

Research Article

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Fecal Colonization Rate of Carbapenem-resistant Enterobacterales among Volunteers in a Community of Raroeng Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand

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Abstract

Carbapenem-resistant Enterobacterales (CRE) have emerged as an important global public health problem due to their ability to cause high morbidity and mortality rates. CRE have the potential to spread not only in hospital settings but also in the community. Based on our knowledge, no investigation has been conducted of CRE presence among people in Raroeng Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand. This study aimed to clarify the CRE colonization status among volunteers in the community. This cross-sectional study was conducted in March 2023. A total of 121 fecal samples were obtained from the volunteers participating in this study. CRE screening, identification and characterization were performed using culture methods, matrix-assisted laser desorption-ionization-time of flight mass spectrometry (MALDI-TOF MS) and phenotypic and genotypic assays for carbapenemase production. Our results revealed that the fecal colonization rate of CRE was 1.65% (n=2). CRE isolates were identified as *Klebsiella pneumoniae*. Carbapenemase types were revealed as class B metallo-beta-lactamase and class D serine-carbapenemase, produced by *bl_{NDM}* and *bla_{OXA-48}*, respectively. Additional antimicrobial resistant genes including beta-lactam resistant genes (*bla_{CTX-M}*, *bla_{SHV}*, *bla_{TEM}*) and an aminoglycoside resistant gene (*aac(6')-Ib*), were also found among the CRE isolates. No significant potential factors related to CRE colonization rate were identified. Our study indicated that CRE colonization in this community is worrisome. Surveillance of CRE colonization and strict implementation of infection control practices are required to restrict the spread of CRE in communities.

Keywords: Carbapenem-resistant Enterobacterales, CRE, Colonization, Community

1. Introduction

Enterobacterales refers to a group of gram-negative bacteria that are important causes of infectious diseases in human (1, 2). Due to a dramatic increase in the prevalence of extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases producing Enterobacterales, the carbapenem class of broad-spectrum antibiotics is used to treat multi-drug-resistant (MDR) Enterobacterales (3, 4). Because of high efficacy and safety, carbapenems are considered to serve as the ultimate last resort option for treating MDR. However, the inappropriate use of carbapenem is a major driver for increasing of emerging Carbapenem-resistant Enterobacterales (CRE) worldwide including Thailand (5-9). CRE has acquired the ability to produce enzymes called carbapenemase, which can break down carbapenem antibiotics and render them ineffective. Three classes (class A, B and D) of carbapenemase enzyme have been classified based on the molecular structure classification of Ambler. However, high level amount of AmpC beta-lactamases, belonging to class C, might reduce sensitivity to carbapenems (10, 11). The carbapenemase genes are either localized on chromosome or plasmids, and the transmission of these genes is involved in the outbreak of CRE (12). The four most popular carbapenemase-related genes, encoding different carbapenemases, include *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-beta-lactamase (NDM), Verona integrin-encoded metallo- β -lactamase (VIM), imipenemase (IMP) and oxacillinase-48 (OXA-48) (13). In 1996, KPC was first reported in the US. Since 2001, it has spread widely in various countries. In 2009, NDM was first reported in India. Currently, NDM has spread worldwide, especially in Asia. The highest prevalence of IMP and VIM are found in Southern Europe and Asia whereas OXA-48 is most prevalent in the Mediterranean region, Europe, and India (13-16). In Thailand, the first emerging KPC was found in 2014 but the prevalence of KPC in Thailand remains extremely low with 1.4%. NDM and OXA-48 being the most common in Thailand (17-19). Although CRE is currently primarily associated with infections in healthcare settings, it has the potential to spread to community settings and lead to the community-acquired CRE infection (20, 21). In 2022, one hospital report indicated the prevalence of CRE colonization in patients before admission was 2.8% (22). Reports indicate that community-acquired CRE,

although less common, is on the rise. Infections associated with CRE can be difficult to treat because of its resistance to many commonly used antibiotics. This can lead to severe and potentially life-threatening infections (20, 21).

In March 2023, the Faculty of Allied Health Science at Nakhonratchasima College conducted a community visit to Raroeng Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand. For this opportunity, the study aimed to determine the CRE fecal colonization rate, to characterize carbapenemase production, and to identify potential risk factors associated with CRE colonization.

2. Materials and Experiment

2.1 Study design, ethics approval and questionnaire survey

This study constituted a cross-sectional study conducted in March 2023 at Raroeng Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand, after receiving approval from the Research Ethics Committee, Faculty of Allied Health Science, Nakhonratchasima College, Thailand. (No. NMCEC-0046/2565) The questionnaires were used to obtain demographic data from volunteers such as the sex, age and history of antibiotic use to evaluate risk factors for CRE transmission in the community. Each participant agreed to be included in this study and signed the appropriate consent form before collecting the questionnaires and specimens.

2.2 Specimen collection and CRE screening

Fecal samples were collected and transferred, approximately 0.5 mg from each volunteer, into Cary-Blair transport medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The medium was stored in 4°C and immediately transported to a laboratory for CRE screening tests. CRE screening was performed following the method modified from Davari N, et al (23). Briefly, stool samples were suspended in 1 ml of 0.9% sterile saline and then 10 μ L of the suspension was spread onto the plate of MacConkey agar (MAC) (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Each 10 μ g of carbapenem disks including ertapenem (ETP), imipenem (IMP), meropenem (MEM) and doripenem (DOR) (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was placed on the inoculated

plate. The plates were incubated at 37°C for 24 hours. The isolated colony grown on the inhibition zone of each carbapenem disk were considered as positive screening results. At least one different colony of suspected CRE strain was collected and MAC subculture were stored at –80°C in Tryptic Soy Broth (TSB) (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) containing 20% glycerol for further study.

2.3 Bacterial identification

All suspected CRE strains were subcultured on MacConkey agar (MAC) and incubated at 37°C for 24 hours to obtain pure colonies. Each pure colony was identified using matrix-assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry according to the manufacturer's instructions (Bruker Daltonik GmbH, Bremen, Germany).

2.4 Antimicrobial susceptibility test for carbapenem resistance

Antimicrobial susceptibility testing of all suspected CRE strains against ertapenem (ETP), imipenem (IMP), meropenem (MEM) and doripenem (DOR) following Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines was conducted using the agar disk diffusion method (24). The interpretive criterion for inhibition zone was based on the recommendation of the CLSI 2023 (24). Quality control was included using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

2.5 Phenotypic detection of carbapenemase production

To investigate carbapenemase production in CRE isolates, the modified carbapenem inactivation method (mCIM) and EDTA-modified carbapenem inactivation method (eCIM) were performed and interpreted following the recommendation of CLSI-M100 guidelines (24). Both mCIM and eCIM tests were duplicated to ensure reproducibility. Briefly, a 1 µL loopful of CRE colony was resuspended in a 2 mL tube of TSB. Another 1 µL loopful of CRE colony was also resuspended in a 2 mL tube of TSB supplemented with EDTA (Thermo Fisher Scientific, Carlsbad, CA, USA) at a final concentration of 5 mM. A 10 µg of meropenem disk was placed in each tube, then each tube was incubated for 4 hours ± 15 minutes at 35°C. Afterward, the meropenem disks were moved

and placed on Mueller-Hinton agar (MHA) plates (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) freshly inoculated with a 0.5 McFarland standard suspension (1.5×10^8 CFU/mL) of a *E. coli* ATCC 25922 strain. The MHA plates were incubated for 16 to 20 hours at 35°C. The mCIM and eCIM results were interpreted as described (24). A CRE isolate producing metallo-carbapenemase is considered when the eCIM zone size increases by ≥ 5 mm compared with the zone size appearing for the mCIM and is considered as non-producing metallo-carbapenemase when the zone size increased by < 4 mm.

2.6 Detection of the antimicrobial resistant genes

Antimicrobial resistant genes of CRE isolates were detected using QIAstat-Dx BCID GN plus AMR panel and analyzed with QIAstat-Dx analyzer 1.0 (Qiagen, USA). A 1.0 McFarland standard suspension (3.0×10^8 CFU/mL) using pure colony on MacConkey agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) was prepared with total volume of 1 mL. The 100 µL of the suspension was added to the QIAstat-Dx BCID GN plus AMR panel cartridge and nucleic acid extraction, amplification and detection were performed automatically on the QIAstat-Dx analyzer. This panel comprises a superior tool for the rapid, comprehensive and automated identification of CRE and antimicrobial resistance genes, providing significant advantages over conventional PCR in terms of speed, breadth and ease of use.

2.7 Statistical analysis

Statistical analyses were performed using SPSS Software, Version 25.0 (Chicago, IL, USA). The univariate analysis with the respective 95% CI was used to analyze risk factors associated with CRE colonization. *P*-value < 0.05 in all experiments was considered statistically significant.

3. Results and Discussion

3.1 Demographic data

This study included fecal specimens from 121 volunteers dwelling in Raroeng Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand during March 2023. The demographic data (Supplementary Table S1) revealed that 52 (42.98%) of 121

volunteers were male and 69 (57.02%) were female at an average age of 49.85 years. A total of 121 fecal specimens were screened to detect CRE colonization.

3.2 CRE screening and bacterial identification

Overall, 8/121 (6.61%) fecal specimens appeared with resistant colonies grown in the inhibition zone of ETP, MEM, IMP or DOR with zone size < 22 mm. In contrast, 113/121 (93.39%) fecal specimens eliminated the CRE resistant colony in the inhibition zone with >22 mm of ETP, MEM, IMP or DOR. A total of eight resistant colonies were collected from eight individuals. These resistant colonies were identified and revealed as two suspected CRE strains and six non-suspected CRE strains. Two suspected CRE comprised *K. pneumoniae* whereas six non-suspected CRE comprised *P. aeruginosa* (n=4) and *A. baumannii* (n=2).

3.3 Determination of antimicrobial susceptibility tests

The two suspected CRE isolates underwent antimicrobial susceptibility tests and were revealed as CRE strains (Table 1). Both isolates (100%) were resistant to ETP and DOR. One isolate (50%) was resistant to IMP and MEM while another was intermediately susceptible to them.

Table 1 Antimicrobial resistance of carbapenem-resistant Enterobacterales isolates.

Antimicrobial agents	Number of isolates (%)		
	Resistant	Intermediate	Susceptibility
Meropenem	1 (50)	1 (50)	0 (0)
Imipenem	1 (50)	1 (50)	0 (0)
Ertapenem	2 (100)	0 (0)	0 (0)
Doripenem	2 (100)	0 (0)	0 (0)

3.4 Phenotypic and genotypic characterization of the CRE

The results of the phenotypic and genotypic detection of the CRE are displayed in Table 2. Of two CRE isolates, one isolate produced metallo-beta-lactamase due to a positive result for mCIM and eCIM. In contrast, another produced serine beta-lactamases because it showed only a positive result for mCIM. For genotypic results, QIAstat-Dx BCID GN plus AMR panel revealed that *K. pneumoniae* RSC054 carries *bla_{NDM}* as carbapenemase genes and carries the additional beta-lactamase genes including *bla_{CTX-M}* and *bla_{SHV}*. In contrast, *K. pneumoniae* RSC085 carries the carbapenemase gene as *bla_{OXA-48}* and additional beta-lactamases genes including *bla_{CTX-M}*, *bla_{SHV}* and *bla_{TEM}*. The aminoglycoside acetyltransferase gene (*aac(6')-Ib*) was also found in this strain.

Table 2 Phenotypic and genotypic characterization of the CRE isolates.

Isolation no.	Bacterial species	Phenotypic confirmatory tests		Carbapenemase gene				B-lactamases gene				Aminoglycoside resistance gene
		mCIM	eCIM	NDM	IMP	VIM	OXA-48	CTX-M	SHV	TEM		<i>aac(6')-Ib</i>
RSC054	<i>K. pneumoniae</i>	+	+	+	-	-	-	+	+	-		-
RSC085	<i>K. pneumoniae</i>	+	-	-	-	-	+	+	+	+		+

3.5 Risk factors of CRE colonization

This study did not find any significant associations between the CRE colonization to host habitat, antibiotic exposures and hand hygiene with *p*-value >0.05 as shown in Table 3.

The interactions between host habitat, antibiotic exposures and hand hygiene were not significantly associated with an increased risk of CRE colonization among the volunteers.

Table 3 Univariate analysis of risk factors associated with CRE colonization.

Risk factors	Univariate analysis		
	Odds Ratio	95% CI	P
Living in municipality area	0.883	0.828 - 0.943	0.785
Previous antibiotic usage	0.808	0.741 - 0.882	0.895
Previous non-prescription antibiotic usage	0.883	0.828 - 0.943	0.928
Previous stopped taking of antibiotics when symptoms improve	0.892	0.838 - 0.949	0.964
Usage of previous antibiotic for new infections	0.892	0.838 - 0.949	0.961
Changing to a broader spectrum antibiotic without physicians' recommendation	0.942	0.901 - 0.985	0.951
Previous usage of anti-inflammatory drugs	0.758	0.685 - 0.839	0.837
Usage of antibiotics without unknown generic name	0.908	0.858 - 0.961	0.970
Taking antibiotic powder for wounds	0.933	0.890 - 0.979	0.974
Usage of lozenge containing antibiotics	0.925	0.879 - 0.973	0.968

4. Discussion

Currently, the global public health system is concerned by the high prevalence of CRE isolates (25-29). Investigation of CRE colonization among healthy people from community settings can promote a better understanding of the emerging CRE, liable for outbreak incidence and contribute to preventing and controlling CRE dissemination. This study revealed that the rate of CRE colonization in fecal samples collected from the volunteers in Raroeng Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province was 1.65%. This colonization rate is less than that of a related report in Thailand, namely, 2.8% (22). However, the colonization rates from the present study and from the report in Thailand in community settings show a higher prevalence than that in reports in India and Cambodia (1% in each) (30, 31). In contrast, the majority of the global reports were undertaken among hospital patients with average colonization rates ranging from 3.73 to 7.42% (29).

CRE are defined as Enterobacterales resistant to at least one of the carbapenem antibiotics based on their antibiotic susceptibility profile (ertapenem, imipenem, meropenem and doripenem) (32). Among the Enterobacterales, *E. coli*, and *K. pneumoniae* are the most commonly detected CRE. Resistance mechanism to carbapenems in CRE is mainly due to the production of carbapenemases enzyme such as KPC (class A), NDM (class B) and OXA (class D). NDM was the most prevalent metallo- β -lactamases (MBL), first identified in *K. pneumoniae* and *E. coli* isolated from a patient traveling from India to Sweden in 2008 (25, 33, 34). Recently, (31) NDM variants

have been identified, and NDM-1 is the most prevalent type (35). Consistent with the above findings, NDM-producing *K. pneumoniae* colonized CRE in the fecal samples of our study carried the NDM gene. In addition to NDM, *K. pneumoniae* colonized CRE strain produced oxacillin-hydrolyzing enzymes (OXA) was also identified in the present study. Over 40 existing carbapenemase variants have been reported. However, the most prevalent OXA is OXA-48 (35). In 2001, *K. pneumoniae* carrying OXA-48 was first reported in Turkey (36). The present study also found *K. pneumoniae* colonized CRE carrying OXA-48 that was consistent with the related findings (25, 30). Based on using QIAstat-Dx BCID GN plus the AMR panel, other antimicrobial resistant genes were also detected. Similar to relate reports,(37-39) our study found that each CRE revealed the concomitant carriage of carbapenemases gene with other beta-lactam resistance genes including CTX-M, SHV and TEM. In addition, an aminoglycoside resistant gene (aac(6')-Ib) was also found as reported by Argente M, et al (40). This finding suggested that our CRE isolates were multidrug resistant strains. Moreover, the association between risk factors and colonization was investigated in this study. Interestingly, no any risk factors have been associated with the CRE colonization among volunteers. In contrast, several studies revealed that risk factors associated with CRE colonization were significantly related to inappropriate antibiotic use but did not reach significance in our study, possibly owing to sample size (41-44). Substantially, the World Health Organization reported that subjects with CRE colonizing in fecal samples are considered

as high risk groups, that can spread CRE by contact and travel (45, 46). CRE colonization will significantly contribute to the spread and the occurrence of CRE isolates in community settings through physical contact with other people in their community and have the tendency to acquire mobile genetic materials containing antimicrobial resistant genes via horizontal gene transfer (46-49). Therefore, surveillance of CRE colonization and strict implementation of infection control practices are required to restrict the spread of CRE in communities.

5. Conclusions

In summary, our data were used to determine the CRE colonization rate in fecal samples from volunteers living in the community of Raroeng Subdistrict, Wang Nam Khiao District, Nakhon Ratchasima Province, Thailand. CRE colonization enables the transmission of CRE throughout the community. Therefore, strategies to prevent and control the dissemination of CRE isolates should be implemented. Future studies should consider larger sample sizes and explore additional factors that may contribute to CRE colonization in the community.

Declaration of Conflicting Interests

The authors declare they have no conflicts of interest for this article, and they alone are responsible for the content.

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Supplementary Materials**Supplementary Table S1** Demographic characteristics of volunteers with and without CRE colonization (n = 121)

	Category	Number of volunteers, N (%)
Gender	Male	52 (42.98%)
	Female	69 (57.02%)
Age (years)	1-10	2 (1.65)
	11-20	9 (7.44)
	21-30	6 (4.96)
	31-40	12 (9.92)
	41-50	29 (23.97)
	51-60	25 (20.66)
	61-70	26 (21.49)
	71-80	9 (7.44)
Occupation	>80	3 (2.48)
	Farmer	45 (37.19)
	Merchant	11 (9.09)
	Student	13 (10.74)
	Employee	31 (25.62)
	Government officer	9 (7.44)
Educational background	Unemployed	12 (9.92)
	Primary education	60 (49.59)
	High school education	43 (35.54)
	Vocational certificate	2 (1.65)
	Bachelor's degree	6 (4.96)
Income (bath/month)	Unlettered	10 (8.26)
	< 5,000 baht	35 (28.93)
	5,000 – 10,000 baht	41 (33.88)
	10,001 – 15,000 baht	6 (4.96)
	>15,000 baht	39 (32.23)