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Nationwide Surveillance and Molecular Characterization of Critically Drug-Resistant Gram-Negative Bacteria: Results of the Research University Network Thailand Study

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ABSTRACT A large-scale surveillance is an important measure to monitor the regional spread of antimicrobial resistance. We prospectively studied the prevalence and molecular characteristics of clinically important Gram-negative bacilli, including *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* complex (ABC), and *Pseudomonas aeruginosa*, from blood, respiratory tract, urine, and sterile sites at 47 hospitals across Thailand. Among 187,619 isolates, 93,810 isolates (50.0%) were critically drug resistant, of which 12,915 isolates (13.8%) were randomly selected for molecular characterization. *E. coli* was most commonly isolated from all specimens, except the respiratory tract, in which ABC was predominant. Prevalence of extended-spectrum cephalosporin resistance (ESCR) was higher in *E. coli* (42.5%) than *K. pneumoniae* (32.0%), but carbapenem-resistant (CR)-*K. pneumoniae* (17.2%) was 4.5-fold higher than CR-*E. coli* (3.8%). The majority of ESCR/CR-*E. coli* and *K. pneumoniae* isolates carried bla_{CTX-M} (64.6% to 82.1%). bla_{NDM} and $bla_{OXA-48-like}$ were the most prevalent carbapenemase genes in CR-*E. coli*/CR-*K. pneumoniae* (74.9%/52.9% and 22.4%/54.1%, respectively). In addition, 12.9%/23.0% of CR-*E. coli*/CR-*K. pneumoniae* cocarried bla_{NDM} and $bla_{OXA-48-like}$. Among ABC isolates, 41.9% were extensively drug resistant (XDR) and 35.7% were multidrug resistant (MDR), while *P. aeruginosa* showed XDR/MDR at 6.3%/16.5%. *A. baumannii* was the most common species among ABC isolates. The major carbapenemase gene in MDR-*A. baumannii*/XDR-*A. baumannii* was $bla_{OXA-23-like}$ (85.8%/93.0%), which had much higher rates than other ABC species. bla_{IMP}, bla_{VIM}, $bla_{OXA-40-like}$, and $bla_{OXA-58-like}$ were also detected in ABC at lower rates. The most common carbapenemase gene in MDR/XDR-*P. aeruginosa* was bla_{IMP} (29.0%/30.6%), followed by bla_{VIM} (9.5%/25.3%). The findings reiterate an alarming situation of drug resistance that requires serious control measures.

KEYWORDS drug resistance, Gram-negative bacteria, surveillance, *bla* gene, Thailand

Critically drug-resistant Gram-negative bacilli (CDRGNB) constitute a significant cause of high morbidity and mortality worldwide. The most common CDRGNB in clinical practice include *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* complex (ABC), and *Pseudomonas aeruginosa*. Among *E. coli* and *K. pneumoniae*, serious concerns are specifically for extended-spectrum cephalosporin-resistant (ESCR) and carbapenem-resistant (CR) isolates. The most important species of ABC is *A. baumannii*. Some isolates of ABC and *P. aeruginosa* demonstrate high resistance to several antimicrobial classes, defined as multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacteria (1). Infections due to these CDRGNB likely contribute to significant long-term stays and high mortality rates (2). The World Health Organization announced that these bacteria were critical priorities for research and new antimicrobial agent development (3). A hospital surveillance system is a proactive approach to taking action on antimicrobial resistance and nosocomial infection. At the international level, surveillance data have been employed to monitor the emergence of resistant bacteria and prevent global spread.

Due to antimicrobial resistance, the mortality rate has strikingly exceeded 54 per 100,000 population in Thailand (4). The availability of over-the-counter drugs and overprescribing in hospitals of many antibacterial agents have provoked the situation. The survey revealed that 29.7% of Thai people were self-medicated with antibacterial agents, and most of them had symptoms resembling viral infection (5). The significant correlation between carbapenem consumption and the increase of CR-*E. coli* and CR-*K. pneumoniae* has also been demonstrated (6). While CDRGNB infection is on the rise in most Southeast Asian countries, large-scale surveillance of clinically significant isolates with molecular analysis remains very limited (7). This study aimed to conduct a nationwide prospective survey of CDRGNB prevalence in Thailand and investigate the molecular characteristics of beta-lactamase genes. CDRGNB isolates were classified into critically drug-resistant (CDR) groups, including CDR-*E. coli* (ESCR-*E. coli* and CR-*E. coli*), CDR-*K. pneumoniae* (ESCR-*K. pneumoniae* and CR-*K. pneumoniae*), CDR-ABC (MDR-ABC and XDR-ABC), and CDR-*P. aeruginosa* (MDR-*P. aeruginosa* and XDR-*P. aeruginosa*). The project was a part of the Research University Network Thailand's collaborative effort in partnership with government, military, and private hospitals to address the threatening health issues.

RESULTS

Prevalence of CDRGNB from clinical specimens. There were 47 hospitals across all Thailand regions that participated in this study, including university, government, military, and private hospitals of various sizes and types of services, as shown in Table 1. A total of 187,619 isolates of four clinically significant Gram-negative bacteria (*E. coli*, *K. pneumoniae*, ABC, and *P. aeruginosa*) from blood, respiratory tract, urine, and sterile site samples were reported during the study period. ABC isolates were collected as a complex according to biochemical-based identification commonly

TABLE 1 Hospitals participating in this study

Type of hospital	Level of care	Size (no. of beds)	No.
Study center and study hubs			
University	Tertiary care	>1,000	4
		>500–1,000	2
		≤500	1
Satellite hospitals			
Government	Tertiary care	>1,000	2
		>500–1,000	19
		≤500	7
	Secondary care	≤500	8
Private	Secondary care	≤500	3
Military	Tertiary care	>500–1,000	1
Total			47

TABLE 2 Prevalence of Gram-negative bacteria in this study

Organism	No. of isolates from clinical specimen (%)				
	All	Blood	Respiratory	Urine	Sterile site
E. coli	63,991 (34.1)	14,548 (56.7)	6,760 (7.4)	39,540 (65.4)	3,143 (31.3)
K. pneumoniae	48,013 (25.6)	6,053 (23.6)	27,618 (30.2)	11,786 (19.5)	2,556 (25.4)
ABC	42,616 (22.7)	3,201 (12.5)	33,741 (36.9)	3,490 (5.8)	2,184 (21.7)
P. aeruginosa	32,999 (17.6)	1,853 (7.2)	23,330 (25.5)	5,647 (9.3)	2,169 (21.6)
Total	187,619 (100)	25,655 (100)	91,449 (100)	60,463 (100)	10,052 (100)

used in hospital laboratories with limited species differentiation. These Gram-negative bacteria were mainly isolated from the respiratory tract (48.7%), followed by urine (32.2%), blood (13.7%), and sterile sites (5.4%). *E. coli* was the most predominant organism, followed by *K. pneumoniae*, ABC, and *P. aeruginosa* (Table 2). *E. coli* was most commonly isolated from blood, urine, and sterile sites, while ABC was most commonly isolated from the respiratory tract. *K. pneumoniae* was the second most common pathogen from all specimen types. Isolates in eight CDR groups comprised half of the study isolates, indicating the overall prevalence of CDRGNB at 50% from clinical specimens (Table 3). CDRGNB had high rates in all specimens, especially for the respiratory tract, urine, and sterile site samples, which were shown in over 50% of isolates. ESCR-*E. coli* was the most prevalent CDRGNB in overall specimens and was found at the highest rates from urine and blood, respectively, while ESCR-*K. pneumoniae* was most detected from the respiratory tract samples. XDR-ABC had a higher ratio over MDR-ABC and were most predominant in respiratory tract samples, followed by sterile sites. *P. aeruginosa* had less prevalence of MDR and XDR isolates than ABC and was also commonly found in the respiratory tract samples.

The distribution of each CDR group in clinical specimens is shown in Fig. 1. Overall, the prevalence of ESCR-*K. pneumoniae* was lower than ESCR-*E. coli*, but the CR-*K. pneumoniae* rate was 4.5-fold higher than that for CR-*E. coli*, resulting in a CDR-*K. pneumoniae* rate (49.2%) slightly higher than the CDR-*E. coli* rate (46.3%). A combination of ESCR-*E. coli* and CR-*E. coli* constituted more than half of *E. coli* isolates from the respiratory tract (54.7%) and sterile site (51.6%) samples and at high rates in urine (47.4%) and blood (38.0%) samples. ESCR-*K. pneumoniae* and CR-*K. pneumoniae* were detected in more than half of *K. pneumoniae* isolates from urine (59.6%) and sterile site (53.0%) samples and also at high rates in the respiratory tract (47.0%) and blood (37.1%) samples. CR-*K. pneumoniae* was at a higher rate than CR-*E. coli* in all specimen types.

TABLE 3 Prevalence of CDRGNB in various specimens

Organism	CDR group	No. of isolates from clinical specimen (%)				
		All (*N* = 187,619)	Blood (*N* = 25,655)	Respiratory (*N* = 91,449)	Urine (*N* = 60,463)	Sterile site (*N* = 10,052)
E. coli	ESCR-*E. coli*	27,169 (14.5)	5,246 (20.4)	3,295 (3.6)	17,167 (28.4)	1,461 (14.5)
	CR-*E. coli*	2,404 (1.3)	274 (1.1)	403 (0.4)	1,566 (2.6)	161 (1.6)
K. pneumoniae	ESCR-*K. pneumoniae*	15,378 (8.2)	1,364 (5.3)	9080 (9.9)	4,160 (6.9)	774 (7.7)
	CR-*K. pneumoniae*	8,220 (4.4)	886 (3.5)	3893 (4.3)	2,860 (4.7)	581 (5.8)
ABC	MDR-ABC	15,222 (8.1)	969 (3.8)	11,975 (13.1)	1,481 (2.5)	797 (7.9)
	XDR-ABC	17,792 (9.5)	1,156 (4.5)	14,491 (15.9)	1,265 (2.1)	880 (8.8)
P. aeruginosa	MDR-*P. aeruginosa*	5,509 (2.9)	232 (0.9)	3,766 (4.1)	1,223 (2.0)	288 (2.9)
	XDR-*P. aeruginosa*	2,030 (1.1)	69 (0.3)	929 (1.0)	942 (1.6)	90 (0.9)
Total		93,823 (50.0)	10,169 (39.6)	47,832 (52.3)	30,664 (50.7)	5,032 (50.1)
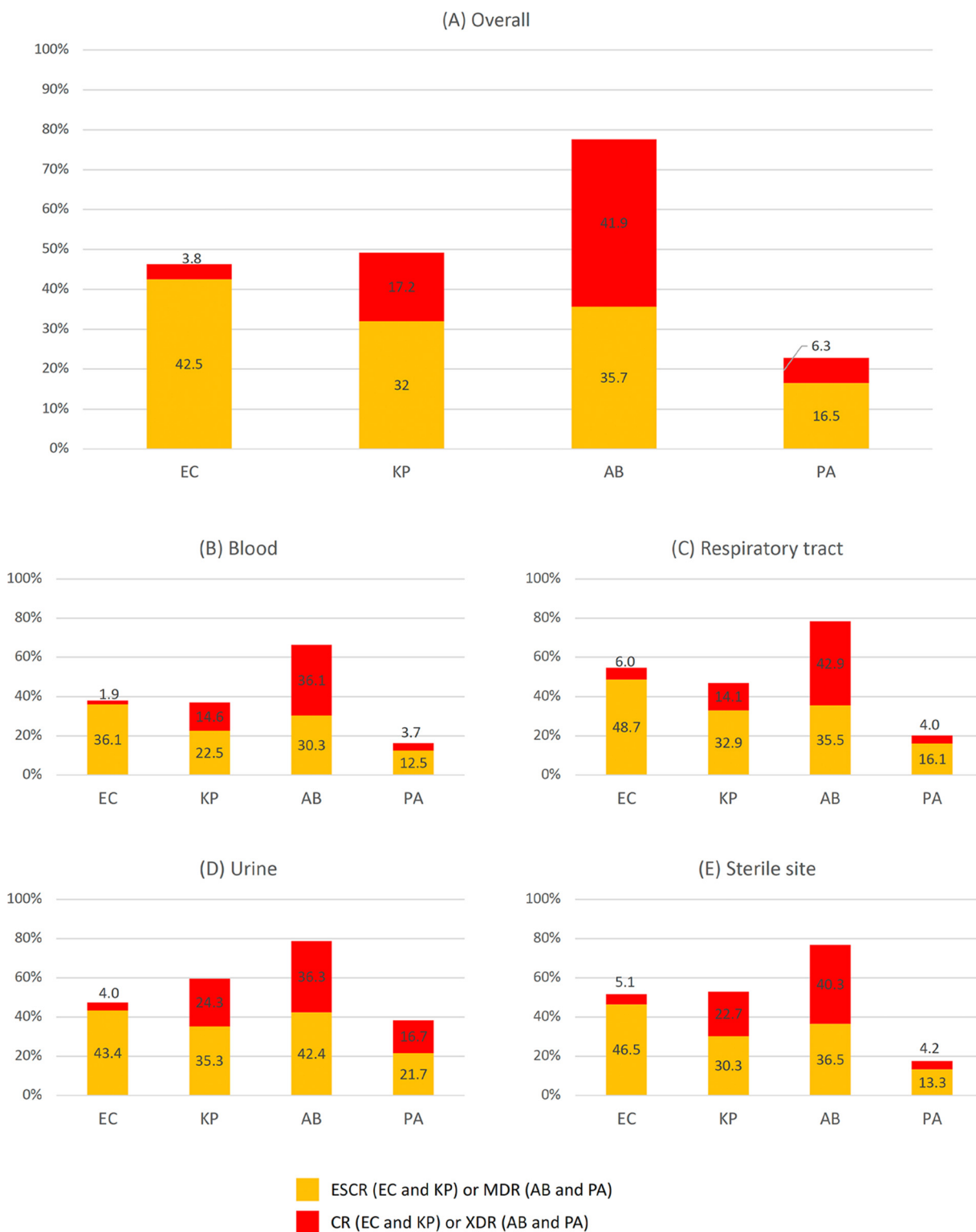
FIG 1 Prevalence of CDRGNB in different clinical specimens. Percentages of each CDRGNB group in overall (A), blood (B), respiratory tract (C), urine (D), and sterile site (E) samples are shown.

CDR-ABC were predominant among ABC isolates in all specimens, ranging from 66.4% to 78.7%. XDR-ABC had the highest rate in respiratory tract samples and higher rates than MDR-ABC in all specimen types except urine. MDR-*P. aeruginosa* and XDR-*P. aeruginosa* were less prevalent overall than other CDRGNB. CDR-*P. aeruginosa* was

TABLE 4 Molecular characteristics of *bla* genes among CDRGNB

CDR group (*n*)	% Detection								
	bla_{CTX-M}	bla_{KPC}	bla_{NDM}	bla_{IMP}	bla_{VIM}	bla_{OXA-23}	bla_{OXA-40}	bla_{OXA-48}	bla_{OXA-58}
ESCR-*E. coli* (2,952)	64.6	—	—	—	—	—	—	—	—
CR-*E. coli* (722)	66.9	0.8	74.9	2.9	4.6	—	—	22.4	—
ESCR-*K. pneumoniae* (3,391)	79.9	—	—	—	—	—	—	—	—
CR-*K. pneumoniae* (1,591)	82.1	0.5	52.9	1.8	2.8	—	—	54.1	—
MDR-ABC									
A. baumannii (1,158)	—	—	—	2.0	2.9	85.8	1.0	—	7.9
Non-*A. baumannii*[b] (181)	—	—	—	12.2	1.7	10.5	1.1	—	14.9
XDR-ABC									
A. baumannii (1,364)	—	—	—	0.8	1.3	93.0	0	—	3.9
Non-*A. baumannii*[b] (160)	—	—	—	6.9	3.1	11.9	3.1	—	8.8
MDR-*P. aeruginosa* (854)	—	—	—	29.0	9.5	—	—	—	—
XDR-*P. aeruginosa* (542)	—	—	—	30.6	25.3	—	—	—	—

[a]—, not determined.
[b]Species of *Acinetobacter baumannii* complex other than *A. baumannii* based on the absence of bla_{OXA-51}.

exceptionally high in urine samples, with a larger ratio of XDR-*P. aeruginosa* than in other specimens.

Molecular characteristics of resistance genes among CDRGNB. A total of 12,915 isolates, or approximately 13.8% of CDRGNB isolates, were randomly selected for resistance gene detection. The results of *bla* genes detected among these isolates are shown in Table 4. bla_{CTX-M} was highly prevalent among CDR-*E. coli* (65.9%) and CDR-*K. pneumoniae* (80.6%) at nearly similar rates between ESCR and CR groups of each organism. Among CR-*E. coli* isolates, bla_{NDM} was the predominant carbapenemase gene, followed by $bla_{OXA-48-like}$. However, both bla_{NDM} and $bla_{OXA-48-like}$ were common among CR-*K. pneumoniae*, with a slightly higher prevalence for $bla_{OXA-48-like}$; 12.9% and 23.0% of CR-*E. coli* and CR-*K. pneumoniae*, respectively, carried both bla_{NDM} and bla_{OXA-48}. Lesser numbers of CR-*E. coli* and CR-*K. pneumoniae* carried bla_{VIM}, bla_{IMP}, and bla_{KPC}, which were slightly more common in CR-*E. coli* than in CR-*K. pneumoniae*. Based on the detection of $bla_{OXA-51-like}$, 86.5% and 89.5% of ABC isolates were determined to be *A. baumannii*, and the rest were other species of ABC. $bla_{OXA-23-like}$ was the most prevalent carbapenemase gene in both MDR-*A. baumannii* and XDR-*A. baumannii* isolates and was shown at a much higher rate than in other ABC species. Metallo-β-lactamase (MBL) genes (bla_{IMP} and bla_{VIM}) and other OXA genes ($bla_{OXA-40-like}$ and $bla_{OXA-58-like}$) were also found with much less prevalence. Notably, bla_{IMP} and $bla_{OXA-58-like}$ were more common among other ABC species than in *A. baumannii*. In addition to $bla_{OXA-51-like}$, 67 (5.8%) and 38 (2.8%) isolates of MDR-*A. baumannii* and XDR-*A. baumannii*, respectively, carried both $bla_{OXA-23-like}$ and $bla_{OXA-58-like}$. Three MDR-*A. baumannii* isolates (0.3%), but not XDR-*A. baumannii*, had four *bla*$_{OXA}$ genes ($bla_{OXA-23-like}$, $bla_{OXA-40-like}$, $bla_{OXA-51-like}$, and $bla_{OXA-58-like}$). However, all MDR-ABC and XDR-ABC isolates with an MBL gene carried either bla_{IMP} or bla_{VIM}, i.e., none of them had both genes. bla_{IMP} was prominent at a similar rate in both MDR-*P. aeruginosa* and XDR-*P. aeruginosa*, followed by bla_{VIM}, which was more pronounced in XDR-*P. aeruginosa* than in MDR-*P. aeruginosa*. Eight MDR-*P. aeruginosa* (0.9%) and 21 XDR-*P. aeruginosa* (3.9%) isolates had both bla_{IMP} and bla_{VIM}.

Antimicrobial susceptibility of CDRGNB. Table 5 shows susceptibility rates of CDRGNB groups to alternative antimicrobial agents. XDR-ABC and XDR-*P. aeruginosa* are not shown due to their resistance to all tested drugs. Carbapenems and amikacin were highly effective against ESCR-*E. coli* and ESCR-*K. pneumoniae*, and amikacin remained moderate against CR-*E. coli* and CR-*K. pneumoniae*. Piperacillin-tazobactam had good activity for ESCR-*E. coli* but not ESCR-*K. pneumoniae*. ESCR-*E. coli* and ESCR-*K. pneumoniae* isolates were moderately susceptible to netilmicin and cefoxitin, but most were resistant to ciprofloxacin, trimethoprim-sulfamethoxazole, and tetracycline. CR-*E. coli* and CR-*K. pneumoniae* were resistant to most drugs, except aminoglycosides may have moderate activity against them. *A. baumannii* isolates were slightly less susceptible than other ABC species, except for trimethoprim-sulfamethoxazole. All XDR-*A. baumannii* and 98.3% of MDR-*A. baumannii*

TABLE 5 Antimicrobial susceptibility of CDRGNB

CDR group	% Susceptibility[a]												
	AMC	TZP	FOX	ETP	IMP	MEM	DOR	AMK	GEN	NET	CIP	SXT	TET
ESCR-*E. coli*	49.7	89.0	75.6	100	100	100	100	98.2	46.7	86.8	25.0	33.2	19.1
ESCR-*K. pneumoniae*	24.9	59.5	74.6	100	100	100	100	96.0	55.2	84.0	31.5	23.4	20.4
CR-*E. coli*	0.6	2.4	3.2	0.7	7.8	5.5	10.1	77.9	30.6	64.8	3.6	8.9	6.8
CR-*K. pneumoniae*	0.1	1.1	5.7	0.5	6.2	4.1	5.2	57.1	66.5	34.3	2.4	10.6	10.1
MDR-ABC													
A. baumannii	NA	2.5	NA	NA	3.3	3.0	3.0	55.3	26.5	NA	3.4	52.4	NA
Non-*A. baumannii*[b]	NA	12.7	NA	NA	8.2	9.2	5.5	57.5	26.5	NA	23.8	33.8	NA
MDR-*P. aeruginosa*	NA	25.0	NA	NA	4.4	5.9	6.8	65.3	19.9	43.3	12.7	NA	NA

[a]AMC (amoxicillin-clavulanate), TZP (piperacillin-tazobactam), FOX (cefoxitin), ETP (ertapenem), IMP (imipenem), MEM (meropenem), DOR (doripenem), AMK (amikacin), GEN (gentamicin), NET (netilmicin), CIP (ciprofloxacin), SXT (trimethoprim-sulfamethoxazole), TET (tetracycline), NA (not applicable).
[b]Species of *Acinetobacter baumannii* complex other than *A. baumannii* based on the absence of bla_{OXA-51}.

isolates were resistant to at least a carbapenem, resulting in the rate of carbapenem resistance among *A. baumannii* of 77.0%. Among *P. aeruginosa*, 96.8% of MDR-*P. aeruginosa* and all XDR-*P. aeruginosa* isolates were resistant to at least a carbapenem, given *P. aeruginosa*'s carbapenem resistance rate of 22.3%. Amikacin showed moderate activity against MDR-ABC and MDR-*P. aeruginosa*. Trimethoprim-sulfamethoxazole and netilmicin had partial action against MDR-*A. baumannii* and MDR-*P. aeruginosa*, respectively.

DISCUSSION

Infection due to CDRGNB has raised a serious concern worldwide and requires urgent solutions to reduce the wide spread of these organisms. Systematic surveillance of drug-resistant bacteria plays a vital role in monitoring and controlling the situation (8). This study conducted large-scale surveillance of four clinically significant Gram-negative bacteria in eight CDR groups. CDR-*E. coli* and CDR-*K. pneumoniae* were common causes of various infections, especially in the urinary tract, intra-abdominal area, and bloodstream, with high rates of extended-spectrum β-lactamase production and low susceptibility to fluoroquinolones, indicating multiple resistance mechanisms (9–11). The prevalence of CR-*E. coli* and CR-*K. pneumoniae* in Thailand has rapidly increased in the past decade. Studies in the late 2000s and early 2010s showed that *E. coli* and *K. pneumoniae* isolates from Thai patients were less than 1% and 5% resistant to carbapenems, respectively (12, 13). In this study, the CR-*E. coli* prevalence has risen to 3.8% and varied in different specimen types, ranging from 1.9% to 6.0%. An average proportion of CR-*K. pneumoniae* was 4.5-fold (ranging from 2.4- to 7.7-fold) higher than CR-*E. coli*. Therefore, within less than a decade, CR-*E. coli* and CR-*K. pneumoniae* prevalences in Thailand have risen more than three times. Inappropriate consumption of carbapenems was shown to contribute to an increased prevalence of CDRGNB, including CR-*E. coli* and CR-*K. pneumoniae* (6, 14). The increasing trends of CR-*E. coli* and CR-*K. pneumoniae* have also been reported from many countries in Southeast Asia, in which CR-*K. pneumoniae* often showed higher prevalence (15). Malaysia, Myanmar, Indonesia, and Vietnam were countries with a high prevalence of CR-*E. coli* (1.9% to 10.0%) and CR-*K. pneumoniae* (7.9% to 9.5%). Our study was among the most extensive and recent studies in Thailand. It revealed significantly higher CR-*E. coli* and CR-*K. pneumoniae* rates than what was shown in previous reports from Thailand and neighboring countries, raising a critical need for infection control actions. Aminoglycosides seemed to be the only class that is active for CR isolates but with modest activities. With a rise of CR isolates among *Enterobacteriaceae*, polymyxins, tigecycline, and fosfomycin were often choices for the alternative or last-line drug. However, these agents still have limitations due to toxicity or availability in different regions (16).

The most common carbapenemase genes in CR-*E. coli* and CR-*K. pneumoniae* detected in this study were bla_{NDM} and $bla_{OXA-48-like}$. In contrast to North America,

Latin America, some European countries, and China, where bla_{KPC} appears commonly, bla_{KPC} has rarely been detected in Thailand and Southeast Asia (17–19). bla_{NDM} has been endemic to South Asia and later widespread in Southeast Asia (15, 17). A study in Myanmar also revealed that all carbapenemase gene-carrying *Enterobacteriaceae* clinical isolates had bla_{NDM}, of which bla_{NDM-5} was the most predominant (20). bla_{NDM} was first reported in Thailand in 2012 (21), and this study, conducted approximately 6 years later, showed a very high prevalence of bla_{NDM} in both CR-*E. coli* and CR-*K. pneumoniae*, suggesting a successful spreading of the gene in this country. Various mobile genetic elements mediate its rapid dissemination, such as transposons and conjugative plasmids. bla_{NDM} was also found in CR-*E. coli* and CR-*K. pneumoniae* isolates from the environment, likely related to contracting these organisms through traveling in the region of endemicity (18). While bla_{IMP} and bla_{VIM} were found at a low prevalence, $bla_{OXA-48-like}$, a class D carbapenemase, was strikingly high among CR-*E. coli* and CR-*K. pneumoniae*. Originating in and primarily endemic to European and Middle East countries, $bla_{OXA-48-like}$ and its derivatives have rapidly spread to Africa, South America, and Asia (17, 18). OXA-48 and OXA-48-like carbapenemases display unique characteristics of a modest hydrolytic activity for penicillins and carbapenems but not for cephalosporins, making it problematic for *in vitro* detection (22). Although OXA-48 usually confers weak resistance to carbapenems in *Enterobacteriaceae*, it may be cocarried with an MBL gene, e.g., bla_{NDM}, as shown in this study, resulting in high-level carbapenem resistance (23).

CDR-ABC was shown to be the main portion of ABC isolates in all specimen types. The most common gene that mediates carbapenem resistance in ABC is bla_{OXA}, which produces a class D OXA β-lactamase. The majority species of ABC was *A. baumannii*, based on the presence of intrinsic $bla_{OXA-51-like}$ (24). Although $bla_{OXA-51-like}$ may not be the most accurate marker for species confirmation, it is practical to screen for *A. baumannii* in an extensive survey. Besides chromosomally encoded $bla_{OXA-51-like}$, we investigated three mainly plasmid-borne bla_{OXA} carbapenemase-related genes, $bla_{OXA-23-like}$, $bla_{OXA-40-like}$ (formerly known as bla_{OXA-24}), and $bla_{OXA-58-like}$, commonly present among ABC in Asian countries (25–28). All four OXA carbapenemases and their derivatives are transferable by a mobile genetic element. Either OXA-23 or OXA-51 is sufficient to confer carbapenem resistance in ABC, but generally, other carbapenemases and other mechanisms, e.g., efflux pump, often collectively coproduce high-level resistance. We showed that $bla_{OXA-23-like}$ was most common among MDR-*A. baumannii* and XDR-*A. baumannii* but was less prevalent among other species of ABC. $bla_{OXA-40-like}$ and $bla_{OXA-58-like}$ are less common and usually confer low activity against carbapenems. This study reported that ABC isolates carry up to four bla_{OXA} genes, making them highly resistant to carbapenems. Although other resistance mechanisms were not investigated, it is likely that a combination of resistance determinants attributed to the extreme resistance phenotype in ABC.

The worldwide SENTRY surveillance program demonstrated that CDR-*P. aeruginosa* had a declining rate and was most susceptible to colistin, followed by amikacin (29). A previous study in Thailand among hospitalized patients during 2014 showed a higher rate of XDR-*P. aeruginosa* but a lower rate of MDR-*P. aeruginosa* compared to this study, suggesting a decreasing trend of XDR-*P. aeruginosa* but not MDR-*P. aeruginosa* (30). Amikacin was shown in the SENTRY report and this study to be most active against MDR-*P. aeruginosa*, yet with a modest susceptibility rate. Most CDR-*P. aeruginosa* isolates were resistant to carbapenem. The carbapenem resistance rate among *P. aeruginosa* isolates tends to be stable or decreasing over time in many regions (29, 31). *P. aeruginosa* often intrinsically harbors several β-lactam resistance mechanisms, including membrane modification, efflux pump, and AmpC enzyme hyperproduction (32). MBL enzymes were sporadically studied among carbapenem-resistant *P. aeruginosa*, of which IMP and VIM were the most common carbapenemases (33, 34). We reported that bla_{IMP} was detected at approximately similar rates in both MDR-*P. aeruginosa* and XDR-*P. aeruginosa*, while bla_{VIM} was detected 2.7-fold more often in XDR-*P.*

aeruginosa than MDR-*P. aeruginosa*. In addition, XDR-*P. aeruginosa* isolates that carried both bla_{IMP} and bla_{VIM} genes were 4.3-fold more common than MDR-*P. aeruginosa*. Therefore, bla_{VIM} could contribute to extended resistance to multiple drugs among *P. aeruginosa* isolates, especially when cocarrying bla_{IMP}. Despite a lesser proportion of resistant isolates than other CDRGNB, both MDR-*P. aeruginosa* and XDR-*P. aeruginosa* remain a severe threat because of their high resistance to commonly used antipseudomonal drugs and their critical role in nosocomial infection. Carbapenem resistance in *P. aeruginosa* is often mediated by MBL genes acquirable through a mobile plasmid and is significantly associated with high mortality (34).
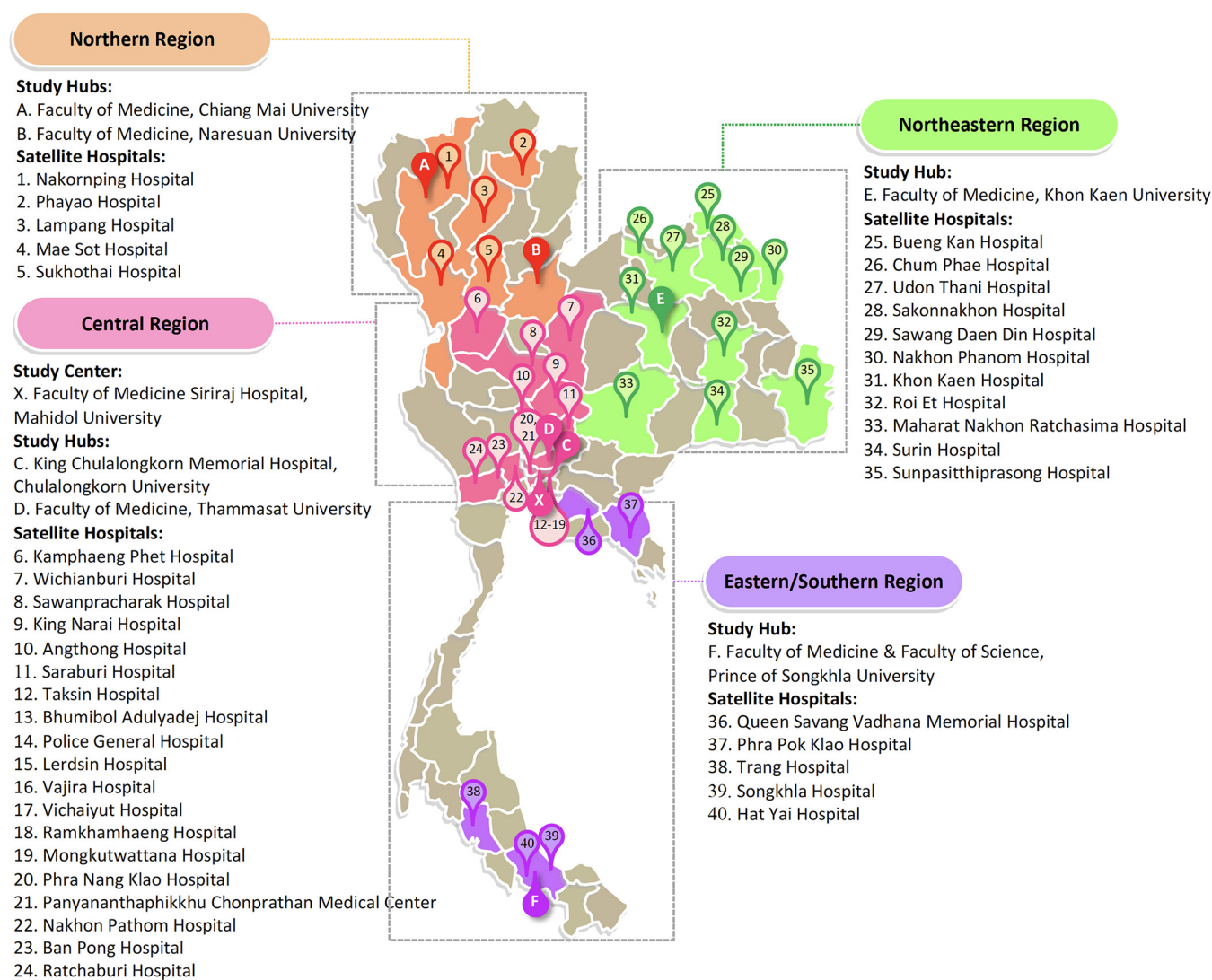
This study has limitations in exploring specific and additional resistance genes and other mechanisms that could contribute to resistance due to a large number of study isolates. In addition, susceptibility to some alternative drugs, e.g., colistin, was not tested because the recommended testing methods were not available at all participating hospitals. Although *A. baumannii* is the most common species of ABC, this study used $bla_{OXA-51-like}$ as a marker for *A. baumannii* but did not specifically differentiate all species. Therefore, phenotypic and genotypic resistance profiles of each species of ABC were not analyzed separately. These limitations would be further studied in selected groups to understand the evolving resistance among CDRGNB and their possible relatedness of resistance clones in various geographic regions. We emphasize in this report a high prevalence of CDRGNB in Thailand in eight CDR groups, raising concern for a severe threat in the Asia-Pacific region. These CDRGNB isolates are usually encountered in various infections, mainly associated with hospitalized patients. The threat of bacterial resistance should be critically addressed in Thailand and in the region for the necessity of appropriate antimicrobial prescription in clinical practice. In addition, the public policy regarding the self-consumption of antibacterial agents should be emphasized to reduce the risk of acquiring drug-resistant infections. Continuously prospective surveillance is an active measure to monitor and control the spread of resistance and to support the importance of antimicrobial stewardship programs. Further studies are recommended to investigate the impact of CDRGNB on various clinical aspects, such as community-acquired infections, nosocomial infections, and treatment outcomes. Multiple organizations and professionals are needed to build a scholarly society to prevent spreading resistance.

MATERIALS AND METHODS

Study design and study sites. Forty-seven hospitals participated as study sites were located across all regions of Thailand (Fig. 2). The Faculty of Medicine, Siriraj Hospital, Mahidol University (Bangkok), served as the study center, and an additional six major university hospitals served as the study hubs. The other 40 hospitals were designated satellite hospitals. Isolates and data from four clinically significant Gram-negative bacteria (*E. coli*, *K. pneumoniae*, ABC, and *P. aeruginosa*) were collected from blood, respiratory, urine, and sterile site (including cerebrospinal fluid, pleural fluid, abdominal fluid, joint fluid, and other fluids from sterile body parts) samples from patients between October 2017 and January 2019. Isolate collection was inclusive for all patient groups at each hospital without stratification according to disease onset or illness history. Isolates were evaluated for prevalence, antimicrobial susceptibility, and molecular characteristics. Each hospital performed bacterial identification and antimicrobial susceptibility testing, including quality control testing, at its laboratory based on standardized microbiological procedures, and additional external quality assessment was provided. Repeated isolates from the same patient were excluded. Random CDRGNB isolates were sent to a study hub or selected study centers for molecular study. The molecular study included the first three isolates of the month, if available, for each CDRGNB group in every clinical specimen from all hospitals for up to 12 months during the collection period. This procedure ensured that isolates were enrolled without selection bias of patient demography, geographic area, or time of collection. This study was approved by the Institution Review Board or Ethical Committee of all participating hospitals.

Resistance definitions. CDRGNB isolates were divided into eight CDR groups, including ESCR-*E. coli*, CR-*E. coli*, ESCR-*K. pneumoniae*, CR-*K. pneumoniae*, MDR-ABC, XDR-ABC, MDR-*P. aeruginosa*, and XDR-*P. aeruginosa*. As defined by the Centers for Disease Control and Prevention, ESCR isolates of *E. coli* and *K. pneumoniae* were resistant to at least one of these cephalosporin agents: ceftriaxone, cefotaxime, ceftazidime, and cefepime. CR isolates were resistant to at least one of these carbapenem agents: imipenem, meropenem, doripenem, and ertapenem (35). *A. baumannii* and *P. aeruginosa* were classified according to their resistance as MDR and XDR. MDR isolates were nonsusceptible to at least one agent in at least

Northern Region

Study Hubs:
A. Faculty of Medicine, Chiang Mai University
B. Faculty of Medicine, Naresuan University

Satellite Hospitals:
1. Nakornping Hospital
2. Phayao Hospital
3. Lampang Hospital
4. Mae Sot Hospital
5. Sukhothai Hospital

Central Region

Study Center:
X. Faculty of Medicine Siriraj Hospital, Mahidol University

Study Hubs:
C. King Chulalongkorn Memorial Hospital, Chulalongkorn University
D. Faculty of Medicine, Thammasat University

Satellite Hospitals:
6. Kamphaeng Phet Hospital
7. Wichianburi Hospital
8. Sawanpracharak Hospital
9. King Narai Hospital
10. Angthong Hospital
11. Saraburi Hospital
12. Taksin Hospital
13. Bhumibol Adulyadej Hospital
14. Police General Hospital
15. Lerdsin Hospital
16. Vajira Hospital
17. Vichaiyut Hospital
18. Ramkhamhaeng Hospital
19. Mongkutwattana Hospital
20. Phra Nang Klao Hospital
21. Panyananthaphikkhu Chonprathan Medical Center
22. Nakhon Pathom Hospital
23. Ban Pong Hospital
24. Ratchaburi Hospital

Northeastern Region

Study Hub:
E. Faculty of Medicine, Khon Kaen University

Satellite Hospitals:
25. Bueng Kan Hospital
26. Chum Phae Hospital
27. Udon Thani Hospital
28. Sakonnakhon Hospital
29. Sawang Daen Din Hospital
30. Nakhon Phanom Hospital
31. Khon Kaen Hospital
32. Roi Et Hospital
33. Maharat Nakhon Ratchasima Hospital
34. Surin Hospital
35. Sunpasitthiprasong Hospital

Eastern/Southern Region

Study Hub:
F. Faculty of Medicine & Faculty of Science, Prince of Songkhla University

Satellite Hospitals:
36. Queen Savang Vadhana Memorial Hospital
37. Phra Pok Klao Hospital
38. Trang Hospital
39. Songkhla Hospital
40. Hat Yai Hospital

FIG 2 Location of study sites. Forty-seven hospitals across Thailand were enrolled in this study, including in the Northern ($n = 7$), Central ($n = 22$), Northeastern ($n = 12$), and Eastern/Southern ($n = 6$) regions. Hospitals were categorized into study center ($n = 1$), study hubs ($n = 6$), and satellite hospitals ($n = 40$).

three antibacterial agent categories, and XDR isolates were nonsusceptible to at least one agent in all but two or fewer antimicrobial categories (1).

Molecular characterization of *bla* genes. Eight groups of CDRGNB from various specimens were collected for further molecular characterization. Each CDRGNB group was randomized monthly to ensure suitable distribution and that isolates collected each month during the study period were included. Bacterial DNA was extracted from colonies using the boiling method. PCR was used for the detection of beta-lactamase (*bla*) genes, including bla_{CTX-M}, bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, $bla_{OXA-23-like}$, $bla_{OXA-40-like}$, $bla_{OXA-48-like}$, $bla_{OXA-51-like}$, and $bla_{OXA-58-like}$. The multiplex PCR primer sets (M1, M2, M3, and M4) and simplex PCR primer (S) were used to detect various specific genes, as detailed in Table 6. According to the corresponding reference, PCR was performed under the relevant conditions except where indicated, and the amplified targets were verified by DNA sequencing if necessary.

Antimicrobial susceptibility testing. Susceptibility testing was performed by using a standard disk diffusion assay or an automated system, either Sensititre (Thermo Fisher Scientific, OH, USA), Vitek2 (bioMérieux, Marcy-L'Etoile, France), or Phoenix (Becton, Dickinson, MD, USA), available at each study site. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (36). All bacterial identification and antimicrobial susceptibility test results were submitted electronically and verified according to the resistance definitions.

TABLE 6 PCR primers used in this study

Primer set	Target organism	Target gene	Primer sequence	Amplicon size (bp)	Reference
M1	ESCR-*E. coli* and ESCR-*K. pneumoniae*	bla_{CTX-M}	(F) 5'-GCGATGTGCAGCACCAGTAA-3'	605	37
			(R) 5'-GGTTGAGGCTGGGTGAAGTA-3'		
		16S rRNA	(F) 5'-AGAGTTTGATCCTGGCTCAG-3'	802	38
			(R) 5'-GCGTGGACTACCAGGGTATC-3'		
M2	CR-*E. coli*, CR-*K. pneumoniae*, MDR-*A. baumannii*, XDR-*A. baumannii*, MDR-*P. aeruginosa* and XDR-*P. aeruginosa*	bla_{IMP}	(F) 5'-GGAATAGAGTGGCTTAAYTCTC-3'	188	39
			(R) 5'-CCAAACYACTASGTTATCT-3'		
		16S rRNA	Similar to M1	802	38
M3[a]	ESCR-*E. coli* and ESCR-*K. pneumoniae*	bla_{NDM}	(F) 5'-GGTTTGGCGATCTGGTTTTC-3'	621	40
			(R) 5'-CGGAATGGCTCATCACGATC-3'		
		bla_{KPC}	(F) 5'-CGTCTAGTTCTGCTGTCTTG-3'	798	40
			(R) 5'-CTTGTCATCCTTGTTAGGCG-3'		
		$bla_{OXA-48-like}$	(F) 5'-GCGTGGTTAAGGATGAACAC-3'	438	40
			(R) 5'-CATCAAGTTCAACCCAACCG-3'		
M4	MDR-*A. baumannii* and XDR-*A. baumannii*	$bla_{OXA-23-like}$	(F) 5'-GATCGGATTGGAGAACCAGA-3'	501	41
			(R) 5'-ATTTCTGACCGCATTTCCAT-3'		
		$bla_{OXA-40-like}$	(F) 5'-GGTTAGTTGGCCCCCTTAAA-3'	246	41
			(R) 5'-AGTTGAGCGAAAAGGGGATT-3'		
		$bla_{OXA-51-like}$	(F) 5'-TAATGCTTTGATCGGCCTTG-3'	353	41
			(R) 5'-TGGATTGCACTTCATCTTGG-3'		
		$bla_{OXA-58-like}$	(F) 5'-AAGTATTGGGGCTTGTGCTG-3'	599	41
			(R) 5'-CCCCTCTGCGCTCTACATAC-3'		
S[b]	CR-*E. coli*, CR-*K. pneumoniae*, MDR-*P. aeruginosa*, XDR-*P. aeruginosa*, MDR-*A. baumannii*, and XDR-*A. baumannii*	bla_{VIM}	(F) 5'-GATGGTGTTTGGTCGCATA-3'	390	25
			(R) 5'-CGAATGCGCAGCACCAG-3'		

[a]The PCR conditions were 94°C for 5 min, 30 cycles of 95°C for 45 s/56°C for 45 s/72°C for 1 min, and 72°C for 5 min.
[b]Only for *A. baumannii*, the PCR conditions were 94°C for 5 min, 30 cycles of 94°C for 25 s/62°C for 40 s/72°C for 50 s, and 72°C for 6 min.

Prince Hospital (Sitthipong Dumrongpiwat, Chonteera Charoenchai), Sawanpracharak Hospital (Wittaya Wangsomboonsiri, Kwanjai Ketwong), Songkhla Hospital (Sujinda Ruangchan, Benjamat Jareeyaphadub), Sukhothai Hospital (Sarawut Promsen), Sunpasitthiprasong Hospital (Jiraporn Nilsakul), Surin Hospital (Sunee Chayangsu, Saowarat Deekae), Taksin Hospital (Huttaya Thuncharoon, Patcharee Choochur), Thammasat University Hospital (Narissara Mungkornkaew), Trang Hospital (Chitprasong Malaisri, Yaowares Panjakun), Udon Thani Hospital (Adisak Sangchankoom, Sarochin Pangnakorn), Vajira Hospital (Tananun Tanpaibule, Daranee Nutalai), Vichaiyut Hospital (Pannawadee Uppathamnarakorn, Warattaya Ueaphathanaphanich), Wichianburi Hospital (Nutwariya Homsukon, Narumol Boonchaiyang).

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