odds ratios (ORs) and corresponding 95% confidence interval (CIs). Covariates found to have a P-value <0.10 on univariant analysis and resulted in a ≥ 10% change in the parameter estimate of variable were retained in the final multivariable logistic regression models for each outcome. All test was 2-tailed, and P values ≤0.05 were used for statistical significance testing. Analyses were performed using the STATA 16.0 (Stata Corp) statistical package.

Efficacy

Results

Patient Demographics and CRE Characteristics

In this study, all CREs are carbapenemase producers. Demographics and baseline characteristics for patients with CP-CRE infection are summarized in Table 1. Only 121 non-duplicate CP-CRE isolates from the patients with CRE infection that met the study inclusion criteria were included in the analysis. Ventilator-associated pneumonia (VAP) and urinary tract infections were the most frequent sites of infection, with 58 (47.9%) and 37 (30.63%) cases, respectively. The highest mortality were found in 20% of the patients with Hospital-acquired pneumonia (HAP) and VAP.

The most common type of sample was sputum (30.6%) followed by urine (24.8%), blood (23.1%), ascites (12.4%), and others (9.1%). The predominant CP-CRE infection species were *Klebsiella* spp. (92/121; 76%), *E. coli* (21/121; 17%) and other Enterobacterales (8/121; 6%).

The majority of CP-CRE isolates carried $bla_{\text{NDM-1}}$ (44%), followed by $bla_{\text{OXA-48}}$ (28%) and $bla_{\text{NDM-1/OXA-48}}$ (28%). Other carbapenemase genes (bla_{IMP}, bla_{VIM}, and bla_{KPC}) and the *mcr-1* gene were not identified in these isolates. Overall, the CP-CRE isolates were more likely to require meropenem MICs of >16 mg/L (75.21%) for growth inhibition and colistin MICs of ≥2 mg/L (20.66%). We found that the NDM-1 group was more likely to require meropenem MICs of >16 mg/L (47/121; 88.68%) and colistin MICs of ≥2 (9/121; 16.98%) for growth inhibition. The characteristic of meropenem MIC distribution in the NDM-1/OXA-48 group was similar to that in the NDM-1 group since there were 31 out of 121 isolates (91.18%) which required meropenem MICs of >16 mg/L. In addition, the NDM-1/OXA-48 group had colistin MICs of ≥2 (6/121; 17.65%).

Although most isolates in the OXA-48 group required meropenem MICs of >16 mg/L (13/121; 38.24%), we found that meropenem MIC distribution in this group was more varied than that in NDM-1 and NDM-1/OXA-48 groups as shown in Table 1. The OXA-48 group had colistin MICs of ≥2 mg/L (10/121; 29.41%).

Antimicrobial Susceptibility

A total of 121 strains of CRE from unique patients were isolated. The susceptibility result of antimicrobial is shown in Table 2. Among 121 isolates, the most susceptible agent was amikacin (90% for NDM-1, 97.1% for OXA-48, and 97.1% for NDM-1/OXA-48). However, we found that all CP-CRE groups had MIC_{90} of 16 mg/L, which is a borderline of amikacin MIC cutoff value. Tigecycline had a good susceptibility to CP-CRE and was second only to amikacin. The susceptible rates to NDM-1, OXA-48, and NDM-1/OXA-48 are 93.6%, 80.7%, and 82.29%, respectively. For Beta-lactams, all CP-CRE groups were not susceptible to ertapenem and ceftazidime, except for OXA-48 (susceptibility rate of 5.88%). NDM-1 (1.9%) and NDM-1/OXA-48 (2.94%) had low susceptibility to meropenem and imipenem.

Ceftazidime/Avibactam showed susceptibility rate of 70.6% for OXA-48. However, ceftazidime-avibactam was susceptible to NDM-1 type for 3.77% and to NDM-1/OXA-48 type for 2.94%. Among these isolates, colistin MIC ≤ 2 mg/L, which was interpreted as intermediate susceptibility, exhibited susceptibility rate of 90.6% for NDM-1 and 85.3% for NDM-1/OXA-48, whereas the susceptibility rate of colistin to OXA-48 was only 79.4%.

The NDM-1 and NDM-1/OXA-48 isolates were more likely to require meropenem MICs of ≥16 mg/L for growth inhibition, while the OXA-48 isolates were more likely to require meropenem MICs of <16 mg/L.

Clinical Outcomes and Risk Factors Associated with the 14-Day Mortality

Among 121 patients with CP-CRE infection, a total of 40 (33%) patients died within 14 days. In this study, most patients were mainly treated with combination therapy. All patients were treated with currently standard doses of drugs and adjusted according to creatinine clearance for patients with chronic kidney disease.[36–38] The regimens in this medical treatment had at least one active antibiotic based on in-vitro susceptibility testing. However, the antibacterial activities of some regimens in the study had some overlap. The most overlap was detected in meropenem combined colistin (24%). Additionally, 19 patients (15.7%) received active monotherapy treatment based on in-vitro susceptibility testing. These patients were usually diagnosed with UTI. We found mortality in only 2 patients who received active monotherapy treatment. Overall, there was no significant difference in the mortality outcomes of patients with CP-CRE infection using different antimicrobial agents. However, in the analysis of differences in specific carbapenemase genes, we compared 2 patient groups, the NDM-1 with

Table 1 Demographic and Baseline Characteristics Data of 121 Patients and Clinical Isolates with Carbapenemase-Producing Carbapenem-Resistant Enterobacterales Infections

Variable	Isolates Carrying Carbapenemase Genes			
	Total N=121 (100%)	**NDM-1 N=53 (43.80%)**	**OXA-48 N=34 (28.10%)**	**NDM-1/OXA-48 N=34 (28.10%)**
Male	75 (61.98)	37 (69.81)	19 (55.88)	19 (55.88)
Age (Mean, SD)	67 (17.73)	67 (18.55)	66 (15.83)	66 (18.68)
Acquisition of infection ≥ 48 hr	103 (85.12)	41 (77.36)	30 (88.24)	32 (94.12)
Ward at the onset of Infection				
Intensive care unit, day 1	18 (14.88)	4 (7.55)	4 (11.76)	10 (29.41)
General care unit, day1	103 (85.12)	49 (92.45)	30 (88.24)	24 (70.59)
Preexisting medical conditions				
Cancer	56 (46.28)	26 (49.06)	15 (44.12)	15 (44.12)
Diabetes	32 (26.45)	14 (26.42)	8 (23.53)	10 (29.41)
Chronic kidney disease	13 (10.74)	2 (3.77)	5 (14.71)	6 (17.65)
Chronic lung disease ie COPD	7 (5.79)	5 (9.43)	2 (5.88)	0
Respiratory failure	11 (9.09)	6 (11.32)	2 (5.88)	3 (8.82)
Cirrhosis	9 (7.44)	3 (5.66)	3 (8.82)	3 (8.82)
Cardiovascular disease	21 (17.36)	12 (22.64)	5 (14.71)	4 (11.76)
Congestive heart failure	11 (9.09)	6 (11.32)	1 (2.94)	4 (11.76)
Immunocompromised				
Chemotherapy within the previous 6 months	17 (14.05)	8 (15.09)	6 (17.65)	3 (8.82)
Human immunodeficiency virus infection	2 (1.65)	2 (3.77)	0	0
Chronic corticosteroid therapy	15 (12.40)	9 (16.98)	1 (2.94)	5 (14.71)
ANC < 200 cells/mL on day 1 of CRE infection	13 (10.74)	6 (11.32)	0	0
APACHE-II score (Mean, SD)	18 (8.37)	18 (7.37)	19 (9.93)	20 (8.27)
qSOFA score ≥ 2	92 (76.03)	38 (71.70)	25 (73.53)	29 (85.29)
Septic shock	20 (16.53)	7 (13.21)	7 (20.59)	6 (17.65)
Pathogens				
Klebsiella pneumoniae	92 (76.03)	31 (58.49)	29 (85.29)	32 (94.12)
Escherichia coli	21 (17.36)	18 (33.96)	3 (8.82)	0
Serratia marcescens	1 (0.83)	0	1 (2.94)	0
Proteus mirabilis	2 (1.65)	0	0	2 (5.88)
Enterobacter spp.	4 (3.31)	4 (7.55)	0	0
Klebsiella aerogenes	1 (0.83)	0	1 (2.94)	0
Source of infections				
Pneumonia (all)	39 (32.23)	17 (32.08)	12 (35.29)	10 (29.41)
Community acquire pneumonia	3 (2.48)	2 (3.77)	1 (2.94)	0
Hospital acquire pneumonia	15 (12.40)	8 (15.09)	2 (5.88)	5 (14.71)
Ventilator acquire pneumonia	21 (17.36)	7 (13.21)	9 (26.47)	5 (14.71)
Urinary tract	34 (28.10)	20 (37.74)	4 (11.76)	10 (29.41)
Intra-abdominal	21 (17.36)	5 (9.43)	9 (26.47)	7 (20.59)
Biliary	2 (1.65)	2 (3.77)	0	0
Catheter-related	5 (4.13)	0	1 (2.94)	4 (11.76)
Skin and soft tissue	3 (2.48)	2 (3.77)	1 (2.94)	0
Surgical site	5 (4.13)	2 (3.77)	2 (5.88)	1 (2.94)

(*Continued*)

Table 1 (Continued).

Variable	Isolates Carrying Carbapenemase Genes			
	Total N=121 (100%)	NDM-1 N=53 (43.80%)	OXA-48 N=34 (28.10%)	NDM-1/OXA-48 N=34 (28.10%)
Bacteremia	8 (6.61)	3 (5.66)	3 (8.82)	2 (5.88)
Others	4 (3.31)	2 (3.77)	2 (5.88)	0
Treatment				
Monotherapy	47 (38.84)	25 (47.17)	9 (26.47)	13 (38.24)
Combination therapy	74 (61.16)	28 (52.83)	25 (73.53)	21 (61.76)
Regimens				
Carbapenems	49 (40.55)	18 (33.96)	17 (50)	14 (41.18)
Colistin[a]	64 (52.89)	21 (39.62)	23 (67.65)	20 (58.82)
Aminoglycosides	22 (18.18)	12 (22.64)	4 (11.76)	6 (17.65)
Fosfomycin	21 (17.36)	8 (15.09)	6 (17.65)	7 (20.59)
Tigecycline	7 (5.79)	2 (3.77)	3 (8.82)	2 (5.88)
Minimum inhibitory concentration, mg/L				
Colistin, MIC ≥ 2[b]	25 (20.66)	9 (16.98)	10 (29.41)	6 (17.65)
Meropenem, MIC ≤ 0.25	3 (2.48)	-	3 (8.82)	-
Meropenem, MIC 0.5	4 (3.31)	1 (1.89)	3 (8.82)	-
Meropenem, MIC 1	7 (5.79)	-	6 (17.65)	1 (2.94)
Meropenem, MIC 2	5 (4.13)	1 (1.89)	4 (11.76)	-
Meropenem, MIC 4	2 (1.65)	-	2 (5.88)	-
Meropenem, MIC 8	5 (4.13)	1 (1.89)	3 (8.82)	1 (2.94)
Meropenem, MIC 16	4 (3.31)	3 (5.66)	-	1 (2.94)
Meropenem, MIC > 16	91 (75.21)	47 (88.68)	13 (38.24)	31 (91.18)

Notes: [a]Colistin containing regimens based on dosing guidance for Intravenous colistin to achieve an adequate plasma concentration of colistin with colistin MIC of 1–2 mg/L.[52] [b]Susceptibility to colistin is defined as MIC ≤ 2 mg/L and resistance to colistin is MIC > 2 mg/L.

Abbreviations: CP-CRE, carbapenemase-producing carbapenem-resistant Enterobacterales; SD, standard deviation; APACHE-II score, Acute Physiology and Chronic Health Evaluation II score; qSOFA, quick SOFA score; ANC, absolute neutrophil count.

NDM-1/OXA-48 and OXA-48 to differentiate MBLs and non-metallo-beta-lactamases (non-MBLs). The analysis suggested that patients in the NDM-1 with NDM-1/OXA-48 groups have a higher mortality rate using either carbapenem-containing regiment or colistin-containing regiment than those in the OXA-48 groups (Table 3). The univariate analysis results revealed statistically significant risk factors, whereas the multivariate analysis indicated that, only Acute Physiology and Chronic Health Evaluation II score ≥15 (odds ratio (OR) 4.49, 95% confidence interval (CI) 1.00–20.03), and Meropenem MIC ≥16 (OR 8.40, 95% CI 1.71–41.18) were significant predictors for death (Table 4).

Discussion

In this study, the most common type of sample was sputum followed by urine, blood, ascites, and others. The predominant CRE infection species were *Klebsiella* spp. Meropenem was selected as a representative of the carbapenem class since, compared with other medications in the class, this medication was frequently to treat patients with CP-CRE infection. The results suggest that patients with CP-CRE harboring NDM-1 or NDM-1 combined OXA-48 were more likely to require meropenem MICs of ≥16 mg/L for growth inhibition, while the OXA-48 group was more likely to require meropenem MICs of ≤16 mg/L. Infection types of most patients in this study were VAP and UTI. We found that the highest mortality was found in 20% of the patients with HAP and VAP. Overall, there was no significant difference in the mortality outcomes of patients with CP-CRE infection treated with different antimicrobial agents. However, the analysis of differences in specific carbapenemase genes, compared 2 patient groups, namely, NDM-1 with NDM-1/OXA-48 and OXA-48 to differentiate MBLs and non-MBLs. The analysis suggested that patients in the NDM-1 with NDM-1

Table 2 Antimicrobial Susceptibility in CP-CRE Producing NDM-1, OXA-48 or NDM-1/OXA-48

Drug	Breakpoint Susceptibility (mg/L)	CP-CRE N (%S)	NDM-1 Producers (n=53)					OXA-48 Producers (n=53)					NDM-1/OXA-48 Producers (n=53)				
			MIC Range		MIC50	MIC90	%S	MIC Range		MIC50	MIC90	%S	MIC Range		MIC50	MIC90	%S
			Min	Max				Min	Max				Min	Max			
Aminoglycosides																	
GEN	≤4	93 (76.86)	≤0.5	>8	1	>8	79.25	≤0.5	>8	1	>8	55.88	≤0.5	>8	1	1	94.12
AMK	≤16	114 (94.21)	≤4	>32	8	16	90.57	≤4	>32	8	16	97.06	≤4	>32	8	16	97.06
Beta-lactams																	
MEM	≤1	15 (12.40)	0.5	>16	>16	>16	1.89	≤0.25	>16	4	>16	38.24	1	>16	>16	>16	2.94
IMP	≤1	18 (14.88)	1	>16	>16	>16	1.89	≤0.5	>16	2	>16	47.06	1	>16	>16	>16	2.94
ETP	≤0.5	2 (1.65)	>2	>2	>2	>2	0	≤0.12	>2	>2	>2	5.88	>2	>2	>2	>2	0
CAZ	≤4	2 (1.65)	>16	>16	>16	>16	0	≤0.5	≥16	>16	>16	2.94	>16	>16	>16	>16	0
Beta-lactam/beta-lactamase inhibitor																	
TZP	≤16/4	1 (0.83)	>32/4	>32/4	>32/4	>32/4	0	16/4	>32/4	>32/4	>32/4	2.94	>32/4	>32/4	>32	>32	0
CZA	≤8/4	28 (23.14)	2/4	>16/4	>16	>16	3.77	≤0.5	>16	1	>16	70.59	≤0.5	>16	>16	>16	2.94
Cyclines																	
TGC	≤1	108 (89.26)	≤0.25	4	0.5	1	93.62	≤0.25	4	0.5	2	80.65	≤0.25	4	0.5	1	82.29
Fluoroquinolones																	
CIP	≤0.25	6 (4.96)	≤0.06	>2	>2	>2	5.66	≤0.06	>2	>2	>2	50	1	>2	>2	>2	0
Monobactams																	
ATM	≤4	6 (4.96)	≤0.5	≥32	>16	>16	5.66	≤0.5	>32	>16	>16	5.88	≤0.5	>32	>16	>16	2.94
Polymyxins[a]																	
CST	≤2	104 (85.95)	1	>8	1	2	90.57	1	>8	1	>8	79.41	1	>8	1	4	85.29
Other antibiotic																	
FOF	≤64	94 (77.69)	0.25	>512	>32	>32	48.94	0.5	>512	>512	>512	21.28	2	>512	>512	>512	29.79
SXT	≤2/38	23 (19.01)	≤1/9	>8/152	>8/152	>8/152	50	≤1/9	>8/152	>8/152	>8/152	29.41	>8/152	>8/152	>8/152	>8/152	0

(*Continued*)

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Table 2 (Continued).

Drug	Breakpoint Susceptibility (mg/L)	CP-CRE N (%S)	NDM-1 Producers (n=53)					OXA-48 Producers (n=53)					NDM-1/OXA-48 Producers (n=53)				
			MIC Range		MIC50	MIC90	%S	MIC Range		MIC50	MIC90	%S	MIC Range		MIC50	MIC90	%S
			Min	Max				Min	Max				Min	Max			
Carbapenem susceptibility of NDM, OXA-48 or NDM-1/OXA-48																	
Drugs	**Breakpoint Susceptibility (mg/L)**	**NDM-1 Producers (n=53)**					**OXA-48 –Producers (n=34)**					**NDM-1/OXA-48 -Producers (n=34)**					
		MIC < 16		**MIC ≥16**			**MIC < 16**		**MIC ≥16**			**MIC < 16**		**MIC ≥16**			
MEM	≤1	6		47			21		13			3		31			
IPM	≤1	2		51			22		12			2		32			

Note: [a]Susceptibility to colistin is defined as MIC ≤ 2 mg/L and resistance to colistin is MIC > 2 mg/L.

Abbreviations: AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CZA, ceftazidime-avibactam; CIP, ciprofloxacin; CST, colistin; ETP, ertapenem; FOF, Fosfomycin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; SXT, trimethoprim-sulfamethoxazole; TGC, tigecycline; TZP, piperacillin-tazobactam.

Table 3 Outcome of Patients with Different Carbapenemase-Producing-CRE According to Treatment Regimens

Regimens	CP-CRE	NDM-1+NDM-1/OXA-48		OXA-48		*P*-value
	Total	Survived N, (%)	Died N, (%)	Survived N, (%)	Died N, (%)	
Carbapenem-containing regimen	49	18 (22.9)	14 (9.1)	17 (12.1)	0 (4.9)	0.001
Colistin-containing regimen	64	26 (31.4)	15 (9.6)	23 (17.6)	0 (5.4)	< 0.001
Aminoglycoside-containing regimen	22	14 (14.7)	4 (3.3)	4 (3.3)	0 (0.7)	0.554
Fosfomycin-containing regimen	21	12 (12.9)	3 (2.1)	6 (5.1)	0 (0.9)	0.526
Tigecycline-containing regimen	7	3 (3.4)	1 (0.6)	3 (2.6)	0 (0.4)	1.000

Table 4 Factors Associated with an All-Cause 14-Day Mortality of 121 Patients with CP-CRE Infections

Covariate	Odds Ratio (95% CI)	*P*-value	Adjusted Odds Ratio (95% CI)	*P*-value
Septic shock	3.91 (1.50–10.19)	0.008		
Pitt bacteremia score ≥ 4 on day 1	3.76 (1.69–8.38)	0.001		
APACHE-II score ≥ 15	10.90 (3.66–32.45)	< 0.001	4.49 (1.00–20.03)	0.049
Cancer	2.69 (1.24–5.83)	0.020		
Cirrhosis	4.59 (1.19–17.63)	0.058		
Chemotherapy within the previous 6 months	9.27 (3.19–26.96)	<0.001		
Chronic corticosteroid therapy	3.63 (1.24–10.58)	0.036		
ANC < 200 cells/mL on day 1 of infection	5.59 (1.76–17.72)	0.009		
Hospital acquire pneumonia	2.64 (0.90–7.72)	0.086		
Urinary tract	0.26 (0.09–0.70)	0.009		
Catheter-related	8.89 (1.35–58.65)	0.041		
ICU setting	5.36 (1.96–14.64)	0.002		
Meropenem, MIC ≥ 16	4.25 (1.45–12.48)	0.008	8.40 (1.71–41.18)	0.009

Abbreviations: CP, carbapenemase-producing; CRE, carbapenem-resistant Enterobacterales; APACHE-II score, Acute Physiology and Chronic Health Evaluation II score; ANC, absolute neutrophil count; ICU, intensive care unit.

combined OXA-48 group had a higher mortality rate to either carbapenem-containing regiment or colistin-containing regiment than those in the OXA-48 group.

In this study, we found that 17 isolates (14%) of CRE were not susceptible to colistin (MIC>2). However, 11 patients were still treated with colistin-based regimen even CRE isolates were not susceptible to colistin. We searched for mechanism of colistin resistance by using detection of *mcr* genes, since these genes are related to colistin resistance. However, in the study, we did not find *mcr-1* genes in all CP-CRE isolates, possibly due to NDM-1 and OXA-48 types of carbapenemase. These gene types caused *crrB* gene transformation which resulted in colistin resistance, since there was a modification in lipopolysaccharide of the outer membrane of gram-negative bacteria.[39] Other gene types such as *mgrB* could be the cause,[40,41] however, we did not include them in our study.

The result indicated that patients with APACHE II score ≥15 had about 4 times the odds of mortality within 14 days after the severity of illness on day 1 of CRE infection has been accounted for. The CP-CRE with meropenem MIC ≥ 16 had about 8 times the odds of mortality within 14 days after antibiotic treatment was administered.

This finding suggests that CP-CRE isolates, especially MBLs, are of particular concern about medical treatment rather than those in non-MBLs group since meropenem MICs of ≥16 mg/L were identified in NDM-1 and NDM-1/OXA-48 groups. In addition, the study suggests that, regardless of whether carbapenem-containing regiment or colistin-containing regiment was used, the mortality rate was high.

Previous studies have demonstrated mortality from CRE infection ranging from approximately 20% to 70%. Moreover, many studies have evaluated the risk factors related to bloodstream infections with multidrug-resistant Enterobacterales. For example, Carbapenemase-production, bacteremia, Pitt bacteremia score ≥4, polymyxin therapy administered, and APACHE-II score ≥15 have been considered independent risk factors for CRE infections.[19,42,43]

Although new medications such as ceftazidime-avibactam, meropenem-vaborbactam, imipenem-cilastatin-relebactam, and cefiderocol are employed to treat CRE, epidemiological data, especially genotypes, should be considered when making treatment decisions regarding these medications. Several previous studies have attempted to test for the most appropriate treatment option for CRE infection.[44–47] However, due to the differences in factors influencing outcomes, those studies do not provide similar outcomes possibly. As a result, at present, there are no clear conclusions on the optimal medicine for the treatment of CRE. In addition, the previous studies are limited, since no description of the mechanism of carbapenem resistance is included and genotypic identification especially MBLs predominates. Moreover, only a few studies have investigated this; thus, additional data gathering is required to achieve better treatment outcomes.

The distribution of carbapenemase genes in Thailand also differs from those from other parts of the world. The most common genes were $bla_{\text{NDM-1}}$, $bla_{\text{IMP-14}}$, and bla_{OXA}, regardless of the organism.[11–13,48] These results support previous studies, showing that NDM is the most common gene in South and Southeast Asia.[1,48,49] However, the results differ from the United States, the most commonly identified carbapenemase is KPC.[1,49]

To our knowledge, this is the first study that has correlated clinical outcomes to the genotype and phenotype of carbapenemase genes among clinical Enterobacterales isolated. With regard to treatment options using either carbapenem-containing regimens or colistin-containing regimens, certain isolates demonstrated colistin MIC of ≤2. However, the appropriate dosage regimens needed to achieve adequate plasma concentrations were colistin MIC of 1–2 mg/L. Moreover, the 14 days mortality rate in patients with NDM-1 or NDM-1/OXA-48 was higher than the rate in those with OXA-48. Although the bacteria were susceptible to aminoglycoside and tigecycline, using these medications in the treatment did not yield different mortality outcomes among the different genotypes.

Ceftazidime-avibactam has previously demonstrated in vitro activities against non-MBL, including isolates that carry AmpC and ESBL enzymes.[50,51] However, in this study only the presence of the MBL gene, NDM-1, and NDM-1 combined with OXA-48, were associated with in vitro resistance to ceftazidime-avibactam. In addition, 70.5% of OXA-48-producing isolates were susceptible to ceftazidime-avibactam. The current study identified 10 (29.5%) OXA-48 producing CP-CRE isolates with reduced ceftazidime-avibactam susceptibility. However, the patients in the current study did not receive ceftazidime-avibactam treatment. The mechanism of reduced susceptibility has not been determined for those isolates but might be attributable to changes in porin or penicillin-binding protein or the presence of avibactam-insensitive beta-lactamases that were not detected by the current testing.

In this study, all CREs are carbapenemase producers, and we found a total of 40 (33%) patients died within 14 days, which is considered as high mortality rate. Thus, we hold the view that in addition to submitting specimens for bacterium identified and antimicrobial susceptibility testing, which is a lab routine activity, carbapenemase detection test should also be combined. At the present time, CP-CRE can be detected in both genotypic and phenotypic carbapenemase detection tests. When that the specimen arrives in the lab or a blood culture broth becomes positive. When the specimens are delivered to the lab or a blood culture broth becomes positive, genotypic carbapenemase detection test takes one day or usually within 2 hours to get the result. Alternatively, phenotypic carbapenemase test can also be used to detect CP-CRE such as mCIM or EDTA-modified carbapenem inactivation method (eCIM), which affords the differentiation of serine and MBLs. This test could be helpful in making therapeutic decisions. In our view, the acknowledgement that patient had a CP-CRE could lead to a more effective treatment and to a use of antimicrobial agents (eg ceftazidime-avibactam, ceftazidime-avibactam plus aztreonam, and cefiderocol etc.) for certain classes of carbapenemases. These agents could be administered almost immediately when a particular carbapenemase is identified.

There are several limitations to this work. Firstly, the limited sample size could impact the analysis of risk factors for mortality. Hence, the risk factors reported in the study might not represent the true or complete range of factors. Secondly, the patients were from a single center in Thailand where carbapenemase genes mainly $bla_{\text{NDM-1}}$ and $bla_{\text{OXA-48}}$

predominate, and differs from those in other regions of the world where bla_{KPC} predominate. Third, we did not evaluate the contribution of the major OMPs to CRE isolates which could cause increased the carbapenem MIC in this study.

Finally, this study is a retrospective study in which we could not provide a clear explanation of rationale in the case of clinical judgment.

Conclusion

In conclusion, the study suggests that the consideration of microbial characteristics may have an important role in making treatment decisions and in treatment of disease with variability in genes. Thus, we suggest that in treatment decisions involving the use of either carbapenem-containing regiment (especially when meropenem MICs of $\geq$ 16) or colistin-containing regiment in patients with CP-CRE infection, especially those in the NDM-1 and both NDM-1 and OXA-48 groups, the patient symptoms should be closely monitored.

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Disclosure

The authors report no conflicts of interest in relation to this work.

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