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Original Article

Clinical and molecular characteristics and risk factors for patients acquiring carbapenemase-producing and non-carbapenemase-producing carbapenem-nonsusceptible-*Enterobacterales* bacteremia

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Risk factor

Abstract *Background/purpose:* Carbapenem-nonsusceptible *Enterobacterales* (CNSE) are a growing global threat. Carbapenemases are often produced by plasmids, which allow rapid transmission. This study aimed to investigate (1) the bacterial type (2) resistant genes (3) antimicrobial susceptibility and (4) risk factors for acquisition of carbapenemase-producing carbapenem-nonsusceptible *Enterobacterales* (CP-CNSE) and non-carbapenemase-producing carbapenem-nonsusceptible *Enterobacterales* (non-CP-CNSE) bacteremia.
Methods: There were a total of 113 isolates of *Enterobacterales* from 2013 to 2018. After excluding nonblood isolates and including only one sample from each patient, 99 isolates were analyzed and the medical charts of these patients were reviewed. Carbapenemase genes, β-lactamase genes and antimicrobial susceptibility of the isolates were determined. Multilocus

Abbreviations: CNSE, Carbapenem-nonsusceptible *Enterobacterales*; CP-CNSE, Carbapenemase-producing carbapenem-nonsusceptible *Enterobacterales*; Non-CP-CNSE, Non-carbapenemase-producing carbapenem-nonsusceptible *Enterobacterales*; MLST, Multilocus sequence typing.

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sequence typing (MLST) was performed on CP-CNSE isolates.
Results: CP-CNSE carried more bla_{SHV} (P = 0.004) and were more resistant to imipenem than non-CP-CNSE (P < 0.001). In the univariate analyses, we found that CP-CNSE bloodstream infection was associated with patient <65 years of age (odds ratio, 3.90; 95% confidence interval [CI], 1.16 to 13.10; P = 0.027), mechanical ventilation at the time of bloodstream infection (BSI) (odds ratio, 3.85; 95% CI, 1.16–12.78; P = 0.028) and exposure to piperacillin/tazobactam (odds ratio, 3.96; 95% CI, 1.09–14.38; P = 0.037). However, on multivariate analyses, no independent predictor for CP-CNSE was identified in this study.
Conclusion: CP-CNSE carried more bla_{SHV} and were more resistant to imipenem when compared to non-CP-CNSE. No independent predictor for CP-CNSE was identified after multivariate analysis. This is the first study conducted in Taiwan comparing risk factors between CP-CNSE and non-CP-CNSE from both clinical and molecular aspects.

Introduction

Carbapenem-nonsusceptible *Enterobacterales* (CNSE) infection is an emerging global public health crisis associated with in-hospital mortality as high as 50–94.2%.[1,2] Having multiple antimicrobial resistance and limited treatment options, CNSE are a growing threat and were ranked as "critical priority pathogens" by World Health Organization (WHO) in 2017.[3] According to a national nosocomial infection surveillance report by Taiwan Centers for Disease Control (Taiwan CDC), the prevalence rates of CNSE rose from 8.6% in 2011 to 26.1% in 2020[4] while the Centers for Disease Control and Prevention of United States reported that CNSE caused 11,800 nosocomial infections in 2012 and 13,100 in 2017.[5]

Depending on their phenotypic resistance, carbapenem-nonsusceptible *Enterobacterales* (CNSE) can be classified into two main subgroups: carbapenemase-producing CNSE (CP-CNSE) and non-carbapenemase-producing CNSE (non-CP-CNSE).[6] CP-CNSE carry carbapenemases which act by hydrolyzing carbapenems. Examples of these carbapenemases include: *Klebsiella pneumoniae* carbapenemase (KPC) in Ambler class A, New Delhi Metallo-β-lactamase (NDM), Verona Integron-encoded Metallo-β-lactamase (VIM), imipenemase (IMP) in Ambler class B and Oxacillinase type carbapenemases such as OXA 48 in Ambler class D.[7] Non-CP-CRE have alternative mechanisms: porin mutations, drug efflux pumps and different types of β-lactamases such as the AmpC cephalosporinase (AmpC) which prevent carbapenems from binding to their targets.[8]

Carbapenemase-producing CNSE (CP-CNSE) were found to be independently associated with mortality in some studies.[9–11] and are often produced by plasmids, allowing easy transfer between bacteria.[12]

Our aim in this study was to investigate the molecular characteristics and risk factors associated with CP-CNSE compared to non-CP-CNSE bloodstream infection (BSI).

Materials and methods

Microbiological method

This study was conducted at MacKay Memorial Hospital, a 2200- bed tertiary medical center in northern Taiwan from January 2013 to December 2018. Identification and antimicrobial susceptibilities on all isolates of *Enterobacterales* during this period were performed using the VITEK 2 system (bioMérieux Vitek Systems, Hazelwood, MO, USA). Isolates were kept frozen at −80 °C in tryptic soy broth containing 20% glycerol (v/v) until further testing. Carbapenem non-susceptibility was defined as having ertapenem MIC ≥1 according to Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial susceptibility testing; thirtieth informational supplement (CLSI document M100-S30, January 2020 update).

Molecular analysis

Bacteria isolates were boiled in sterile water for 10 min, and the supernatants were collected and used for PCR as DNA sources. The 25 μl reaction mix consisted of 1X S-T Gold buffer, 1.5 mM MgCl2, each dNTP at 0.2 mM, 20 pmol of each primer, and 0.4 units of Super-Therm polymerase. The isolates were screened by PCR for the presence of the carbapenemase genes (bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{SPM}, bla_{GIM}, bla_{SIM}, bla_{KPC}, bla_{OXA-48}, bla_{BIC}, bla_{AIM}, bla_{DIM}) and β-lactamase genes (bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{AmpC}).[9,13] Products were visualized on agarose gel and equence analysis of the resulting amplicons was carried out using DNA analyzer. Sequence similarity searches were performed with an ABI Prism 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The primers used for PCR amplification are listed in previous studies (see Supplement Table 1).[13–17]

MLST genotyping

MLST of isolates was performed by amplifying and sequencing internal fragments derived from seven specific housekeeping genes of *K. pneumoniae* (rpoB, gapA, mdh, pgi, phoE, infB, and tonB). Housekeeping genes primers were designed by Institute Pasteur MLST and whole genome MLST databases. The allelic profiles were compared with those included in the electronic database of the Pasteur Institute to identify the sequence type (https://bigsdb.pasteur.fr/index.html). Multilocus sequence typing (MLST) analysis was performed only on CP-CNSE. Multi-locus sequence typing (MLST) of isolates was conducted by

amplifying and sequencing internal fragments derived from eight specific housekeeping genes of *E. coli* genes (dinB, icdA, pabB, polB, putP, trpA, trpB and uidA). Housekeeping genes primers were designed by Institute Pasteur MLST and whole genome MLST databases. The allelic profiles were compared with those included in the electronic database of the Pasteur Institute to identify the sequence type (https://bigsdb.pasteur.fr/index.html).

Pulsed-field gel electrophoresis (PFGE)

The whole genomic DNA of 13 bacteremic isolates of CP-CNSE were digested with *Xba* I (Biolabs) and typed by PFGE.[18] We applied a CHEF MAPPER apparatus (Bio-Rad, Hercules, CA) with 6 V/cm, pulsed from 2.16 s to 54.17 s, for 20 h at 14 °C, to divide DNA fragments on 1% (w/v) SeaKem GTG agarose gels in 0.5% Tris-borate-EDTA (TBE) buffer. Ethidium bromide was added for at least 30 min for gel staining and ultraviolet light for molecular imaging (Molecular imager Gel Doc XR+, Bio-Rad, CA). We examined the restriction profile on gels visually in accordance with Tenover et al.[19] and used BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium) for computer-assisted analysis. As for cluster analysis, unweighted pair group method with mathematical averaging was used. We analyzed DNA relatedness using band-based Dice coefficient with a tolerance setting of 1.0% band tolerance and 2.0% optimization for all profiles. Central analysis was only applied to band size more than 48 kb. The bacteremic isolates were classified into the same PFGE type if there was ≧80% similarity.

Patients' data collection

Medical charts of patients with blood isolates of *Enterobacterales* during the study period were retrospectively reviewed. For patients with more than one CNSE isolate, only the first record was included. The following data were collected: age and sex, preexisting medical conditions (end stage renal disease (ESRD), hematologic conditions, solid tumors, known immunodeficiencies, diabetes mellitus (DM), liver cirrhosis), length of hospitalization (days), operation or invasive procedures (such as catheters insertion) or presence of drains within 3 days before blood stream infection (BSI), intravascular catheter within 3 days prior to BSI, mechanical ventilation (invasive or noninvasive) at the time of BSI, steroid or chemotherapy use within 3 days of BSI, fever and leukocytes and neutrophils counts at the time of BSI. Information on the use of broad-spectrum antibiotics within 30 days was collected, including fluroquinolones, carbapenems, cephalosporins, amoxicillin/clavulanate, ampicillin/sulbactam, tigecycline, colistin, amikacin, gentamicin and TMP/SMX. Patients with age <20 years were excluded. This retrospective study was approved by the MacKay Memorial Institutional Review Broad (protocol numbers 20MMHIS394e).

Statistical analysis

The statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean with standard deviations or median with interquartile range (IQR) for non-parametric variables. Categorical variables were expressed as frequencies with percentages. Statistical tests included Pearson χ^2 test or Fisher's exact test for categorical variables. Univariate and multivariable logistic regressions were performed. All tests were 2-tailed, and p-values < 0.05 was considered significant.

Results

Microbiological characteristics

A total of 113 isolates of carbapenem-nonsusceptible *Enterobacteriales* (CNSE) were isolated from 2013 to 2018. Of these isolates, 17 were *Enterobacter cloaecae*, 21 were *Escherichia coli*, 73 were *K. pneumoniae*, with one each of *Proteus mirabilis* and *Serratia marcensens*.

After eliminating non-blood samples, 99 blood isolates of CNSE were available for analysis (Fig. 1). The bacterial species in blood isolates of CNSE and molecular characteristics including types of carbapenemase and other β-lactamases are summarized in Table 1a and Table 1b. The majority of both CP-CNSE and non-CP-CNSE isolates were from *K. pneumoniae* and there was no significant difference between the two groups (P = 0.292). All CP-CNSE bacteremic isolates had other β-lactamases in addition to carbapenemases and most of them carried two or more β lactamases other than carbapenemases (12/13, 92.3%). The majority of non-CP-CNSE bacteremic isolates (66/86, or 76.7%) were found to have more than one β-lactamase.

When the distribution of bacteremic isolates were compared, there was a significant difference only in the bla_{SHV} gene (P = 0.004) which was more likely to be carried by CP-CNSE. There was no difference between CP-CNSE and non-CP-CNSE for bla_{TEM-1}, bla_{DHA-1}, bla_{CTX-M} and bla_{CIT-2}.

The percentage of CP-CNSE in all CRE (CP-CNSE and non-CP-CNSE) isolated annually from 2013 to 2018 are as follows: 3.22% in 2013, 0% in 2014, 21.43% in 2015, 0% in 2016, 38.89% in 2017 and 18.18% in 2018.

Multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE)

MLST was performed in CP-CNSE to investigate the clonality of the bacteremic isolates and 53.8% were ST11, 7.7% were ST48, 7.7% were ST39, 7.7% were ST859 and 23.1% were unknown. The majority of *K. pneumoniae* belonged to ST11. Comparison of PFGE patterns among CP-CNSE is shown in Fig. 2. The 8 Carbapenemase-producing *K. pneumoniae* with identifiable ST type were classified by 4 PFGE patterns. The 2 Carbapenemase-producing *E. coli* with identifiable ST type were classified by 2 PFGE patterns.

Antimicrobial susceptibility profiles

The susceptibility profiles of CNSE are summarized in Table 2. There were higher rates of resistance to imipenem among CP-CNSE than in non-CP-CNSE (CP-CNSE/non-CP-CNSE: 100%/21.71%, P < 0.001). Although there were higher rates of resistance to several antibiotics in CP-CNSE such as

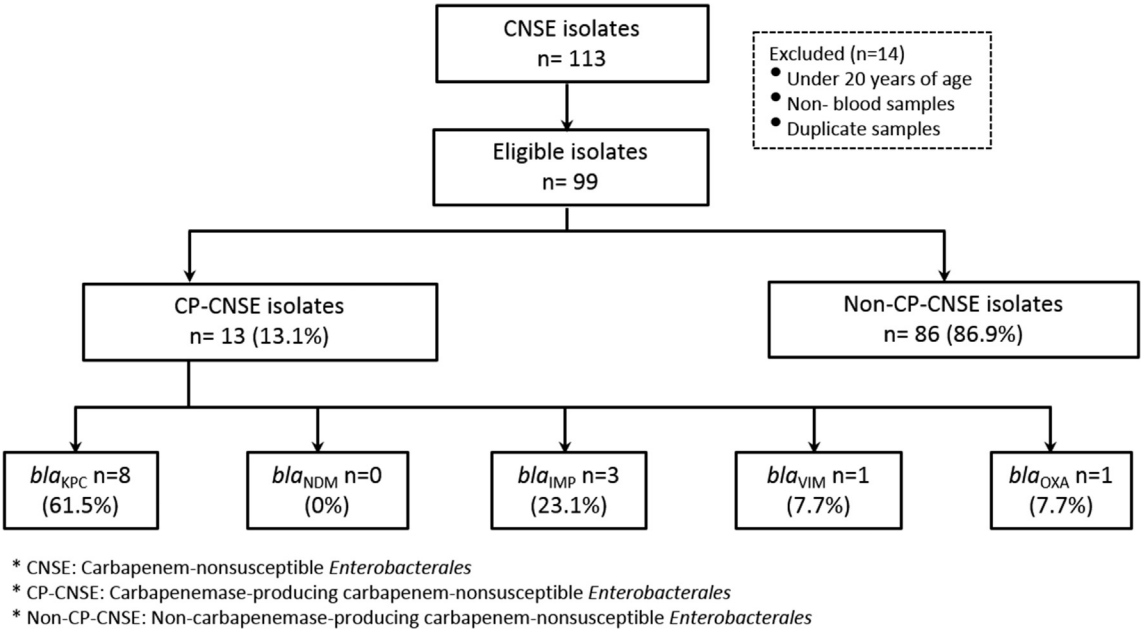

Figure 1. Selection of subjects.

cefpirome (CP-CNSE/non-CP-CNSE: 91.67%/67.27%, P = 0.092), ciprofloxacin (CP-CNSE/non-CP-CNSE: 92.31%/78.18%, P = 0.248), moxifloxacin (CP-CNSE/non-CP-CNSE: 90.91/76%, P = 0.278) and amikacin (CP-CNSE/non-CP-CNSE: 30.77%/14.55%, P = 0.171), none of these showed statistical significance.

Clinical characteristics

After excluding non-blood isolates (pus/wound, ascites, CSF and other body fluids) and including only one sample from each patient, there were 99 patients whose medical charts were reviewed.

The mean age was 70.15 years old, with a standard deviation of 12.46 and the median length of stay was 32 days with an interquartile range of 47. The majority of patients were 65 years or older, (66, 66.7%) compared to those less than 65 years (33, 33.3%). Male patients outnumbered female patients (57 versus 42), 22 patients (22.2%) had used steroids prior to the onset of bacteremia, 37.4% had solid tumors, 43.4% had diabetes mellitus and 20.2% of patients had chronic kidney disease requiring regular dialysis. Slightly over half (52.5%) of the patients had an intravascular catheter inserted prior to the onset of bacteremia, 18.2% underwent recent operation or invasive procedures prior to bacteremia while 27.3% were on mechanical ventilation prior to bacteremia (Table 3).

Baseline characteristics of patients with CP-CNSE bacteremia and non-CP-CNSE bacteremia were comparable in terms of predisposing conditions such as comorbidities (hematologic malignancies, solid tumors, immunodeficiencies, DM, ESRD on H/D, liver cirrhosis), types of antibiotics used, presence of invasive procedures and hospital length of stay.

Using simple logistic regression, those <65 years of age were more likely to harbor CP-CNSE while those ≥65 years of age were more likely to harbor non-CP-CNSE (odds ratio, 3.90; 95% confidence interval [CI], 1.16 to 13.10; P = 0.027) (Table 4). Furthermore, use of mechanical ventilation (invasive or noninvasive) at the time of BSI (odds ratio, 3.85; 95% CI, 1.16—12.78; P = 0.028) was associated with CP-CNSE. Finally, patients having exposure to piperacillin/tazobactam use within 30 days prior to bacteremia were more likely to have CP-CNSE

Table 1a Bacterial species in CNSE bacteremic isolates.

	CP-CNSE (n = 13), No. (%)	Non-CP-CNSE (n = 86), No. (%)	P value
Species			0.292
Klebsiella pneumoniae	11 (84.6)	53 (61.6)	0.208
ST11 *K pneumoniae*[a]	7 (53.8)	—	
Escherichia coli	2 (15.4)	18 (20.9)	>0.99
Enterobacter spp	—	13 (15.1)	
Serratia marcescens	—	1 (1.2)	
Proteus mirabilis	—	1 (1.2)	

[a] The 7 isolates of ST11 *K. pneumoniae* is part of the 11 isolates of *K. pneumoniae in* CP-CNSE group.

Table 1b Molecular characteristics of CNSE bacteremic isolates.

Distribution of resistance and β-lactamase genes			0.357
Ambler class A			
bla_{KPC-2}	8 (61.5)	—	
bla_{CTX-M}	7 (53.8)	47 (54.7)	0.957
$bla_{CTX-M-15}$	2 (15.4)	5 (5.8)	
$bla_{CTX-M-55}$	—	6 (7.0)	
$bla_{CTX-M-14}$	6 (46.2)	36 (41.9)	
$bla_{CTX-M-65}$	1 (7.7)	1 (1.2)	
bla_{SHV}	13 (100)	53 (61.6)	0.004
bla_{SHV-11}	4 (30.8)	38 (44.2)	
bla_{SHV-1}	2 (15.4)	8 (9.3)	
bla_{SHV-27}	—	1 (1.2)	
bla_{SHV-28}	—	1 (1.2)	
bla_{SHV-12}	7 (53.8)	5 (5.8)	
bla_{TEM-1}	7 (53.8)	25 (29.1)	0.110
Ambler class B			
bla_{VIM-1}	1 (7.7)	—	
bla_{IMP-1}	1 (7.7)		
bla_{IMP-8}	2 (15.4)	—	
bla_{NDM}	0 (0)		
Ambler class C			
bla_{DHA-1}	5 (38.5)	46 (53.5)	0.312
bla_{CIT-2}	1 (7.7)	15 (17.4)	0.687
Ambler class D			
bla_{OXA-48}	1 (7.7)		

infection (odds ratio, 3.96; 95% CI, 1.09—14.38; P = 0.037). Carbapenem exposure (odds ratio, 1.74; 95% CI, 0.52—5.87; P = 0.371) was statistically similar between the two groups, although there was a higher odd of exposure among those with CP-CNSE. The above factors were not found to be independently associated with CP-CNSE bacteremia on multivariate logistical regression (Table 5).

Discussion

According to previous studies, the carbapenemase production rate of CNSE in Taiwan was 5—41.2%.[2,20] This is in contrast to other countries which reported CP-CNSE prevalence as high as 45.4—80%.[20] In this study, we found that the prevalence of carbapenemase among the CNSE isolates in our institution was 15% in total, with the majority of them being *Klebsiella pneumoniae* harboring bla_{KPC-2} gene (61.5%), and most belonged to ST11 clone (76.9%). This is similar to previous surveys which found that *K. pneumoniae* carbapenemase-2-producing *K. pneumoniae* sequence type 11 was the predominant clone in Taiwan.[21,22] According to the national antimicrobial resistance surveillance report from the Taiwan CDC, the incidence of CNSE isolates in intensive care units increased annually. In contrast, the percentage of CNSE isolates in our institution was 5.7% in 2009 and 5.2% in 2018 and did not show an annual increase. Last but not least, ST 859 *K. pneumoniae* and ST 39 *E. coli* identified in this study were the first isolates reported in Taiwan to the best of our knowledge. These two ST types have been sporadically reported in China.[23,24] ST 48 *E. coli* which carried VIM-1 has been reported in Taiwan previously.[25] This differed from our ST 48 isolate which did not carry VIM-1 but IMP-1.

In our study, CP-CNSE was more likely to carry bla_{SHV} gene comparing to non-CP-CNSE (Table 1). Three out of seven ST 11 carbapenemase-producing *K. pneumoniae* isolates carried both bla_{SHV-11} and bla_{KPC-2} (Fig. 2a). In 2012, a

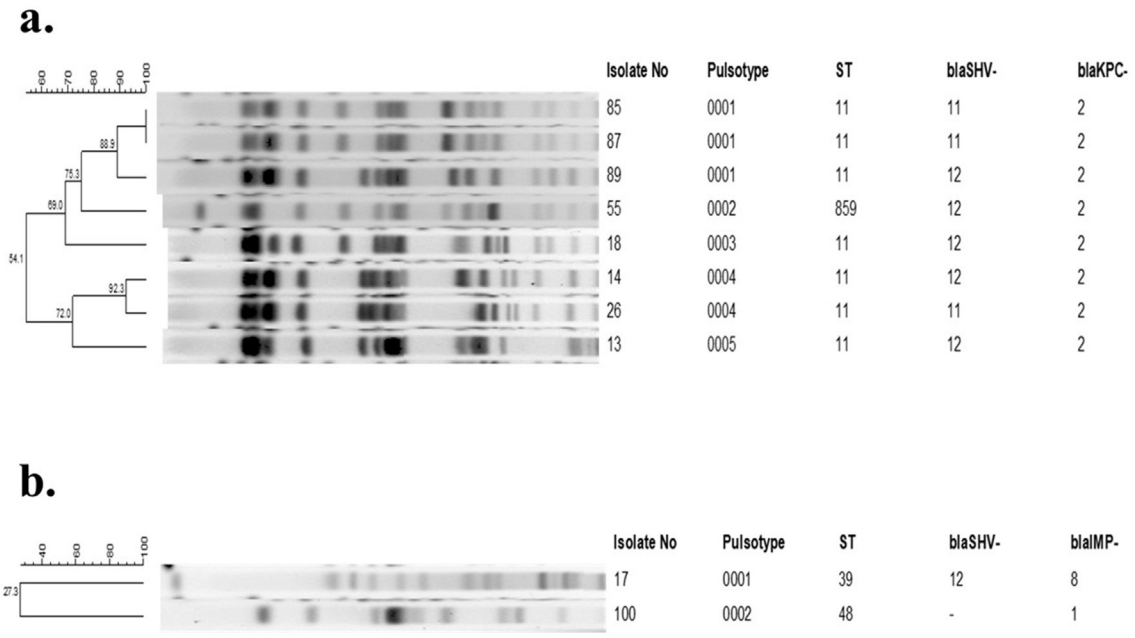

Figure 2. Pulse-field gel electrophoresis of carbapenemase-producing carbapenem-nonsusceptible *Enterobacterales:* (a) *K. pneumoniae* (b) *E. coli*.

Table 2 Susceptibility profiles of bacteremic carbapenem-nonsusceptible *Enterobacterales* included in this study[a].

	CP-CNSE**				Non-CP-CNSE**				P value
	Total	S	R	R (%)	Total	S	R	R (%)	
Ertapenem	13	0	13	100%	86	0	86	100	—
Imipenem	12	0	12	100%	85	21	64	21.71%	<0.001
Meropenem	0	0	0	—	31	29	2	6.45%	—
Ampicillin	12	0	12	100%	43	1	42	97.67%	0.597
Ampicillin/sulbactam	12	0	12	100%	37	0	37	100%	—
Cefuroxime	13	0	0	100%	53	1	52	98.11%	0.620
Flomoxef	13	1	12	92.31%	85	6	79	92.94%	0.935
Ceftazidime	13	0	13	100%	55	1	54	92.73%	0.320
Cefoxitin	3	0	3	100%	39	1	38	97.44%	0.782
Cefpirome	11	1	10	91.67%	55	18	37	67.27%	0.092
Ciprofloxacin	13	1	12	92.31%	55	12	43	78.18%	0.248
Moxifloxacin	11	1	10	90.91%	50	12	38	76%	0.278
Levofloxacin	6	1	5	83.33	19	3	16	84.21%	0.960
Amikacin	13	9	4	30.77%	55	47	8	14.55%	0.171
Gentamicin	12	6	6	50%	49	28	21	42.86%	0.658
Colistin	9	8	1	11.11%	67	61	6	8.96%	0.835
Tigecycline	12	10	2	16.67%	86	59	27	31.4%	0.300
TMP/SMX	13	2	11	84.62%	55	18	37	67.27%	0.221

[a] Resistance rate includes resistant and intermediate isolates, based on CLSI criteria, unless otherwise noted.
** CP-CNSE: CNSE isolates containing carbapenemase producing genes: bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{OXA-48} and bla_{NDM}.
** Non-CP-CNSE: CNSE isolates that did not contain the above listed carbapenemase producing genes.

Table 3 Demographics.

Age (mean, SD), years		70.15 (12.46)
Age group, years, No. (%)	<65	33 (33.3)
	>or = 65	66 (66.7)
Gender	Male	57 (57.6)
	Female	42 (42.4)
Current Hospitalization, No. (%)		
Length of stay (median, 25th - 75th percentile), days		32 (15.5—62.0)
Steroid use within 3 days prior to Bloodstream infection (BSI)		22 (22.2)
Chemotherapy within 3 days prior to BSI		5 (5.1)
OP or invasive procedures (drain) or presence of drains within 3 days before BSI		18 (18.2)
Intravascular catheter within 3 days prior to BSI		52 (52.5)
Mechanical ventilation (invasive or noninvasive) at time of BSI		27 (27.3)
Underlying diseases, No. (%)		
Hematologic malignancies		5 (5.1)
Solid tumors		37 (37.4)
Known immunodeficiencies		2 (2.0)
Type 2 diabetes mellitus		43 (43.4)
End-stage renal disease receiving hemodialysis		20 (20.2)
Liver cirrhosis		7 (7.1)
Prior use of antibiotics within 30 days, No. (%)		
Fluoroquinolones		97 (98.0)
Carbapenems		37 (37.4)
Cephalosporins		62 (62.6)
Tigecycline		12 (12.1)
Colistin		12 (12.1)
Amikacin		2 (2.0)
Gentamicin		0 (0.0)
Piperacillin/tazobactam		18 (18.2)
Amoxicillin/clavulanate or ampicillin/sulbactam		30 (30.3)

Table 4 Association between factors and types of carbapenem-nonsusceptible *Enterobacterales*.

	Categories	CP-CNSE, No. (%)	Non-CP-CNSE, No. (%)	*P* value	Odds ratio (95%CI)
Age group, years;	<65	8 (61.5)	25 (29.1)	0.027	3.90 (1.16–13.10)
	≥65	5 (38.5)	61 (70.9)		
Gender	Male	9 (69.2)	48 (55.8)	0.366	1.78 (0.51–6.23)
	Female	4 (30.8)	38 (44.2)		
Length of hospitalization, days[a]	≤30	4 (33.3)	42 (51.9)	0.239	2.15 (0.60–7.72)
	>30	8 (66.7)	39 (48.1)		
Hematologic malignancies		0 (0.0)	5 (5.8)	>0.99	NA
Solid tumors		4 (30.8)	33 (38.4)	0.599	0.71 (0.20–2.51)
Known Immunodeficiencies		0 (0.0)	2 (2.3)	>0.99	NA
Type 2 diabetes mellitus		3 (23.1)	40 (46.5)	0.125	0.35 (0.09–1.34)
End-stage renal disease receiving hemodialysis		4 (30.8)	16 (18.6)	0.315	1.94 (0.53–7.11)
Liver cirrhosis		1 (8.3)	6 (7.0)	0.864	1.21 (0.13–11.04)
Absolute Neutrophil Count, cells/μL	≤1500	0 (0)	8 (9.4)	0.947	1.08 (0.12–9.54)
	>1500	13 (100)	77 (90.6)		
Leukocyte Count (WBC), cells 10^9/L	≥10000	8 (61.5)	60 (69.8)	0.552	0.69 (0.21–2.32)
	<10000	5 (38.5)	26 (30.2)		
Current or prior hospitalization within 14 days of bloodstream infection (BSI)		11 (84.6)	58 (67.4)	0.224	2.66 (0.55–12.80)
Length of hospitalization before the onset of BSI[a]	≤14 days	6 (50.0)	43 (53.1)	0.842	1.13 (0.34–3.81)
	>14 days	6 (50.0)	38 (46.9)		
Fever or hypothermia at time of BSI		9 (69.2)	68 (79.1)	0.430	0.60 (0.16–2.16)
Operation or presence of drains within 3 days before BSI		3 (23.1)	15 (17.4)	0.625	1.42 (0.35–5.79)
Intravascular catheter within 3 days prior to BSI		7 (53.8)	45 (52.3)	0.919	1.06 (0.33–3.42)
Mechanical ventilation (invasive or noninvasive) at time of BSI		7 (53.8)	20 (23.3)	0.028	3.85 (1.16–12.78)
Steroid use within 3 days prior to BSI		1 (7.7)	21 (25.0)	0.195	0.20 (0.03–2.04)
Chemotherapy within 3 days prior to BSI		0 (0.0)	5 (5.9)	>0.99	NA
Antibiotics use within 30 days prior to bacteremia					
Fluoroquinolones		6 (50.0)	27 (31.8)	0.219	2.15 (0.63–7.28)
Carbapenems		6 (50.0)	31 (36.5)	0.371	1.74 (0.52–5.87)
Cephalosporins		8 (66.7)	54 (62.8)	0.794	1.19 (0.33–4.25)
Tigecycline		3 (25.0)	9 (10.6)	0.170	2.82 (0.64–12.34)
Colistin		2 (16.7)	10 (11.8)	0.631	1.50 (0.29–7.85)
Amikacin		0 (0.0)	2 (2.4)	>0.99	NA
Gentamicin		0 (0.0)	0 (0.0)		NA
Piperacillin/tazobactam		5 (41.7)	13 (15.3)	0.037	3.96 (1.09–14.38)
Amoxicillin/clavulanate or ampicillin/sulbactam		6 (50.0)	24 (28.2)	0.600	1.841 (0.188–17.998)

[a] Patients who died, transferred or discharged on the same days they arrived at emergency room were excluded.

plasmid pKPC-LK30 carrying bla_{SHV-11} and bla_{KPC-2} was identified in ST11 carbapenemase resistant *K. pneumoniae* in Taiwan. Although the bla_{KPC} genes are mostly found on transferable plasmids such as those containing mobile transposons Tn4401.[20] It is interesting that this pKPC-LK30 lacks one of the replication origins and cannot conjugate, which means clonal spread was more likely than plasmid conjugation.[26] In the CRACKLE-2 study, a prospective cohort study performed in the United States with the predominant strain ST258 *K. pneumoniae*; CP-CNSE were also more likely to carry the bla_{SHV} gene.[27] It is worth mentioning that ST11 and ST258 are phylogenetically related.[21] Non-CP-CNSE was more associated with bla_{CTX-M} gene in the CRACKLE-2 study, but this was not found in our present study. In the CRACKLE-2 study, bla_{Ampc} gene was more likely to be harbored by non-CP-CNSE. We checked

Table 5 Multivariate analyses of risk factors for CP-CNSE.

	CP-CNSE compared with Non-CP-CNSE	
	Adjusted OR (95% CI)	P value
Age	0.34 (0.09−1.24)	0.101
Piperacillin/tazobactam use within 30 days prior to bacteremia	3.22 (0.82−12.66)	0.094
Mechanical ventilation (invasive or noninvasive) at time of BSI	2.31 (0.61−8.71)	0.218

the AmpC gene family in this study, including the bla_{DHA-1} and bla_{CIT-2}, but they were no differences between CP-CNSE and non-CP-CNSE.

As for antimicrobial susceptibilities comparing CP-CNSE and non-CP-CNSE (Table 2), CP-CNSE was significantly more resistant to imipenem than non-CP-CNSE. This is compatible with the SMART surveillance program: only 2.3% of KPC-producing *Enterobacterales* isolates showed *in vitro* susceptibility to imipenem.[28] In addition, CP-CNSE showed a trend of higher resistance to cefpirome, the 4th generation cephalosporin, than non-CP-CNSE (P = 0.092). These results were consistent with the conclusion made by Jean et al., who investigated the *Enterobacteriales* isolates causing intra-abdominal infections in the Asia−Pacific region and found that imipenem non-susceptibility and cefepime MIC >8 μg/mL were independent predictors of CP-CNSE.[29] In another research, cefepime was shown to be an effective treatment option against non-CP-CNSE isolates in intra-abdominal infections.[30]

While the risk factors for CNSE include indwelling catheters and use of antibiotics,[1] risk factors for CP-CNSE and non-CP-CNSE has been found to differ. CP-CNSE bacteremia was associated with male gender, intensive care unit stay and hospitalization within one year; while hematological malignancies and carbapenem exposure were associated with non-CP-CNSE infection.[31,32] In one study, the odds of prior 30-day carbapenem exposure was three times higher among non-CP-CNSE than CP-CNSE patients,[31] suggesting differential antibiotic selection pressure. This is in contrast to our present study which did not reveal that carbapenem use in the last 30 days was a risk factor for the acquisition of non-CP-CNSE as opposed to CP-CNSE. One reason for this observation could be that the antibiotic selection pressure which led to the CNSE bacteremia may have occurred prior to 30 days. Indeed, multiple studies have found that patients generally become infected by endogenous strains of CNSE already colonizing in their gut.[33−35] Gorrie et al. reported that approximately 50% of MDR-KP infections were caused by *K. pneumoniae* isolates compatible with the patients's own microbiota, while 48% of patients with infections were found with prior colonization.[33] Solter reported that the duration of CNSE carriage is more than one month in most cases.[36] Hence, what our present study suggested was that the antibiotic selection pressure for CP versus non-CP CNSE occurred at a time prior to the last 30 days. This is further corroborated in our finding that those aged <65 years of age tended to harbor CP-CNSE while those ≥65 years of age tended to harbor non-CP-CNSE (Table 4) in univariate analysis. It is likely that older patients (≥65 years of age) were more likely to have had multiple hospital admissions and more antibiotic use in the past. Hence, carbapenem use that occurred remotely in the past might have resulted in non-CP-CNSE carriage in the gut, which resulted in bacteremia in our patients ≥65 years of age. Besides, CNSE carriage was common in residents from the long-term care facilities in Taiwan.[37] Non-CP-CNSE acquisitions were reported to be associated with asymptomatic carriage among the population ≥65 years of age with recent antibiotic exposure, not necessarily carbapenems.[38] Further survey for CP-CNSE and non-CP-CNSE in local long-term care facilities may answer the questions about why the elderly is prone to have non-CP-CNSE infection.

In univariate analyses, we found that CNSE type was associated with age, exposure to piperacillin/tazobactam and mechanical ventilation at the time of BSI. However, on multivariate logistical analysis these factors did not stand out as being independently associated with CP-CNSE. In this population, older age was significantly associated with the absence of mechanical ventilation but not with exposure to piperacillin/tazobactam. Patients not on mechanical ventilation were more likely to be older, perhaps as older patients were more probably to both be under a Do-Not-Resuscitate order and to harbor non-CP-CNSE bacteria. Hence while older age was significantly associated with non-CP-CNSE in this study, it was heavily confounded by the absence of mechanical ventilation in our elderly population. These findings were partially in line with previous studies. In one Japanese study, age was found to be associated with types of CNSE type in univariate analyses. However, similar to our study, significance was lost in multivariate analyses. Furthermore, in the same study, endotracheal intubation was not more prevalent among IMP carbapenemase-producing Enterobacteriaceae compared to non-CP-CNSE.[39] More research is needed to elucidate the relationship between age and CP-CNSE.

The main limitation in our study was the small sample size, can be a confounding factor. In addition, the prevalence of CP-CNSE in our population was low, preventing significant differences from being detected. Moreover, this study was conducted in a single medical center in Taiwan, and there may be different carbapenemase-gene distributions in other institutions. Our study results may thus not be generalizable to other regions with larger prevalence or different distributions of carbapenemase. Continuation of the present work to attain a larger sample size by including more institutions or expanding the duration of data collection may be helpful.

In conclusion, CP-CNSE harbored more bla_{SHV} and were more resistant to imipenem than non-CP-CNSE were. No independent predictor for CP-CNSE was identified in this study. Further studies are needed to investigate the difference between CP-CNSE and non-CP-CNSE.

Declaration of competing interest

All authors declare that they have no relevant conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.10.008.