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MECHANISMS OF RESISTANCE

Antecedent Carbapenem Exposure as a Risk Factor for Non-Carbapenemase-Producing Carbapenem-Resistant *Enterobacteriaceae* and Carbapenemase-Producing *Enterobacteriaceae*

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ABSTRACT Carbapenem-resistant *Enterobacteriaceae* (CRE) can be mechanistically classified into carbapenemase-producing *Enterobacteriaceae* (CPE) and non-carbapenemase-producing carbapenem nonsusceptible *Enterobacteriaceae* (NCPCRE). We sought to investigate the effect of antecedent carbapenem exposure as a risk factor for NCPCRE versus CPE. Among all patients with CRE colonization and infection, we conducted a case-control study comparing patients with NCPCRE (cases) and patients with CPE (controls). The presence of carbapenemases was investigated with phenotypic tests followed by PCR for predominant carbapenemase genes. We included 843 unique patients with first-episode CRE, including 387 (45.9%) NCPCRE and 456 (54.1%) CPE. The resistance genes detected in CPEs were bla_{NDM} (42.8%), bla_{KPC} (38.4%), and

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$bla_{OXA-48-like}$ (12.1%). After adjusting for confounders and clustering at the institutional level, the odds of prior 30-day carbapenem exposure was three times higher among NCPCRE than CPE patients (adjusted odds ratio [aOR], 3.48; 95% confidence interval [CI], 2.39 to 5.09; $P < 0.001$). The odds of prior carbapenem exposure and NCPCRE detection persisted in stratified analyses by *Enterobacteriaceae* species (*Klebsiella pneumoniae* and *Escherichia coli*) and carbapenemase gene (bla_{NDM} and bla_{KPC}). CPE was associated with male gender (aOR, 1.45; 95% CI, 1.07 to 1.97; $P = 0.02$), intensive care unit stay (aOR, 1.84; 95% CI, 1.24 to 2.74; $P = 0.003$), and hospitalization in the preceding 1 year (aOR, 1.42; 95% CI, 1.01 to 2.02; $P = 0.05$). In a large nationwide study, antecedent carbapenem exposure was a significant risk factor for NCPCRE versus CPE, suggesting a differential effect of antibiotic selection pressure.

KEYWORDS carbapenem resistance, carbapenem-resistant *Enterobacteriaceae*, carbapenemase-producing *Enterobacteriaceae*, risk factors

The emergence and rapid spread of carbapenem-resistant *Enterobacteriaceae* (CRE) with associated limited antimicrobial options for treatment and poor clinical outcomes (1) is a major threat to safe health care delivery. Mechanisms of carbapenem nonsusceptibility can be divided broadly into carbapenemase production (carbapenemase-producing *Enterobacteriaceae* [CPE]) and a combination of β-lactamase (extended-spectrum β-lactamase [ESBL] and AmpC) production and dysregulation of porin channels (non-carbapenemase-producing carbapenem nonsusceptible *Enterobacteriaceae* [NCPCRE]) (2). Carbapenemase genes are diverse and can be located chromosomally or within plasmids, with the latter conferring ease of transmissibility within and between bacterial species.

Orsi and colleagues (3) demonstrated that compared to *Klebsiella pneumoniae* carbapenemase (KPC)-producing CRE, NCPCRE were associated with prior antibiotic exposure, demonstrating that patient-level risk factors may differ according to mechanisms of resistance. On the other hand, existing evidence suggests that the fitness cost renders NCPCRE less transmissible than CPE (4). The successful Israeli national intervention for CRE control, which instituted more stringent infection prevention and control (IPC) measures (without additional antibiotic stewardship interventions) for CPE than for NCPCRE (5), suggests that CPE may be more likely to emerge through horizontal bacterial or gene transmission than NCPCRE. A more recent study by Simner and colleagues (6) described an association between overnight stay in foreign health care facilities and CPE detection in the United States. However, the authors did not explore the impact of prior carbapenem exposure.

Prior antibiotic exposure, specifically, carbapenem exposure, has long been associated with the emergence of CRE. However, considering the diverse mechanisms of resistance, it is possible that the impact of antibiotic exposure on the occurrence of CRE may vary depending on the resistance mechanism. Identifying potentially different impacts of carbapenem exposure, especially in settings where the bacteria are endemic, is essential for the optimal implementation of infection control and antimicrobial stewardship programs.

To this effect, we aimed to study the role of antecedent carbapenem exposure as a risk factor for NCPCRE and CPE. We hypothesized that antecedent carbapenem exposure was associated more with NCPCRE than with CPE.

RESULTS

Between September 2010 and April 2015, 843 patients with CRE were recruited. Of these, 456 CPE and 387 NCPCRE patients fulfilled the inclusion criteria (Fig. 1). More than 80% of CPE patients had bla_{NDM} and bla_{KPC} carbapenemase genes. CRE was identified through active surveillance cultures (stool samples, rectal swabs, or perianal swabs) (52.8%), followed by urine (18.4%) and blood (13.8%) (Table 1). *K. pneumoniae* was the most common *Enterobacteriaceae* (50.9%), followed by *E. coli* (25.2%), and

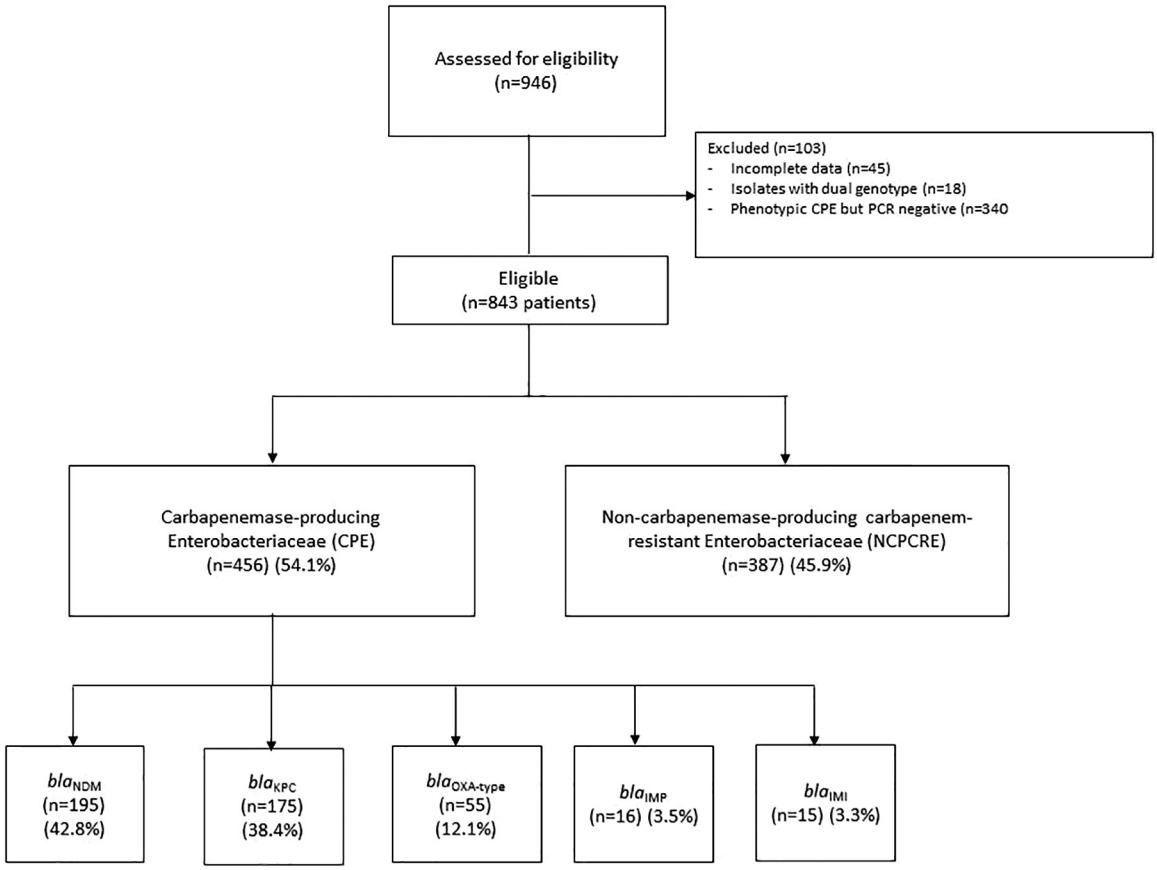

FIG 1 Selection of study subjects.

Enterobacter sp. (17.6%). Of the CRE isolates, 97.2% were not susceptible to meropenem and/or imipenem and 2.9% were not susceptible to ertapenem only.

Impact of carbapenem exposure on NCPCRE and CPE. As summarized in Table 2, on univariate analysis, NCPCRE had higher frequencies of carbapenem exposure than CPE (odds ratio [OR], 2.97; 95% confidence interval [CI], 2.22 to 3.97; $P < 0.001$). On multivariate analysis, NCPCRE had three times higher odds of carbapenem exposure (adjusted OR [aOR], 3.48; 95% CI, 2.39 to 5.09; $P < 0.001$) (Table 2). This association strengthened with increasing duration of carbapenem exposure. On multivariate analysis, patients receiving more than 3 days of carbapenems had the highest odds of NCPCRE (aOR, 3.67; 95% CI, 2.44 to 5.54; $P < 0.001$), followed by patients receiving 1 to 3 days of carbapenems (OR, 2.59; 95% CI, 1.44 to 4.66; $P = 0.002$) (see Table S1 in the supplemental material).

As summarized in Table 3, on stratified analysis by *Enterobacteriaceae* species, the odds of carbapenem exposure was higher among NCPCR *K. pneumoniae* (aOR, 5.34; 95% CI, 2.96 to 9.64; $P < 0.001$) (see Table S2 in the supplemental material) and NCPCRE *E. coli* (aOR, 5.58; 95% CI, 2.37 to 13.15; $P < 0.001$) (see Table S3 in the supplemental material) than carbapenemase-producing (CP) *K. pneumoniae* and CP *E. coli*, respectively. On stratified analysis by genotype, the odds of carbapenem exposure was higher among KPC-producing *Enterobacteriaceae* (aOR, 7.04; 95% CI, 3.55 to 13.99; $P < 0.001$) (see Table S4 in the supplemental material) and NDM-producing *Enterobacteriaceae* (aOR, 3.74; 95% CI, 2.31 to 6.05; $P < 0.001$) (see Table S5 in the supplemental material). Carbapenem exposure emerged an independent risk factor for NCPCRE in stratified analysis by specimen type (see Table S6 and S7 in the supplemental material).

Other risk factors for NCPCRE and CPE. In addition to carbapenems, the odds of antecedent trimethoprim-sulfamethoxazole exposure was 2.5 times higher among

TABLE 1 *Enterobacteriaceae* species, sources, and susceptibility patterns[a]

	No. (%) of patients with:		
Enterobacteriaceae	**CRE (*n* = 843)**	**NCPCRE (*n* = 387)**	**CPE (*n* = 456)**
Specimen			
Stool or rectal swabs	445 (52.8)	186 (48.1)	259 (56.8)
Blood	116 (13.8)	42 (10.9)	74 (16.2)
Urine	155 (18.4)	86 (22.2)	69 (15.1)
Wound	40 (4.7)	20 (5.2)	20 (4.4)
Intra-abdominal and hepatobiliary specimens	39 (4.6)	31 (8.0)	8 (1.8)
Others	48 (5.7)	22 (5.7)	26 (5.7)
Bacterial species			
Klebsiella pneumoniae	429 (50.9)	241 (62.3)	188 (41.2)
Escherichia coli	212 (25.2)	63 (16.3)	149 (32.7)
Enterobacter sp.	148 (17.6)	59 (15.3)	89 (19.5)
Citrobacter sp.	22 (2.6)	1 (0.3)	21 (4.6)
Klebsiella sp.	7 (0.8)	5 (1.3)	2 (0.4)
Proteus sp.	14 (1.7)	12 (3.1)	2 (0.4)
Others[b]	11 (1.3)	6 (1.6)	5 (1.1)
Susceptibility			
Not susceptible to meropenem and/or imipenem	819 (97.2)	378 (97.7)	441 (96.7)
Not susceptible to ertapenem only	24 (2.9)	9 (2.3)	15 (3.3)

[a]Abbreviations: CRE, carbapenem-resistant *Enterobacteriaceae*; NCPCRE, non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*; CPE, carbapenemase-producing *Enterobacteriaceae*.
[b]Others include *Morganella morganii* and *Serratia marcescens*.

NCPCRE than CPE (aOR, 2.44; 95% CI, 1.27 to 4.69; $P = 0.01$) (Table 2). Additionally, independent risk factors for CPE were male gender (aOR, 1.45; 95% CI, 1.07 to 1.97; $P = 0.02$), hospitalization during preceding 1 year (aOR, 1.42; 95% CI, 1.01 to 2.02; $P = 0.05$), and being in the intensive care unit (ICU) at the time of culture (aOR, 1.84; 95% CI, 1.24 to 2.74; $P = 0.003$).

DISCUSSION

In the current analysis involving 843 subjects over 5 years, first-episode NCPCRE detection was associated with carbapenem exposure compared with first-episode CPE detection, an association strengthened by the dose-response relationship with increasing duration of carbapenem exposure. Additionally, carbapenem exposure remained a risk factor for first-episode NCPCRE detection in analyses stratified by bacterial species (*E. coli* and *K. pneumoniae*), CP genotype (bla_{NDM} and bla_{KPC}), and specimen type (surveillance cultures and clinical cultures). Conversely, residence in the ICU, hospitalization in the preceding 12 months, and male gender increased the likelihood of first episode CPE detection.

Consistent with our study findings, available epidemiologic studies suggest potentially differing mechanisms of acquisition between NCPCRE with CPE, with NCPCRE possibly arising from *de novo* mutations or genetic reassortment of carbapenem-sensitive *Enterobacteriaceae* under antimicrobial selection pressure and CPE being acquired via clonal bacterial spread or horizontal gene transfer (mainly plasmid-mediated), as carbapenemase genes are not endogenously generated *in vivo* in patients (7–9). Since the occurrence and propagation of resistance mechanisms could be affected by bacterial species and prevailing dominant genotypes, we conducted multiple stratified analyses that showed an association between carbapenem exposure and NCPCRE regardless of the genomic resistance mechanism and *Enterobacteriaceae* species, as reported before by Cheng et al. (10).

Currently, IPC measures (e.g., hand hygiene, environmental hygiene, and early detection and isolation of CRE carriers) and antibiotic stewardship (ASP) are considered the main pillars of CRE control strategies (11–15), regardless of the prevailing resistance mechanisms. Our findings suggest that antimicrobial stewardship may play a more significant role in preventing NCPCRE than CPE. Although important for the allocation

TABLE 2 Association between antecedent carbapenem exposure and types of carbapenem-resistant *Enterobacteriaceae*[a]

Variable	Patients with:		Values by analysis			
			Univariate		Multivariate[d]	
	NCPCRE[b] ($n = 387$)	CPE[bc] ($n = 456$)	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
Exposure of interest						
Carbapenem exposed	197 (50.9)	118 (25.9)	2.97 (2.22–3.97)	<0.001	**3.48 (2.39–5.09)**	**<0.001**
Demographics						
Gender, male	200 (51.7)	273 (59.9)	0.72 (0.55–0.94)	0.02	**0.69 (0.51–0.94)**[e]	**0.02**
Age, median years (IQR)	68 (57–80)	67 (58–77)		0.55		
Medical history (1 year before culture)						
Charlson score, median (IQR)	5 (3–8)	6 (3–8)		0.05	0.97 (0.92–1.01)	0.15
Hospitalization	255 (65.9)	327 (71.7)	0.76 (0.57–1.02)	0.07	**0.70 (0.50–0.99)**[e]	**0.05**
Upper gastrointestinal scopes	80 (20.7)	108 (23.7)	0.84 (0.61–1.16)	0.30		
Lower gastrointestinal scopes	47 (12.1)	53 (11.6)	1.05 (0.69–1.60)	0.82		
Surgery	241 (62.3)	305 (66.9)	0.82 (0.62–1.08)	0.16		
Multidrug-resistant organisms[f]	152 (39.3)	167 (36.6)	1.12 (0.85–1.48)	0.43		
Current hospitalization						
Time at risk[g], median days (IQR)	12 (2–31)	8 (1–23.5)		<0.001	1.00 (0.99–1.00)	0.34
ICU at the time of culture	85 (22.0)	124 (27.2)	0.75 (0.55–1.03)	0.08	**0.54 (0.37–0.81)**[e]	**0.003**
Feeding tube[h]	144 (37.2)	157 (34.4)	1.13 (0.85–1.50)	0.40		
Central venous line[h]	164 (42.4)	160 (35.1)	1.36 (1.03–1.80)	0.03	0.99 (0.69–1.43)	0.97
Antibiotic exposure during 30 days before culture (proportions)						
Third- and fourth-generation cephalosporins	122 (31.5)	125 (27.4)	1.22 (0.91–1.64)	0.19	1.00 (0.67–1.49)	>0.99
Extended-spectrum penicillins	228 (58.9)	258 (56.6)	1.10 (0.84–1.45)	0.49	1.09 (0.80–1.48)	0.60
Fluoroquinolones	100 (25.8)	87 (19.1)	1.48 (1.07–2.05)	0.02	1.15 (0.80–1.68)	0.45
Aminoglycosides	66 (17.1)	52 (11.4)	1.60 (1.08–2.36)	0.02	1.02 (0.64–1.63)	0.93
Tigecycline	9 (2.3)	9 (1.9)	1.18 (0.46–3.00)	0.73		
Trimethoprim-sulfamethoxazole	37 (9.6)	17 (3.7)	2.73 (1.51–4.93)	0.001	**2.44 (1.27–4.69)**	**0.01**
Metronidazole	94 (24.3)	89 (19.5)	1.32 (0.95–1.84)	0.10	1.20 (0.78–1.84)	0.42
Specimen type						
Clinical cultures (versus surveillance cultures)	224 (57.9)	201 (44.1)	1.74 (1.33–2.29)	<0.001	1.03 (0.74–1.43)	0.87

[a]Abbreviations: NCPCRE, non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*; CPE, carbapenemase-producing *Enterobacteriaceae*; ICU, intensive care unit; IQR, interquartile range; OR, odd ratio; aOR, adjusted odds ratio.

[b]Unless otherwise indicated, values are *n* (%).

[c]CPE is the reference comparison group (control group) unless stated otherwise.

[d]Random effect logistic regression model allowing for clustering within hospitals and adjusted for month of CRE sample collection. Statistically significant values are shown in boldface.

[e]For ease of interpretation of risk factors for CPE, the logistic regression model was repeated with NCPCRE as the reference group and the results were as follows: male gender (aOR,1.45; 95% CI, 1.07 to 1.97; $P = 0.02$); hospitalization during preceding 1 year (aOR, 1.42; 95% CI, 1.01 to 2.02; $P = 0.05$), and being in the ICU at the time of culture (aOR, 1.84; 95% CI, 1.24 to 2.74; $P = 0.003$).

[f]Multidrug-resistant organisms includes methicillin-resistant *Staphylococcus aureus* (MRSA), carbapenem-resistant *Acinetobacter baumannii* (CRAB), vancomycin-resistant enterococci (VRE), and carbapenem-resistant *Pseudomonas aeruginosa* (CRPA).

[g]Days from admission to collection of culture.

[h]During time at risk.

of scarce resources, the impact of CRE resistance mechanisms on the efficacy of different infection prevention and antimicrobial stewardship interventions remains largely undetermined. In the often-cited successful experience of CRE control in Israel, CPE carriers were cohorted with dedicated staff, while NCPCRE carriers were placed under contact precautions without cohorting (5).

In our current analysis, male gender was a risk factor for CPE carriage. Prior studies have demonstrated an association between male gender and methicillin-resistant *Staphylococcus aureus* (MRSA) (16), as well as health care-associated infections (17). As a first, we found that male gender is also a risk factor for CPE, specifically, NDM-producing *Enterobacteriaceae*. Community studies of hand hygiene in public restrooms (18) and motorway station restrooms (19) suggest that women show better compliance to hand hygiene and soap use than men. Further studies are needed among hospitalized patients to understand the difference in the occurrence CRE among male and female patients.

TABLE 3 Association between antecedent carbapenem exposure and the occurrence of carbapenem-resistant *Enterobacteriaceae* stratified by species and carbapenemase types[a]

Stratified analyses by comparison	Multivariate analysis[b] aOR (95% CI)
NCPCR *K. pneumoniae* vs CP *K. pneumoniae*[c]	5.34 (2.96–9.64)
NCPCR *E. coli* vs CP *E. coli*[d]	5.58 (2.37–13.15)
NCPCRE vs KPC-producing *Enterobacteriaceae*[e]	7.04 (3.55–13.99)
NCPCRE vs NDM-producing *Enterobacteriaceae*[f]	3.74 (2.31–6.05)

[a]Abbreviations: NCPCR, non-carbapenemase-producing carbapenem-resistant; CP, carbapenemase-producing; NCPCRE, non-carbapenemase-producing carbapenem-resistant *Enterobacteriaceae*; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo-beta-lactamase.
[b]Random effect logistic regression model allowing for clustering within hospitals and adjusted for month of CRE sample collection. Note: carbapenemase-producing organisms (CP *K. pneumoniae*, CP *E. coli*, KPE-producing *Enterobacteriaceae*, NDM-producing *Enterobacteriaceae*) are the base group for odds ratio calculations. All comparisons have *P* values of <0.001.
[c]Details of univariate and multivariate analyses are available in Table S2.
[d]Details of univariate and multivariate analyses are available in Table S3.
[e]Details of univariate and multivariate analyses are available in Table S4.
[f]Details of univariate and multivariate analyses are available in Table S5.

Our study has several limitations. First, the lack of a non-CRE control group limits the generalizability of the findings of this study. The association found between NCPCRE and carbapenem exposure is conditional upon the control group being a CPE. Thus, we were unable to investigate the possibility of both NCPCRE and CPE having more carbapenem exposure than a non-CRE or noninfected control groups. Second, we were unable to calculate and include colonization pressure, which is a known risk factor for CRE acquisition (20). Third, molecular typing (e.g., whole-genome sequencing) would have given information regarding the number and sizes of clusters (if any) of CPE and NCPCRE, and this would have strengthened (or weakened) the argument that CPE is acquired exogenously by horizontal transmission, while NCPCRE is acquired endogenously and driven mainly by antibiotic pressure. Fourth, risk factor analyses for antimicrobial resistance at the patient-level and population-level are prone to selection bias and ecological bias, respectively (21); hence, our findings should be interpreted with full consideration of these biases.

To conclude, our study adds credence to the need to identify the heterogeneous resistance mechanisms of CRE to effectively balance antimicrobial stewardship versus infection prevention and control measures in areas with prevalent CPE or NCPCRE. Further studies are needed to explore affordable options for laboratory diagnosis of CRE resistance mechanisms and also the biological and genomic differences between CPE and NCPCRE.

MATERIALS AND METHODS

Study design, setting, and participants. We conducted a case-control study by using data from the Carbapenemase-Producing *Enterobacteriaceae* in Singapore (CaPES) network between January 2010 and May 2015. The CaPES study recruited all hospitalized adult patients with colonization and/or infection with CRE. All seven government-funded multidisciplinary hospitals in Singapore, which provide >80% of inpatient medical care in Singapore, participated in this network. Interim results and the methodology of the prospective CaPES study have previously been published and showed that all major genotypes of CPE were endemic in Singapore (22).

Definitions and data collection. Cases were patients with NCPCRE isolates, which were not susceptible to meropenem, imipenem, and/or ertapenem; were phenotypically noncarbapenemase producers; and tested negative for carbapenemase genes. Controls were defined as patients with CPE isolates, which tested positive for carbapenemases, as described below, and were not susceptible to meropenem, imipenem, and/or ertapenem. For patients with multiple CRE cultures, only the first isolate (clinical or surveillance cultures) was included. All participating hospitals implemented active surveillance for CRE as part of IPC measures. We excluded patients with both CPEs and NCPCREs, mixed CPE genotypes ($n = 18$), incomplete case record forms, and CRE isolates that were phenotypically carbapenemase producers but were negative for CPE by PCR. Carbapenem exposure (meropenem, imipenem, and ertapenem) within the preceding 30 days was the main exposure of interest.

We documented patient demographics, underlying medical conditions, Charlson comorbidity index score, exposure to third and fourth generation cephalosporins (ceftriaxone, ceftazidime, and cefepime), extended-spectrum penicillins (amoxicillin-clavulanate, ampicillin-sulbactam, and piperacillin-tazobactam), fluoroquinolones (ciprofloxacin and levofloxacin), aminoglycosides (gentamicin and amikacin), tigecycline, metronidazole, trimethoprim-sulfamethoxazole, and polymyxin B during 30 days prior to the CRE

culture collection date. Data were collected on history of hospitalization, surgical procedures, upper and/or lower gastrointestinal scopes, and carriage of multidrug-resistant organisms during 12 months before the CRE culture collection date. We also collected data on ICU stay at the time of culture and the presence of a feeding tube and central venous line (CVL) during the time at risk (time from admission to the isolation of CRE). All data were collected from electronic medical records.

Microbiological methods. Antibiotic susceptibility testing and organism identification of isolates from clinical cultures were conducted at participating institutions. Microbiology laboratories at participating institutions used either Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) methods to detect CREs. Carbapenem MICs were then confirmed by Etest (bioMérieux, Marcy-l'Étoile, France). CREs and isolates suspected of carrying carbapenemase genes were submitted to the National Public Health Laboratory (NPHL) for further phenotypic characterization and PCR-based assays. At NPHL (and at some hospital microbiology laboratories), the presence of carbapenemase production was investigated phenotypically by using diagnostic meropenem tablets of the KPC/metallo-β-lactamase confirmation kit (Rosco Diagnostica A/S, Taastrup, Denmark) and/or Rapidec Carba NP. Characterization of β-lactamase genes was performed by PCR assays targeting class A carbapenemases (bla_{KPC}, bla_{GES}, bla_{IMI}, and bla_{NMC-A}), class B metallo-β-lactamases (bla_{NDM}, bla_{VIM}, and bla_{IMP}) (23), and class D carbapenemases ($bla_{OXA-48-like}$ and bla_{OXA-23} carbapenemases) (24). Carbapenem nonsusceptibility due to porin loss associated with extended-spectrum β-lactamases and AmpC overproduction was not investigated by molecular techniques.

Statistical method. For patient-level analysis, the Student's *t* test or the nonparametric Wilcoxon signed-rank test was used for continuous variables depending on their distribution. The χ^2 or Fisher exact test was used to compare categorical variables.

Covariates identified as apriori confounders were Charlson score, hospitalization during the preceding 1 year, being in ICU at the time of culture, time at risk, presence of central venous line, and exposure to fluoroquinolones, third- or fourth-generation cephalosporins, extended-spectrum penicillins, and aminoglycosides. Additionally, variables with a *P* value of <0.1 on univariate analysis and clinical plausibility were included in the logistic regression model. For correlated variables (pairwise correlation coefficient, >0.7), only one of the covariates was selected for inclusion into the candidate models by the strength of association. We conducted a random effect logistic regression model allowing for clustering at the institution level and adjusted for month (to account for possibility of unknown outbreaks during certain time periods) of isolation of CRE to identify independent factors associated more with NCPCRE than CPE. A two-sided *P* value of <0.05 was considered statistically significant. *P* values were interpreted together with 95% confidence intervals (CIs) for the logistic regression model.

We explored the impact of carbapenem exposure on the occurrence of NCPCRE as a binary variable. We then studied the effect of different durations of carbapenem exposure by grouping patients into three categories (no carbapenem exposure, 1 to 3 days of exposure, and >3 days of exposure). Carbapenem exposure was dichotomized at 3 days, as it is the usual duration for empirical antibiotic therapy and findings would be beneficial for future stewardship application. To explore if the effect of carbapenem exposures on the occurrence of CRE varies by *Enterobacteriaceae* species and genotypes of CPE, we conducted stratified analysis to study the effect of carbapenem exposure (as a binary variable) on the occurrence of non-carbapenemase-producing carbapenem-resistant (NCPCR) *E. coli* compared with carbapenemase-producing (CP) *E. coli*, NCPCR *K. pneumoniae* compared with CP *K. pneumoniae*, and NCPCRE compared with NDM-producing *Enterobacteriaceae* and KPC-producing *Enterobacteriaceae*. Since more than half of the study populations were colonized rather than infected with CPE, we performed stratified analysis by specimen type, comparing surveillance culture to clinical cultures. Clinical cultures were all cultures sent as part of clinical care of patients.

All analyses were done with STATA 14.0 (StataCorp, Texas, USA).

Ethics approval. The CaPES study was reviewed and approved by the ethics institutional review board of National Health Group Singapore (DSRB reference no. 2014/00617).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00845-19.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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