International Journal of Antimicrobial Agents 53 (2019) 657–662

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Risk factors and outcomes associated with the isolation of polymyxin B and carbapenem-resistant Enterobacteriaceae spp.: A case–control study

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ARTICLE INFO

Article history:
Received 10 October 2018
Accepted 9 March 2019

Editor: Jason Roberts

Keywords:
Polymyxin
Colistin
Resistance
Predictors
Enterobacteriaceae

ABSTRACT

Increasing resistance to polymyxin, a last-line antibiotic, is a growing public health concern worldwide. The primary objective of this study was to identify predictors for the isolation of polymyxin-resistant (PR) carbapenem-resistant Enterobacteriaceae (CRE) among hospitalized patients. The secondary objective was to describe the clinical outcomes of patients with PR-CRE infections. A retrospective case–control study including patients admitted to Singapore General Hospital between June 2012 and June 2016 was conducted. Cases were defined as patients who had clinical cultures from which a PR-CRE was isolated. Controls were randomly selected from patients with polymyxin-susceptible (PS) CRE admitted during the same period, and frequency-matched to site of isolation. We included 37 PR cases and 111 PS controls. Polymyxin resistance was detected predominantly in *Enterobacter* spp. (54.1%) and *Klebsiella pneumoniae* (43.2%). Multilocus sequence typing showed little clonal relatedness among the isolates. *mcr-1* was detected in two PR-CRE isolates. Multivariable analyses showed that PR-CRE isolation was associated with prior polymyxins (adjusted odds ratio (OR), 21.31; 95% confidence interval (CI), 3.04–150.96) and carbapenem exposures (OR 3.74; CI 1.13–12.44), when adjusted for time at risk and bacteria species. In PR-CRE patients with infections, the 30-day all-cause in-hospital mortality was 50.0% as compared to 38.1% in patients with PS-CRE ($P = 0.346$). Prior polymyxin and carbapenem exposures were independent risk factors for isolation of PR-CRE. Outcomes of PR-CRE and PS-CRE infections were similar in this study.

1. Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) infections are difficult-to-treat infections with limited therapeutic options. Polymyxins are one of the last-line antibiotics for such infections. However, high rates of polymyxin resistance have been reported in some parts of the world and were found to be independently associated with mortality [1,2].

In our hospital we have been observing an increasing prevalence of CRE, with a corresponding increase in polymyxins usage. Consequently, polymyxin resistance was detected in our CRE isolates, even as early as in 2012 [3]. There are only a few existing studies which investigated the risk factors of polymyxin resistance in CRE [4–7]. Whilst some of these studies have suggested the association between polymyxin use and acquisition of polymyxin resistance, results were not consistent. Hence, the primary objective of our study was to assess the risk factors associated with isolation of polymyxin-resistant (PR) CRE. The secondary objective was to describe the clinical outcomes of patients with PR-CRE infections.

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https://doi.org/10.1016/j.ijantimicag.2019.03.011
0924-8579/

2. Materials and Methods

2.1. Study design

A retrospective matched case–control study was conducted at a large acute care hospital in Singapore between June 2012 and June 2016 to investigate the risk factors associated with the isolation of PR-CRE. All adult inpatients (≥21 years old) with a clinical (non-surveillance) culture yielding PR-CRE, regardless of whether the culture represents a clinical infection or colonization, were defined as cases. Three controls with polymyxin-B-susceptible (PS) CRE cultures were selected for each case. Controls were frequency-matched to cases by specimen type.

Patients with CRE were identified from the databases of the microbiological laboratory. Each patient was included in the study only once, at the time of the first PR/PS-CRE isolation, which is defined as the index date. Clinical cultures yielding Enterobacteriaceae which were intrinsically resistant to polymyxins (e.g. *Proteus* spp., *Providencia* spp., *Morganella morganii, Serratia* spp. and *Burkholderia cepacia*) or more than one type of carbapenem-resistant Gram-negative organism were excluded.

2.2. Data collection

Data were sought from electronic medical records and clinical microbiology laboratory computerized databases. Information was collected in a structured data collection form by medically trained staff. Variables analyzed as risk factors included: (1) demographics (age, gender); (2) presence of comorbid conditions and age-adjusted Charlson comorbidity index; (3) neutropenic status (defined as absolute neutrophil count $<500/mm^3$); (4) severity of illness as determined by APACHE II score; (5) hospitalization history such as previous hospital stay, time at risk (defined as the number of days from the time of admission to the time of index culture), previous intensive care unit (ICU) stay; (6) exposure to invasive interventions (e.g. central venous catheter (CVC), invasive ventilation, dialysis, invasive surgery); (7) receipt of immunosuppressive therapy (defined as receipt of ≥1 dose of chemotherapy or immunosuppressant, or ≥14 days of corticosteroids at an equivalent daily dose of 20 mg prednisolone); and (8) antibiotics exposure (receipt of more than one dose). Daily doses of intravenous (IV) polymyxin B and total duration of use were tabulated. All variables were recorded for a period of 30 days prior to the index date.

Outcomes were evaluated for patients with clinically relevant infections, i.e. if clinical specimens were obtained from sterile sites (e.g. blood or pleural effusion); and/or if there was a presence of clinical symptoms consistent with infection. The primary outcome of interest was 30-day all-cause in-hospital mortality (death occurring for any reason within 30 days of index date during the hospital stay). Secondary outcomes included the following: (1) 30-day infection-related mortality (death occurring due to infection (as indicated as cause of death in hospital medical records) within 30 days of index date during the hospital stay); (2) clinical response (resolution of abnormalities of vital signs and infection markers, or resolution of symptoms specific to the infection); (3) microbiological clearance (documented clearance of index organism from blood cultures (assessed only in patients with positive blood cultures)); (4) length of stay post-infection (number of days that the patient spent in the hospital after the index date); and (5) 30-day readmission (non-elective readmission within 30 days of discharge).

2.3. Microbiological methods

Genus identity was determined using VITEK GNI+ cards (bioMérieux, Hazelwood, MO, USA). Antibiotic susceptibilities were determined using commercial microbroth dilution panels (Trek Diagnostics, East Grinstead, UK), performed according to manufacturer's recommendations. The minimum inhibitory concentrations (MICs) of most antibiotics were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The European Committee on Antimicrobial Susceptibility Testing colistin susceptibility breakpoints for Enterobacteriaceae and the Food and Drug Administration (FDA) criteria for tigecycline were used to interpret the susceptibilities of polymyxin B and tigecycline, respectively. Isolates with a MIC of >2 mg/L for polymyxin B were considered non-susceptible, while isolates which were non-susceptible to at least one carbapenem or where carbapenemase genes were detected were considered CRE.

Presence of carbapenemase and *mcr-1* genes were detected by polymerase chain reaction (PCR) as previously described [8]. In silico analysis of the draft genomes obtained from whole genome sequencing using the multilocus sequence typing (MLST) database (http://pubmlst.org/) was conducted to determine the sequence types of the polymyxin B-resistant isolates.

2.4. Statistical analyses

Data are expressed as mean ± standard deviation for continuous variables with normal distribution, median (interquartile range) for continuous variables with skewed distribution, and *n* (percentage) for categorical variables. Normality of continuous variables was checked by visual inspection of histograms.

Unconditional logistic regression was used to compute the odds ratio (OR) and corresponding 95% confidence intervals (CI). Restricted cubic spline regression was used to examine the linearity of continuous explanatory variables. To identify the risk factors for isolation of PR-CRE, multivariable logistic regression models were constructed. The base model was adjusted for time at risk and bacteria species. Clinically plausible variables with *P*-values <0.10 identified in the univariate analysis were included individually into the base model in a stepwise manner. The likelihood ratio test was used to determine inclusion in the final parsimonious model. The goodness-of-fit of all models were assessed by Hosmer–Lemeshow test and Akaike information criteria (AIC). All statistical analyses were conducted with the software package STATA MP 14.0 (Stata Corp., College Station, TX) and a two-tailed value of $P<0.05$ was considered to be statistically significant.

3. Results

During the four-year study period, a total of 37 PR-CRE cases were identified, and 111 corresponding PS-CRE controls were included. The microbiological characteristics and antibiotic susceptibility profiles are detailed in Tables 1 and 2. Polymyxin resistance was detected predominantly in *Enterobacter* spp. (20/37, 54.1%) and *Klebsiella pneumoniae* (16/37, 43.2%). Most of these PR-CRE isolates were KPC-producers (59.5%) and were highly resistant to all beta-lactams tested. Susceptibilities were retained for amikacin (100%), tigecycline (89.2%), ceftazidime-avibactam (83.8%), fosfomycin (70.3%) and levofloxacin (64.9%). When compared to PS-CRE controls, the distribution of carbapenemases was largely similar. However, it appears that PR-CRE isolates tended to be more susceptible to levofloxacin and amikacin, while susceptibility to fosfomycin was lower.

MLST analyses of the major Enterobacteriaceae species showed diverse STs among the species. PR *K. pneumoniae* belonged to nine different STs (11, 15, 20, 273, 392, 513, 719, 841, 978), of which ST11 (four isolates) and ST20 (three isolates) were the most common. PR *E. cloacae* belonged to 10 different STs (54, 78, 93, 109, 131, 144, 175, 182, 418, 502), of which ST93 (nine isolates) was the most

Table 1
Microbiological characteristics of polymyxin-resistant (PR) carbapenem-resistant Enterobacteriaceae (CRE) and polymyxin-susceptible (PS) CRE isolates.

	PR-CRE ($n=37$) n (%)	PS-CRE ($n=111$) n (%)	P-value
Species			<0.002
Enterobacter spp.	20 (54.1)	26 (23.4)	
Klebsiella spp.	16 (43.2)	63 (56.8)	
Escherichia coli	1 (2.7)	19 (17.1)	
Citrobacter spp.	0 (0)	3 (2.7)	
Specimen type			1.000
Abdominal	4 (10.8)	12 (10.8)	
Blood	7 (18.9)	21 (18.9)	
Respiratory	6 (16.2)	18 (16.2)	
Skin and soft tissue	6 (16.2)	18 (16.2)	
Urinary	14 (37.8)	42 (37.8)	
Carbapenemase type			0.073
KPC	22 (59.5)	49 (44.1)	
Metallobetalactamase	6 (16.2)	22 (19.8)	
OXA-48-like	0 (0)	15 (13.5)	
Co-producers	0 (0)	3 (2.7)	
None	9 (24.3)	22 (19.8)	

common. The one PR *E. coli* was ST354. *mcr-1* was detected only in two PR-CRE isolates.

Table 3 compares the baseline demographics and clinical characteristics of the PR cases and PS controls. Isolation of PR CRE was associated with a longer time at risk (33 vs 15 days, $P=0.021$), previous ICU stay (48.7% vs 20.7%, $P=0.001$), tracheostomy (18.9% vs 6.3%, $P=0.03$), central venous catheter (70.3% vs 44.1%, $P=0.007$), and nasogastric tube (64.9% vs 44.1%, $P=0.03$) use. PR-CRE was more commonly isolated in patients with concurrent infections (70.3% vs 40.5%, $P=0.002$). Previous carbapenem (62.2% vs 35.1%, $P=0.005$) and polymyxin (24.3% vs 1.8%, $p < 0.001$) exposures were more frequent in patients with PR-CRE.

Upon multivariable analysis (Table 4), previous carbapenem and polymyxin exposures were directly associated with PR-CRE isolation. Polymyxin resistance was more likely to occur in *Enterobacter* spp. (compared to *Klebsiella* spp.), and in patients with Charlson comorbidity index <5.

Among the nine patients (eight PR cases, one PS control) who were exposed to IV polymyxins, three were isolated from blood (two PR cases, one PS control), three from respiratory specimens, and one each from urinary, abdominal and soft tissue specimens. In the PR cases, the median IV polymyxin exposure duration prior to index was 13 days (range: 4–19 days). The median dose was 17,785 units/kg per day (range: 13,700–31,250 units/kg per day). All cases were receiving polymyxin as part of antibiotic combination therapy, except for one case who was exposed to five days of polymyxin monotherapy. The most common combination antibiotic was meropenem (six cases). The PS-CRE control received two days of IV polymyxin at 18,200 units/kg per day, which was given concurrently with imipenem/cilastatin.

Outcomes were analyzed for 83 (56.1%) patients who had clinically significant infections (abdominal – 14/16 (87.5%), respiratory – 12/24 (50.0%), skin and soft tissue – 10/24 (41.7%), urine – 20/56 (35.7%), blood – 27/28 (96.4%)). The proportion of patients whose cultures represented clinically significant infections were similar in the PR and PS group [20/37 (54.0%) vs 63/111 (56.8%), $P=0.774$]. The outcomes of patients with PR-CRE and PS-CRE infections are shown in Table 5. Clinical presentation on index date of the two groups were similar, with the exception of higher occurrence of co-infections in patients with PR-CRE (85.0% vs 50.8%, $P=0.012$). Outcomes were similar in the two groups, although microbiological clearance was more often observed in patients with PR-CRE bloodstream infections (100% vs 55.0%, $P=0.059$).

4. Discussion

Polymyxins are one of the last-line antibiotics for CRE and development of polymyxin resistance is of great concern. Monitoring for the emergence and elucidation of predictors for polymyxin resistance might be useful to prevent resistance emergence. Our study analyzed the predictors in the isolation of PR-CRE among hospitalized patients. We demonstrated that previous polymyxins exposure was the strongest predictor for the isolation of PR-CRE in our study population.

The polyclonal nature and the low occurrence of *mcr-1* among PR-CRE suggested that development of polymyxin resistance via horizontal transmission was unlikely in our study. As in many of the previous epidemiological studies, we observed that polymyxin exposure is one of the key factors associated with polymyxin resistance [6,7,9,10]. This is founded on the biological basis that acquired resistance to polymyxin can arise due to selection pressure from excessive or inadequate polymyxin use in patients colonized with previously polymyxin-susceptible strains. Phenotypic adaptation occurs via selection of polymyxin-dependent/polymyxin-resistant populations among a heteroresistant population [11]. However, the breakpoint/threshold for emergence of resistance is not evident in our study, as polymyxin resistance was detected in patients receiving polymyxins for as short as four days, whereas most previous reports documented resistance only with prolonged exposures [10,12]. The relationship of resistance emergence and polymyxin dosing is also not apparent in our study. Dosing of polymyxins is an evolving field and perhaps polymyxin

Table 2
Antimicrobial susceptibilities of polymyxin-resistant (PR) carbapenem-resistant Enterobacteriaceae (CRE) and polymyxin-susceptible (PS) CRE isolates.

	PR-CRE ($n=37$)				PS-CRE ($n=111$)				
	Proportion susceptible (%)	MIC_{50}	MIC_{90}	Range	Proportion susceptible (%)	MIC_{50}	MIC_{90}	Range	P-value
Cefepime	3 (8.1)	≥64	≥64	≤1 to ≥64	8 (7.2)	≥64	≥64	≤1 to ≥64	1.000
Piperacillin-tazobactam	1 (2.7)	≥128	≥128	8 to ≥128	5 (4.5)	≥128	≥128	8 to ≥128	1.000
Aztreonam	2 (5.4)	≥64	≥64	≤0.5 to ≥64	8 (7.2)	≥64	≥64	≤0.5 to ≥64	1.000
Ertapenem	3 (8.1)	≥32	≥32	≤0.5 to ≥32	3 (2.7)	≥32	≥32	1 to ≥32	0.165
Imipenem	9 (24.3)	16	≥32	0.5 to ≥32	17 (15.3)	8	≥32	≤0.25 to ≥32	0.212
Doripenem	9 (24.3)	8	≥32	≤0.25 to ≥32	24 (21.6)	8	≥32	≤0.25 to ≥32	0.732
Meropenem	7 (18.9)	16	≥32	≤0.25 to ≥32	20 (18.0)	8	≥32	≤0.25 to ≥32	0.902
Levofloxacin	24 (64.9)	4	≥32	≤0.5 to ≥32	51 (46.0)	8	≥32	≤0.5 to ≥32	0.046
Amikacin	37 (100)	4	16	1 to 32	99 (89.2)	8	64	≤1 to ≥128	0.037
Tigecycline	33 (89.2)	1	4	≤0.25 to ≥16	98 (88.3)	0.5	4	≤0.25 to 4	1.000
Ceftazidime-avibactam	31 (83.8)	1	≥128	0.25 to ≥128	86 (77.5)	1	≥128	≤0.12 to ≥128	0.414
Fosfomycin	26 (70.3)	64	256	1 to ≥2048	97 (87.4)	32	256	≤0.5 to ≥2048	0.016
Polymyxin B	–	≥16	≥16	4 to ≥16	–	0.5	2	≤0.25 to 2	–

MIC, minimum inhibitory concentration.

Table 3
Clinical characteristics of patients with polymyxin-resistant (PR) carbapenem-resistant Enterobacteriaceae (CRE) and polymyxin-susceptible (PS) CRE isolates.

	PR-CRE (n=37)	PS-CRE (n=111)	OR (95% CI)	P-value
Age	65 (43–85)	68 (37–86)	1.00 (0.97 0–1.02)	0.703
Male	19 (51.3)	60 (54.1)	0.90 (0.43–1.89)	0.775
Medical ward	25 (67.6)	71 (64.0)	1.17 (0.53–2.59)	0.691
Time at risk	33 (12–48)	15 (2–30)	1.02 (1.00–1.03)	0.021
Co-morbidities				
Ischemic heart disease	9 (24.3)	30 (27.0)	0.87 (0.37–2.05)	0.747
Diabetes	15 (40.5)	48 (43.2)	0.89 (0.42–1.91)	0.773
Chronic renal failure	4 (10.8)	24 (21.6)	0.44 (0.14–1.36)	0.154
Malignancy	12 (32.4)	42 (37.8)	0.79 (0.36–1.73)	0.555
Neutropenia	0	7 (6.3)	–	0.130
Charlson comorbidity index ≥ 5	15 (40.5)	66 (59.5)	0.46 (0.22–0.99)	0.048
Concurrent infection	20 (70.3)	45 (40.5)	3.47 (1.56–7.71)	0.002
Severity of illness				
APACHE II ≥15	13 (35.1)	42 (37.8)	0.89 (0.41–1.93)	0.768
Septic shock	5 (13.5)	8 (7.2)	1.99 (0.61–6.52)	0.255
Hospital exposures				
Previous hospitalisation	8 (21.6)	36 (32.4)	0.57 (0.24–1.38)	0.216
Previous ICU stay	18 (48.7)	23 (20.7)	3.62 (1.64–8.00)	0.001
ICU days	0 (0–17)	0 (0–12)	1.11 (1.04–1.19)	0.002
Mechanical ventilation	13 (35.1)	26 (23.4)	1.77 (0.79–3.96)	0.164
Tracheostomy	7 (18.9)	7 (6.3)	3.47 (1.13–10.66)	0.030
Central venous catheter	26 (70.3)	49 (44.1)	2.99 (1.35–6.64)	0.007
Nasogastric tube	24 (64.9)	49 (44.1)	2.34 (1.08–5.05)	0.030
Surgery	18 (48.7)	35 (31.5)	2.05 (0.96–4.39)	0.062
Medication exposures				
Immunosuppresive therapy	12 (32.4)	19 (17.1)	2.32 (1.00–5.42)	0.051
Aminoglycosides	8 (21.6)	14 (12.6)	1.91 (0.73–5.00)	0.187
Fluoroquinolones	11 (29.7)	37 (33.3)	0.84 (0.38–1.90)	0.685
Carbapenems	23 (62.2)	39 (35.1)	3.03 (1.40–6.55)	0.005
Beta-lactam/beta-lactamase inhibitors	29 (78.4)	75 (67.6)	1.74 (0.72–4.19)	0.217
3rd/4th-generation cephalosporins	13 (35.1)	28 (25.2)	1.61 (0.72–3.57)	0.246
Tigecycline	2 (5.4)	4 (3.6)	1.53 (0.27–8.71)	0.633
Intravenous polymyxin B	8 (21.6)	1 (0.9)	30.33 (3.65–252.48)	0.002
Inhaled colistin	4 (10.8)	1 (0.9)	13.33 (1.44–123.44)	0.023
Polymyxin B and/or inhaled colistin	9 (24.3)	2 (1.8)	17.51 (3.58–85.68)	<0.001

Data are expressed as mean ± standard deviation for continuous variables with normal distribution, median (interquartile range) for continuous variables with skewed distribution, and n (%) for categorical variables. ICU, intensive care unit.

Table 4
Multivariable analysis of risk factors for polymyxin-resistant (PR) carbapenem-resistant Enterobacteriaceae (CRE) isolation.

Risk factor	OR (95% CI)*
Time at risk[a]	
4–14 days	0.88 (0.19–4.05)
15–30 days	0.29 (0.06–1.42)
>30 days	1.57 (0.37–6.62)
Bacterial species[b]	
Enterobacter spp.	8.44 (2.86–24.86)
Others	0.15 (0.01–1.47)
Charlson comorbidity index ≥5	0.20 (0.07–0.59)
Previous carbapenem exposure	3.74 (1.13–12.44)
Previous polymyxins exposure	21.31 (3.04–150.96)

CI, confidence interval; OR, odds ratio.
[a] Reference group: time at risk 0–3 days.
[b] Reference group: *Klebsiella* spp.; the other species were grouped as 'Others'.
* The Hosmer–Lemeshow goodness of fit test showed that the model fitted the data well (χ^2=2.84, d.f. 8, P=0.944).

therapeutic drug monitoring may help shed light on the pharmacodynamics/pharmacokinetic optimization of dosing to retard resistance emergence.

Interestingly, almost all patients were receiving polymyxin in combination with another antibiotic, the most common being a carbapenem. Combination therapy can preferentially select for efflux pump-mediated resistance, leading to broad-spectrum resistance [13]. Therefore, there is a necessity to monitor the use of antibiotic combinations and to identify strategies to optimize the administration of combination therapy.

It is also noted that polymyxin exposure was only detected in <25% of the PR-CRE cohort. This is in spite of measuring polymyxin exposure for up to 30 days prior to the index culture, reducing the risk of under detection of polymyxin exposure. While polymyxin exposure remains an important factor for the development of polymyxin resistance, it appears that this development is a complex multifactorial process and may be driven by factors other than polymyxin use. In our study, we observed that prior carbapenem exposure was also associated with the isolation of PR-CRE, which was similar to findings from another study conducted in Turkey [14]. This might be a chance association since carbapenems were often administered in combination with polymyxins or other antibiotics for treatment of previous polymyxin-susceptible infections. On the other hand, there is also mechanistic plausibility supporting the relationship of carbapenem exposure and polymyxin resistance, because resistance may arise due to other de novo mutations triggered by antibacterials other than polymyxin, especially if it involves non-specific drug efflux regulatory pathways. For instance, colistin resistance was associated with *phoPQ* mutations which arose through chlorhexidine adaptation in *K. pneumoniae* [15].

PR-CRE isolation was also associated with lower Charlson comorbidity index in our study, which was contrary to another polymyxin resistance study [7]. This suggests that the comorbidity status might be a chance finding. It might be because PR-CRE flourish in certain gut microbiomes, which are in turn affected by multiple factors not limited to underlying comorbidities, or caused by de novo mutations triggered by other antibacterials exposure, as discussed above. Among the patients with infection, outcomes

Table 5
Outcomes of patients with polymyxin-resistant (PR) carbapenem-resistant Enterobacteriaceae (CRE) and polymyxin-susceptible (PS) CRE infections.

	PR-CRE (n=20)	PS-CRE (n=63)	P-value
Clinical presentation			
Charlson comorbidity index ≥5	10 (50.0)	40 (63.5)	0.283
APACHE II score ≥15	9 (45.0)	32 (50.8)	0.652
Septic shock	4 (20.0)	7 (11.3)	0.449
Intensive care unit stay	9 (45.0)	15 (23.8)	0.069
Concurrent infection	17 (85.0)	32 (50.8)	0.012
Outcomes			
30-day all-cause in-hospital mortality	10 (50.0)	24 (38.1)	0.346
30-day infection-related in-hospital mortality	4 (20.0)	10 (15.9)	0.668
Clinical response	13 (65.0)	37 (58.7)	0.618
Microbiologic clearance*	7 (100.0)	11 (55.0)	0.059
Hospital length of stay post-infection	16 (9–24)	15 (7–26)	0.930
30-day readmission	2 (20.0)	16 (41.0)	0.288

Data are expressed as mean ± standard deviation for continuous variables with normal distribution, median (interquartile range) for continuous variables with skewed distribution, and n (%) for categorical variables.

* Only assessed in patients with positive blood cultures (7 PR-CRE cases and 20 PS-CRE controls).

were similar between those with PR-CRE and PS-CRE. This might be related to PR-CRE isolates still retaining susceptibility to agents such as quinolones.

Our study is limited by its retrospective nature and small sample size. Furthermore, unknown confounders and unmeasured factors could have contributed to our findings. We were unable to include surveillance cultures in our study, as surveillance screening of CRE was not routine in our institution and only limited to patients with certain risk factors such as previous healthcare facility stay or admission to the renal or oncology units. Hence, we cannot exclude that patients included in the study were already colonized with PR-CRE. *mcr* was also the only plasmid gene we studied, and unknown transmissible polymyxin resistance mechanisms could have contributed to the observations of this study. Additional whole-genome sequencing/RNA sequencing are required to elucidate other unknown polymyxin resistance mechanisms.

5. Conclusions

Polymyxin exposure was strongly associated with the emergence of polymyxin resistance. However, polymyxin resistance development appears to be a complex multifactorial process and is unlikely to be controlled with polymyxin stewardship alone. Inadequate usage of other antimicrobials such as carbapenems may also contribute to polymyxin resistance. Owing to the lack of new antibiotics in the pipeline, future research should focus on optimizing polymyxin administration including identifying the optimal dosing regimen and strain-specific combination therapy effective in preventing resistance development.

Acknowledgements

We thank the Carbapenemase-Producing Enterobacteriaceae in Singapore (CaPES) Study Group, Singapore for their kind provision of part of the raw sequences for whole-genome analyses. This study was presented in part at the 27th European Congress of Clinical Microbiology & Infectious Diseases, Vienna, Austria, 22–25 April 2017 and the ASM Microbe 2017 meeting, New Orleans, USA, 1–5 June 2017.

Funding

This work was supported in part by National Medical Research Council, Singapore (NMRC/CG/C005/2017 & NMRC/CG/M011/2017) and Singapore General Hospital Research Grant, Singapore (SRG-NIG #06/2017).

Competing Interests

A.L.H. Kwa has received unrestricted funding for research from Pfizer Inc, Merck Sharp & Dohme (I.A) Corp and Bayer (SEA) Pte Ltd.

Ethical Approval

This study was approved by the Singhealth Centralised Institutional Review Board (CIRB 2016/2476).

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