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Risk Factors for Gastrointestinal Colonization and Acquisition of Carbapenem-Resistant Gram-Negative Bacteria among Patients in Intensive Care Units in Thailand

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ABSTRACT This study was conducted to investigate the prevalence of and risk factors for colonization and acquisition of carbapenem-resistant (CR) Gram-negative bacteria (GNB) among patients admitted to intensive care units (ICUs) in two tertiary care hospitals in northern Thailand. Screening of rectal swab specimens for CR-GNB was performed on patients at ICU admission and discharge. The phenotypes and genotypes of all isolates were determined. Risk factors were analyzed by logistic regression analysis. The overall carriage rate of CR-GNB at admission was 11.6% (32/275), with the most predominant species carried being *Acinetobacter baumannii* ($n = 15$), followed by *Klebsiella pneumoniae* ($n = 9$). The risk factor for CR-GNB colonization was hospitalization within the previous 6 months ($P = 0.002$). During the ICU stay, the rate of CR-GNB acquisition was 25.2% (52/206), with the most predominant species carried being *A. baumannii* ($n = 28$) and *K. pneumoniae* ($n = 13$). Risk factors associated with CR-GNB acquisition were the use of an enteral feeding tube ($P = 0.008$) and administration of third-generation cephalosporins ($P = 0.032$) and carbapenems ($P = 0.045$). The most common carbapenemase genes in *A. baumannii* and *K. pneumoniae* were $bla_{OXA-23/51}$ and bla_{NDM}, respectively. Patient-to-patient transmission was demonstrated in three cases, resulting in the acquisition of CR *A. baumannii* (2 cases) and *K. pneumoniae* (1 case) isolates from other patients who were admitted during the same period of time. This is the first Indochinese study screening patients, examining patients for the carriage of CR-GNB, and further demonstrating the transfer of CR-GNB isolates in ICUs. Our study suggests that effective infection control measures are required to limit the spread of CR-GNB within hospitals.

KEYWORDS carbapenem, Gram-negative bacteria, colonization, acquisition, risk factor, bla_{OXA}, bla_{NDM}, carriage

Carbapenem-resistant (CR) Gram-negative bacteria (GNB), such as *Enterobacteriaceae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, are challenges in the management of high-risk patients, e.g., intensive care unit (ICU) patients, particularly in low- and middle-income countries. Several mechanisms, including alteration of outer membranes, efflux pump overexpression, and production of carbapenemases, contrib-

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ute to carbapenem resistance (1). The last mechanism is worrisome, as genes encoding carbapenemases often reside on mobile genetic elements, facilitating their spread among bacterial pathogens. Several types of carbapenemases have been reported, including serine-type enzymes (KPC, OXA-48) and metallo-β-lactamases (NDM, IMP, VIM) (1).

CR-GNB frequently show resistance to other antibiotic classes, such as aminoglycosides and quinolones, limiting therapeutic options. Infections with CR-GNB among patients in ICUs can be associated with increases in the severity of illness, costs of treatment, length of stay, and mortality (2). The incidence of CR-GNB infection varies, depending on the region and the patient population. In Thailand, infections caused by CR *A. baumannii* and CR *P. aeruginosa* are frequently reported, and this is particularly the case for *A. baumannii*, which is regarded as a key pathogen in Thailand. However, those caused by CR *Enterobacteriaceae* are not so common (3). Recently, data from the National Antimicrobial Resistance Surveillance Thailand (NARST) from January 2017 to December 2017 showed that 70% and 20% of *Acinetobacter calcoaceticus-A. baumannii* complex and *P. aeruginosa* isolates, respectively, were resistant to meropenem. In contrast, the rate of carbapenem resistance in *Enterobacteriaceae* remains low. For instance, the prevalence of resistance to imipenem and meropenem in *Escherichia coli* was approximately 2.5% (http://narst.dmsc.moph.go.th/).

Intestinal colonization with CR-GNB at the time of ICU admission has been shown to be associated with an increased risk of subsequent CR-GNB infections and high mortality rates (4, 5). Furthermore, ICU patients who are positive for carriage on admission may act as a reservoir for further dissemination in ICUs (6). Additionally, the clonal spread of CR-GNB and the horizontal transfer of carbapenemase genes among Gram-negative bacteria within hospitals have previously been reported (7–9). Low CR-GNB carriage rates were reported from South Korea (0.3%) (10) and France (2.1%) (11), whereas those from China and Greece were higher (6.5 to 12.8%) (12, 13). However, the fecal carriage rates of CR-GNB among patients admitted to ICUs in India and Iran were as high as 35 to 37% (14, 15).

Since therapeutic options for CR-GNB infections are limited, infection control measures, such as contact isolation, cleaning and disinfection of medical devices, as well as antibiotic stewardship programs, have become increasingly important to minimize their spread. Previous studies have shown that routine surveillance for CR-GNB in rectal swab specimens on admission contributes to a significant reduction in CR-GNB infections during ICU stays (16, 17). However, data on the rate of colonization with CR-GNB among hospitalized patients in Thai hospitals are very limited. This study was conducted to assess the prevalence of and risk factors for intestinal colonization and acquisition of CR-GNB among patients admitted to ICUs.

RESULTS

Characteristics of studied ICU patients. During the study period, 2,652 patients were admitted to ICUs (2,345 from Buddhachinaraj Hospital [BUH] and 307 from Naresuan University Hospital [NUH]). Of these, 275 ICU patients (213 and 62 from BUH and NUH, respectively) agreed to participate in this study (Fig. 1). The patient median age was 63 years (range, 20 to 97 years), and 54.2% were male. The median length of ICU stay was 6 days (range, 2 to 43 days). The most common reason for ICU admission was respiratory tract disease (25.1%), followed by cardiovascular disease (12.7%). The majority of patients were admitted to an ICU directly from home (36.0%). The most common underlying condition was cardiovascular disease (47.3%). The patients' characteristics are shown in Table 1.

Prevalence of and risk factors for intestinal colonization with CR-GNB among ICU patients at admission. Of the 275 patients, 32 patients carried CR-GNB (11.6%, 95% confidence interval [CI] = 8.1 to 16.0%); 10.8% (23/213) were from BUH and 14.5% (9/62) were from NUH (Fig. 1). Twenty-eight patients carried a single CR-GNB isolate. Two different CR-GNB species were detected in 3 patients, and 3 different species were simultaneously isolated from 1 patient. Overall, 37 CR-GNB isolates were recovered, and

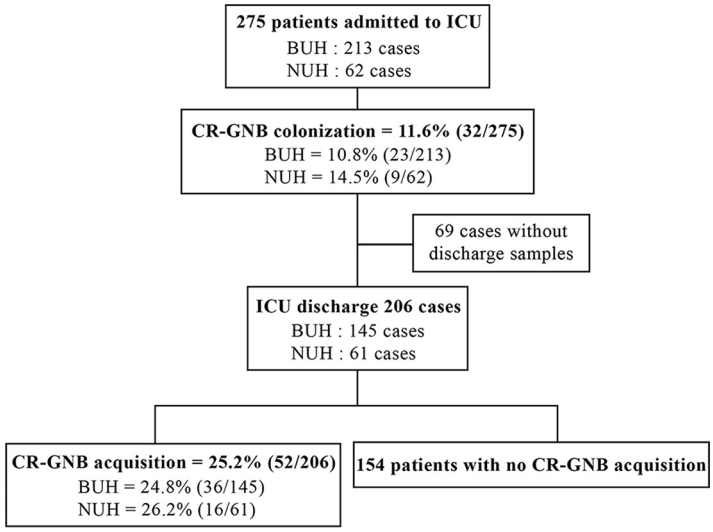

FIG 1 Flowchart of patients included in this study. CR-GNB, carbapenem-resistant Gram-negative bacteria; ICU, intensive care unit; BUH, Buddhachinaraj Hospital; NUH, Naresuan University Hospital.

the predominant species was *A. baumannii* ($n = 15$, 40.5%), followed by *Klebsiella pneumoniae* ($n = 9$, 24.3%), *Acinetobacter* spp. ($n = 5$, 13.5%), and *Enterobacter* spp. ($n = 3$, 8.1%) (Table 2).

Among the 37 CR-GNB isolates, 26 isolates from 26 different patients were carbapenemase-producing GNB (CP-GNB), resulting in a prevalence of CP-GNB carriage of 9.5% (26/275, 95% CI = 6.3 to 13.5%). The majority of CP-GNB isolates were bla_{OXA}-positive *A. baumannii* isolates ($n = 14$), which possessed a variety of OXA-carbapenemase genes, mostly $bla_{OXA-23/51}$. bla_{NDM} was found in *K. pneumoniae*, *Enterobacter* spp., and *A. baumannii*. One *A. baumannii* isolate was positive for bla_{IMP} (Table 2).

The patients' characteristics and history were analyzed to identify risk factors associated with intestinal colonization with CR-GNB. Using univariate analysis, CR-GNB colonization was associated with antibiotic usage within the previous 3 months, hospitalization within the previous 6 months, and prior respiratory tract disease. However, with multiple logistic regression analysis, hospitalization within the previous 6 months was the only risk factor associated with CR-GNB colonization ($P = 0.002$, adjusted odds ratio [aOR] = 3.818, 95% CI = 1.642 to 8.878) (Table 1).

Among the 32 patients who were colonized with CR-GNB, 6 patients developed infections during their ICU stays. However, clinical infection due to CR-GNB was found in only 1 patient (3.13%). This patient was a CR *A. baumannii* carrier at ICU admission, further developed a pulmonary infection caused by CR *A. baumannii* on the 4th day in the ICU, and was discharged alive after 11 days in the ICU. Other causes of clinical infections included extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* ($n = 1$), *K. pneumoniae* ($n = 1$), *Enterobacter aerogenes* ($n = 1$), *Elizabethkingia meningoseptica* ($n = 1$), and a combination of *P. aeruginosa* and *Enterococcus* spp. ($n = 1$).

Prevalence of and risk factors for CR-GNB acquisition during ICU stay. At discharge, we were able to obtain rectal swab specimens from 206 patients. Fifty-two patients acquired CR-GNB isolates, leading to an overall acquisition incidence of 25.2% (52/206, 95% CI = 19.5 to 31.7%), with the acquisition incidence being 24.8% (36/145) at BUH and 26.2% (16/61) at NUH (Fig. 1). In 52 acquisition cases, 43 patients were negative for CR-GNB carriage at admission screening. Only 9 patients who were already a carrier upon admission acquired an additional CR-GNB isolate during their ICU stay. A total of 59 CR-GNB isolates were recovered. The most common species was *A. baumannii* ($n = 28$, 47.5%), followed by *K. pneumoniae* ($n = 13$, 22.0%), *Acinetobacter* spp. ($n = 7$, 11.9%), and *P. aeruginosa* ($n = 5$, 8.5%) (Table 2).

Among the 59 CR-GNB isolates, 27 isolates from 27 different patients were CP-GNB, resulting in an overall CP-GNB acquisition rate of 13.1% (27/206, 95% CI = 8.8 to 18.5%).

TABLE 1 Factors associated with intestinal colonization of CR-GNB among ICU patients[e]

Characteristic	No. (%) of patients in the following groups with the indicated characteristic:			Univariate analysis *P* value	Multivariate logistic regression analysis	
	All patients (*n* = 275)	CR-GNB carriers (*n* = 32)	Non-CR-GNB carriers (*n* = 243)		*P* value[a]	aOR (95% CI)
Male patients	149 (54.2)	14 (43.8)	135 (55.6)	0.199		
Age >65 yr	119 (43.3)	16 (50.0)	103 (42.4)	0.497		
Secondary school education[b]	190 (69.1)	19 (59.4)	171 (70.4)	0.174		
≥5 family members[c]	52 (18.9)	4 (12.5)	48 (19.8)	0.381		
Family income of <10,000 baht[d]	168 (61.1)	18 (56.3)	150 (61.7)	0.405		
Urban living	85 (30.9)	11 (34.4)	74 (30.5)	0.520		
Antibiotic usage within previous 3 mo	93 (33.8)	17 (53.1)	76 (31.3)	0.017	0.278	1.614 (0.679–3.836)
Hospitalization within previous 6 mo	89 (32.4)	19 (59.4)	70 (28.8)	0.001	0.002	3.818 (1.642–8.878)
Referral of patients from:						
Home	99 (36.0)	10 (31.3)	89 (36.6)	0.722		
Another hospital	73 (26.5)	7 (21.9)	66 (27.2)	0.654		
Another ward within hospital	92 (33.5)	12 (37.5)	80 (32.9)	0.434		
Principle diagnosis						
Respiratory tract disease	69 (25.1)	13 (40.6)	56 (23.0)	0.034	0.051	2.309 (0.996–5.353)
Cardiovascular disease	35 (12.7)	6 (18.8)	29 (11.9)	0.271		
Renal disease	19 (6.9)	3 (9.4)	16 (6.6)	0.477		
Sepsis	41 (14.9)	4 (12.5)	37 (15.2)	0.797		
Underlying condition						
Cardiovascular disease	130 (47.3)	14 (43.8)	116 (47.7)	0.665		
Diabetes	71 (25.8)	11 (34.4)	60 (24.7)	0.233		
Respiratory tract disease	35 (12.7)	6 (18.8)	29 (11.9)	0.264		
Renal disease	38 (13.8)	5 (15.6)	33 (13.6)	0.784		
Liver disease	18 (6.5)	1 (3.1)	17 (7.0)	0.704		
Length of ICU stay (day)						
<8 days	165 (60.0)	17 (53.1)	148 (60.9)	0.274		
8–14 days	51 (14.5)	7 (21.9)	44 (18.1)	0.666		
15–21 days	29 (10.5)	5 (15.6)	24 (9.9)	0.362		
>21 days	14 (5.1)	2 (6.3)	12 (4.9)	0.678		
30-day mortality	35 (12.7)	4 (12.5)	31 (12.8)	1.000		

[a]A *P* value of <0.05 was considered statistically significant.
[b]Basic education in Thailand consists of 6 years of primary school education and 6 years of secondary school education.
[c]Average number of household members.
[d]The average minimum income per month is 10,000 baht.
[e]Data are for a total of 275 patients.

The most common CP-GNB was $bla_{OXA-23/51}$-positive *A. baumannii* (*n* = 15), followed by bla_{NDM}-positive *K. pneumoniae* (*n* = 9). Three *P. aeruginosa* isolates were positive for bla_{IMP}.

Univariate analysis and final multiple logistic regression analysis showed that the use of an enteral feeding tube (*P* = 0.008, aOR = 5.386, 95% CI = 1.563 to 18.564) and administration of third-generation cephalosporins (*P* = 0.032, aOR = 2.293, 95% CI = 1.074 to 4.896) and carbapenems (*P* = 0.045, aOR = 2.199, 95% CI = 1.019 to 4.743) were significantly associated with CR-GNB acquisition the during ICU stay (Table 3).

Patient-to-patient transmission. All 52 patients who acquired CR-GNB during their ICU stay were considered at a possible risk of patient-to-patient transmission. Fifty-one and 15 isolates of CR *Acinetobacter* spp. and CR *K. pneumoniae*, respectively, were available for genotypic studies. Pulsed-field gel electrophoresis (PFGE) analysis of *Acinetobacter* spp. and *K. pneumoniae* isolates revealed 31 and 9 different profiles, respectively (see Fig. S1 and S2 in the supplemental material). Among these, 5 and 2 PFGE profiles from *Acinetobacter* spp. and *K. pneumoniae*, respectively, were found to contain >1 isolate. However, the majority of isolates which shared identical or similar PFGE profiles were recovered from patients admitted to an ICU across various time points. Thus, patient-to-patient transmission was unlikely (Table S1).

TABLE 2 Distribution of CR-GNB isolates and types of carbapenemase genes among CR-GNB in carriage and acquisition groups

Organism	No. (%) of isolates	No. of isolates carrying the following carbapenemase gene:									
		bla$_{IMP}$	*bla*$_{NDM}$	*bla*$_{OXA-23}$	*bla*$_{OXA-24}$	*bla*$_{OXA-51}$	*bla*$_{OXA-58}$	*bla*$_{OXA-23/51}$	*bla*$_{OXA-51/58}$	*bla*$_{OXA-23/51/58}$	*bla*$_{OXA-24/51/58}$
CR-GNB carriage isolates	37										
A. baumannii	15 (40.5)	1	1	2	1	2		5	1	3	
K. pneumoniae	9 (24.3)		6								
Acinetobacter spp.	5 (13.5)					1	1				
Enterobacter spp.	3 (8.1)		2								
Other CR-GNB[a]	5 (13.5)										
CR-GNB acquisition isolates	59										
A. baumannii	28 (47.5)					4		15	3	2	1
K. pneumoniae	13 (22.0)		9								
Acinetobacter spp.	7 (11.9)		2	1				1			
P. aeruginosa	5 (8.5)	3									
Enterobacter spp.	1 (1.7)		1								
Klebsiella spp.	1 (1.7)		1								
Other CR-GNB[b]	4 (6.8)										

[a]Other CR-GNB isolates included *P. aeruginosa* ($n = 2$), *Escherichia coli* ($n = 1$), *Serratia marcescens* ($n = 1$), and *Citrobacter freundii* ($n = 1$).
[b]Other CR-GNB isolates included *Klebsiella oxytoca* ($n = 2$), *Serratia* spp. ($n = 1$), and *Shigella* spp. ($n = 1$).

Nevertheless, we found three cases of patient-to-patient transmission (Table S1). In the first case, the patient acquired a CR *A. baumannii* isolate which was previously found in another patient who had been admitted to the ICU 7 days earlier. The *A. baumannii* isolates showed closely related PFGE patterns (only one band difference) (Fig. S1) and identical antimicrobial resistance patterns, indicating that these were the same strain. Moreover, the two patients were placed in adjacent beds, and their ICU stays overlapped by 6 days.

The second case of patient-to-patient transmission involved a patient who acquired a CR *A. baumannii* isolate from another patient who had been admitted to the ICU 2 days earlier. The two *A. baumannii* isolates showed identical PFGE profiles and identical antimicrobial resistance profiles, and the patient ICU stays overlapped by 6 days, confirming patient-to-patient transmission (Table S1 and Fig. S1).

In the third example, the patient acquired a CR *K. pneumoniae* isolate which had previously been found in another patient who was admitted to the ICU during the same period of time. The PFGE and antimicrobial resistance patterns of the 2 isolates were identical, and the ICU stays overlapped by 2 days (Table S1 and Fig. S2).

DISCUSSION

Intestinal colonization with CR-GNB among ICU patients is a major threat for hospitalized patients, as the colonized patients serve as a reservoir for the dissemination of CR-GNB in hospital settings (6, 7). Screening of rectal swab specimens for CR-GNB carriage at the time of ICU admission may help to identify patients who require isolation or who are at high risk of developing endogenous infections and require frequent monitoring during their ICU stay. CR-GNB colonization among patients admitted to an ICU has previously been studied, and various carriage rates have been reported (10–15). In the present study, we found that 11.6% and 9.5% of patients admitted to ICUs were colonized with CR-GNB and CP-GNB, respectively. During their ICU stays, these patients acquired CR-GNB and CP-GNB at rates of 25.2% and 13.1%, respectively. The relatively high rate of CR-GNB acquisition implies that infection control measures may not be optimal, contributing to the spread of carbapenem-resistant organisms in ICUs.

Species identification revealed that the most common CR-GNB was *A. baumannii*, followed by *K. pneumoniae*, in the carriage and acquisition groups. Molecular characterization of carbapenemase genes showed that *bla*$_{OXA}$ (*bla*$_{OXA-23}$, *bla*$_{OXA-24}$, *bla*$_{OXA-51}$, and *bla*$_{OXA-58}$) was commonly found in *A. baumannii*, consistent with the fact that

TABLE 3 Factors associated with acquisition of CR-GNB among patients during ICU stay[c]

Characteristic	No. (%) of patients in the following groups with the indicated characteristic:			Univariate analysis *P* value	Multivariate logistic regression analysis	
	All patients (*n* = 206)	CR-GNB acquisition (*n* = 52)	Non-CR-GNB acquisition (*n* = 154)		*P* value[a]	aOR (95% CI)
Male gender	113 (54.9)	30 (57.7)	83 (53.9)	0.540		
Age >65 yr	84 (40.8)	23 (44.2)	61 (39.6)	0.486		
Mechanical devices						
Central venous catheter	29 (14.1)	8 (15.4)	21 (13.6)	0.690		
Indwelling urinary catheter	168 (81.6)	43 (82.7)	125 (81.2)	0.485		
Enteral feeding tube	153 (74.3)	46 (88.5)	107 (69.5)	0.002	0.008	5.386 (1.563–18.564)
Endotracheal tube	159 (77.2)	46 (88.5)	113 (73.4)	0.007	0.729	0.763 (0.165–3.527)
Antibiotic usage during ICU stay						
First- and second-generation cephalosporins	35 (17.0)	6 (11.5)	29 (18.8)	0.258		
Third-generation cephalosporins	121 (58.7)	37 (71.2)	84 (54.5)	0.017	0.032	2.293 (1.074–4.896)
Carbapenems	42 (20.4)	16 (30.8)	26 (16.9)	0.023	0.045	2.199 (1.019–4.743)
Penicillins[b]	4 (1.9)	2 (3.8)	2 (1.3)	0.254		
β-Lactam–β-lactamase inhibitors	36 (17.5)	10 (19.2)	26 (16.9)	0.629		
Aminoglycosides[b]	2 (1.0)	1 (1.9)	1 (0.6)	0.433		
Fluoroquinolones	18 (8.7)	7 (13.5)	11 (7.1)	0.156		
Macrolides	26 (12.6)	8 (15.4)	18 (11.7)	0.437		
Colistin[b]	7 (3.4)	2 (3.8)	5 (3.2)	0.682		
Vancomycin	11 (5.3)	4 (7.7)	7 (4.5)	0.470		
Clindamycin	21 (10.2)	4 (7.7)	17 (11.0)	0.531		
Metronidazole	12 (5.8)	5 (9.6)	7 (4.5)	0.174		
Fosfomycin[b]	2 (1.0)	1 (1.9)	1 (0.6)	0.433		
Underlying conditions						
Cardiovascular disease	97 (47.1)	23 (44.2)	74 (48.1)	0.870		
Respiratory disease	25 (12.1)	7 (13.5)	18 (11.7)	0.635		
Renal disease	24 (11.7)	8 (15.4)	16 (10.4)	0.264		
Diabetes	52 (25.2)	14 (26.9)	38 (24.7)	0.592		
Liver disease	14 (6.8)	2 (3.8)	12 (7.8)	0.524		
Length of ICU stay (day)						
<8 days	129 (62.6)	24 (46.2)	105 (68.2)	0.008	0.498	0.730 (0.293–1.815)
8–14 days	34 (16.5)	10 (19.2)	24 (15.6)	0.449		
15–21 days	22 (10.7)	9 (17.3)	13 (8.4)	0.055	0.131	2.144 (0.797–5.767)
>21 days	8 (3.9)	4 (7.7)	4 (2.6)	0.100	0.149	3.227 (0.657–15.860)
30-day mortality	16 (7.8)	5 (9.6)	11 (7.1)	0.553		

[a]A *P* value of <0.05 was considered statistically significant.
[b]The rate of use was too low to give an accurate *P* value.
[c]Data are for a total of 206 patients.

carbapenem resistance in *Acinetobacter* spp. is often associated with the production of OXA-type carbapenemases (18). bla_{NDM} was mainly found in *K. pneumoniae*, which is in good agreement with previous reports from countries in Southeast Asia, such as Singapore and Malaysia, where bla_{NDM}-positive *K. pneumoniae* is prevalent among CR-GNB (19, 20). bla_{NDM} and bla_{IMP} were found in other GNB, such as *Acinetobacter* spp., *P. aeruginosa*, and *Enterobacter* spp., although bla_{IMP} was observed more frequently in *P. aeruginosa*. The presence of bla_{OXA}-, bla_{IMP}-, and bla_{NDM}-positive isolates in a patient's intestinal tract is of concern since these genes are commonly associated with a transmissible plasmid; thus, they can be readily transferred to other intestinal flora (21, 22). It should be noted that 8 patients were colonized with *Stenotrophomonas maltophilia*. However, carbapenem resistance in *S. maltophilia* is intrinsic since it possesses a chromosomally encoded metallo-β-lactamase. *S. maltophilia* rarely causes serious disease. Additionally, it is not common among clinically important Gram-negative bacteria; therefore, *S. maltophilia* was not further investigated. In addition, we

identified several isolates of CR-GNB which were carbapenemase negative (Table 2). Carbapenem resistance in these organisms may result from efflux pump overexpression or alterations in the outer membrane protein (1). For *Enterobacteriaceae*, carbapenem resistance may be caused by a combination of AmpC or ESBL production with porin defects (1).

Multivariate logistic regression analysis showed that hospitalization within the previous 6 months was strongly associated with CR-GNB carriage upon ICU admission (Table 1), similar to the findings of previous studies in China and Greece (12, 13). This finding is not totally unexpected, as a previous hospitalization could expose patients to resistant bacteria in the hospital, which might contribute to subsequent CR-GNB colonization.

The relatively high rate of acquiring CR-GNB during an ICU stay could be due to the transmission of CR-GNB between patients via health care workers, ICU environments (inanimate objects/surfaces), or medical devices, since GNB can survive for a long period of time in the environment (23). These explanations were supported by several studies from different countries, including Thailand, which showed that CR-GNB isolates from colonized or infected patients were clonally related to those present on medical devices or in hospital environments (24–26). In our study, one of the risk factors for acquiring CR-GNB during an ICU stay was the use of an enteral feeding tube (Table 3), similar to the findings of a recent study by Yamamoto et al., who reported that the use of an enteral feeding tube was significantly associated with carbapenem-resistant *Enterobacteriaceae* carriage among hospitalized patients in Japan (27). It is possible that the use of an enteral feeding tube provides a convenient way for CR-GNB organisms to enter the patient's intestinal tract, leading to CR-GNB acquisition.

Administration of antibiotics within an ICU could play an important role in CR-GNB acquisition (2). Our study identified the use of third-generation cephalosporins and carbapenems to be independent risk factors for acquiring CR-GNB during an ICU stay (Table 3), consistent with the findings of previous studies (11, 28, 29). This could be due to the selective pressure of the use of antibiotics on the patient's intestinal flora, which corresponded to the previous finding of the inappropriate use of antibiotics in Thai hospitals (30).

Since the intestinal tract is considered an important reservoir for CR-GNB that may result in endogenous infections, many studies have suggested that identification of colonized patients and subsequent patient isolation in a single room should be performed to prevent the spread of CR-GNB within hospitals (6, 16). However, screening of rectal swab specimens and isolation of colonized patients may not be possible for resource-constrained countries, like Thailand. Furthermore, among the 52 acquisition cases, we observed only 3 cases of possible patient-to-patient transmission. Therefore, infection control measures, such as hand hygiene and proper cleaning and disinfection of medical devices, may be adequate to negate the dissemination of CR-GNB and reduce the acquisition rates of CR-GNB within ICUs. These procedures have been effectively used to reduce the number of cases of CR-GNB infection and colonization in ICUs in several countries, including Thailand (31–33).

Previous studies revealed that patients who are CR-GNB carriers have a higher mortality rate than noncarriers (4, 5). In our study, however, we found no significant differences in terms of mortality rates between patients who were colonized with or acquired CR-GNB and noncarriage or nonacquisition groups. These observations were consistent with those previously reported in Israel and China (7, 12). However, we noted that the lengths of ICU stay among patients who were CR-GNB carriers or who acquired CR-GNB were longer than those among noncarriage or nonacquisition groups, although these were not statistically significantly different (Tables 1 and 3).

Our study has some limitations. First, we did not collected samples from hospital environments (sink, bed, table, etc.), health care workers, or medical devices. Investigation of CR-GNB contamination in these sources might be useful to explain the relatively high CR-GNB acquisition rate during an ICU stay in our study. Second, we did not perform risk factor analysis for *Enterobacteriaceae* and nonfermenters (such as

Acinetobacter spp. and *P. aeruginosa*) separately, as the number of CR-GNB isolates was relatively small. Third, we used meropenem-containing agar for the screening of CR-GNB, and so some carbapenemase-producing *Enterobacteriaceae* isolates with low MICs to meropenem may have been missed. Thus, the prevalence of CR-GNB carriage and acquisition could have been underestimated. Fourth, this study was conducted in two hospitals, which may not accurately represent the situation of CR-GNB carriage and acquisition in Thai hospitals. Despite these limitations, our study provides insight into the prevalence of and risk factors for CR-GNB carriage at ICU admission and acquisition, which has important implications for the prevention of CR-GNB dissemination in Thai hospitals.

In conclusion, our study is the first Indochinese study reporting the prevalence of and risk factors for CR-GNB colonization and acquisition among ICU patients as well as demonstrating the transfer of CR-GNB isolates in ICUs. A relatively high acquisition rate highlights the need for more effective infection control measures, including hand hygiene, cleaning and disinfection of medical devices and ICU environments, as well as antibiotic stewardship programs, to limit the spread of CR-GNB within hospitals.

MATERIALS AND METHODS

Study settings and ethical statement. This observational study was prospectively conducted in the ICUs of two tertiary care hospitals, the 1,000-bed Buddhachinaraj Hospital (BUH) and the 400-bed Naresuan University Hospital (NUH), located in Phitsanulok Province, northern Thailand. The present study was approved by the Naresuan University Institutional Review Board (COA no. 130/2013). Written informed consent was obtained from the relatives of the patients participating in the study.

Rectal swab sampling and data collection. From December 2014 to December 2015, a total of 275 ICU patients aged >20 years participated in this study. Rectal swab specimens were collected from each patient within 48 h of ICU admission and at the time of discharge. The collection of samples from the same patients readmitted during the study period and patients who had diarrhea were excluded. Microbiological analysis was carried out within 24 h.

The demographic characteristics of the patients were obtained using a questionnaire and interviews with the patients' relatives. Clinical profiles were obtained from the patient's medical record. The following data were collected: age, gender, education, occupation, the number of family members, place of living, income, underlying conditions, principal diagnosis, previous antibiotic usage, and history of hospitalization.

Microbiological analysis. Rectal swab samples were directly cultured on Chrome UTI agar (Oxoid, Basingstoke, UK) supplemented with 25 μg/ml vancomycin and 0.5 μg/ml meropenem and incubated under aerobic condition for 24 h at 37°C. Colonies of different morphologies were selected and subcultured onto tryptic soy agar for further studies. Species identification was performed by using a RapID One system (Remel Inc., KS, USA), in accordance with the manufacturer's instruction. Isolates that showed doubtful results were further identified by amplification and sequencing of the 16S rRNA gene using previously published primers and conditions (34). Identification of *Acinetobacter* spp. to the species level was performed by multiplex PCR as described previously (35).

All isolates were subjected to testing for susceptibility to carbapenems (imipenem, meropenem, and ertapenem) by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) protocols, and the results were interpreted according to CLSI criteria (36). Isolates that showed resistance to either imipenem, meropenem, or ertapenem were considered CR-GNB.

Molecular studies. All CR-GNB isolates were screened for the presence of carbapenemase genes, i.e., bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{NDM}, and bla_{IMP} (37) and bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, and bla_{OXA-58} (38), by PCR using specific primers, as previously described. The PCR mixture consisted of 1 μl template DNA, 1× PCR buffer, 0.2 mM deoxynucleoside triphosphates, 0.5 μM each primer, and 1 U of *Taq* polymerase in a total volume of 25 μl. PCRs consisted of predenaturation at 95°C for 7 min, followed by 30 cycles of 95°C for 30 s, 52°C (48°C for bla_{KPC}, bla_{OXA-48}, and bla_{VIM}) for 1 min, and 72°C for 1 min and a final extension at 72°C for 10 min. PCR products were analyzed by agarose gel electrophoresis. Selected PCR products were purified and sequenced.

Genotypic characterizations of CR *Acinetobacter* spp. and CR *K. pneumoniae* isolates were performed by pulsed-field gel electrophoresis (PFGE). Briefly, a chromosomal DNA plug was prepared and digested with XbaI (*K. pneumoniae*) and ApaI (*Acinetobacter* spp.) (Fermentas Inc, Hanover, MD, USA) as previously described (39). Electrophoresis was performed in 1% pulsed-field-certified agarose in 0.5× Tris-borate-EDTA buffer using a contour-clamped homogeneous electric field Mapper XA system (Bio-Rad Laboratories, CA, USA). The gels were run at 6.0 V/cm with an angle of 120 degrees at 14°C for 20 h. *Saccharomyces cerevisiae* chromosomal DNA (Bio-Rad Laboratories) was used as a molecular size standard. DNA profiles were analyzed visually and interpreted according to the criteria of Tenover et al. (40).

Definitions. CR-GNB colonization was defined as a positive CR-GNB screening result at admission. CR-GNB acquisition was defined as (i) a negative first screening result at admission with a positive one at discharge or (ii) a positive screening result at admission and a positive one with different CR-GNB isolates at discharge. In all cases, the ICU stay had to have been at least 48 h.

For all acquisition cases, we employed the following criteria to determine whether the CR-GNB isolate was acquired as a result of patient-to-patient transmission: (i) the genotypes of the isolates were identical on the basis of their PFGE profiles, and (ii) the isolates were transmitted from known carriers who had an overlapping ICU stay with the recipient patient. An overlapping stay was defined as the coexistence of both carrier and recipient patient for at least ≥2 days.

Statistical analysis. Statistical analysis was performed using SPSS (version 17) software (SPSS Inc., Chicago, IL, USA). Univariate analysis was performed using the chi-square or the Fisher exact test, when appropriate, for categorical variables. Variables selected by univariate analysis ($P < 0.1$) were included in multivariate logistic regression analysis using a backward stepwise procedure. All statistical tests were two-tailed, and a P value of <0.05 was considered statistically significantly different. Results are presented as the adjusted odd ratios (aOR) and 95% confidence intervals (CI).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00341-18.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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We declare no conflict of interest.

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