Original Article

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Clinical Characteristics and Beta-Lactamase Genes of Carbapenem-Nonsusceptible *Klebsiella Pneumoniae* Strains Which Remains Susceptible to Beta-Lactams of Narrower Spectra

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Objective: Some carbapenem-nonsusceptible *Klebsiella pneumoniae* (CnSKP) isolates are susceptible to β-lactams of narrower spectra. However, their clinical significance and molecular characterization are unknown.

Methods: CnSKPs were screened, and antimicrobial susceptibility test results were retrieved in the database of clinical microbiology laboratory from 2004 to 2019. Patient characteristics and outcomes were collected from the medical records. Polymerase chain reaction for β-lactamases and pulsed-field gel electrophoresis (PFGE) were performed.

Results: The overall prevalence of CnSKP was 0.96% (197/20,465). At least 37 of the 197 isolates remained susceptible to β-lactams of narrower spectra, and a total of 34 CnSKP isolates were included. Carbapenemase genes were detected in nine isolates (26.5%), three of which were bla_{KPC}, bla_{IMP-2}, and bla_{NDM}. A few isolates remained susceptible to β-lactams of narrower spectra, such as cefazolin (5.9%), ceftriaxone (5.9%), ceftazidime (5.9%), cefmetazole (11.8%), cefepime (26.5%), and piperacillin-tazobactam (20.6%). All these isolates had a meropenem minimum inhibitory concentration (MIC) ranging from 2 to 4 mg/L and were susceptible to ceftazidime-avibactam. All these bacteria harbored extended-spectrum β-lactamase (ESBL) genes, and none had carbapenemase genes. The treatment outcomes of patients who carried these bacteria were not associated with appropriate antibiotic use but with the site of isolation. Hospital-acquired infection, nonsusceptibility to cefepime, and meropenem MIC ≥ 16 mg/L were associated with the detection of carbapenemase genes and 14-day mortality in univariate analyses. PFGE results suggested that carbapenemase genes disseminated more readily than ESBL and AmpC β-lactamase genes.

Conclusions: The CnSKP composition is complex. Carbapenemase-producing *Klebsiella pneumoniae* (CPKP) strains have different characteristics from non-CPKP strains.

Key words: carbapenem-nonsusceptible, *Klebsiella pneumoniae*, carbapenemase, extended-spectrum β-lactamase (ESBL), AmpC β-lactamase

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Introduction

Klebsiella pneumoniae (K. pneumoniae) is the leading causative pathogen of healthcare-associated infections in Taiwan.[1] As β-lactam antibiotics are often used to treat *K. pneumoniae* infections, the nonsusceptibility rates of many β-lactams against *K. pneumoniae* have been increasing in Taiwan.[2-7] Although carbapenems are regarded as antibiotics of the last resort,[8] the prevalence of carbapenem-nonsusceptible *K. pneumoniae* (CnSKP) in intensive care units (ICUs) in Taiwan has increased by more than twofold over the last decade.[1] With treatment options dwindling, CnSKP is associated with increased morbidity and mortality.[3,9-12]

Two major mechanisms contribute to carbapenem nonsusceptibility. One is the production of carbapenemases, and the other is the production of extended-spectrum β-lactamases (ESBLs) or AmpC β-lactamases with modification of outer membrane permeability.[8,10,13-15] Carbapenemases represent a family of enzymes which can hydrolyze a broad spectrum of β-lactams; therefore, carbapenemase-producing *K. pneumoniae* (CPKP) is often nonsusceptible to other β-lactams.[15] In Taiwan, the proportion of CPKP among CnSKP ranges from 20% to 70%,[3,7,13] depending on the regions where the bacteria were isolated. Compared to non-CPKP, CPKP is more nonsusceptible to both β-lactams and non-β-lactams,[2,3,16] is more transmissible,[3,10] and contributes to increased mortality.[16] Ceftazidime-avibactam, a novel β-lactam/β-lactamase inhibitor, has been approved for the treatment of complicated intra-abdominal infections, complicated urinary tract infections, and nosocomial pneumonias in Taiwan.[17] Ceftazidime-avibactam is active against multidrug-resistant Gram-negative bacteria producing ESBLs, *K. pneumoniae* carbapenemase (KPC), AmpC β-lactamases, and some class D β-lactamases (OXA-48).[17]

In our institution, a few clinical CnSKP isolates remained susceptible to β-lactams of narrower spectra and had more indeterminate results on the automated antimicrobial susceptibility testing (AST) system. We postulated that these isolates were non-CPKP, but their clinical significance and treatment options were unknown. Herein, we studied the clinical and molecular characterization of CnSKP which remains susceptible to antibiotics of narrower spectra. Susceptibility to ceftazidime/avibactam was also examined.

Materials and Methods

Hospital settings, isolates, and ethics

This study was conducted in a 1,000-bed university-affiliated medical center in Taiwan and was approved by the Institutional Review Board of E-Da Hospital (EMRP-108-088). A database of the clinical microbiology laboratory was searched for *K. pneumoniae* isolates obtained between April 2004 and December 2019. *K. pneumoniae* isolates that were interpreted as nonsusceptible to imipenem or meropenem but susceptible to other antibiotics were screened (eFigure 1 in the supplement). All these isolates were stored as glycerol stocks at −80˚C until use. The need for informed consent was waived because this retrospective study involved no more than a minimal risk of harm to patients from which bacterial isolates were collected.

Data collection and definitions

Patient characteristics and clinical outcomes were collected from the medical records. An isolate was designated as hospital-acquired if the patient had been hospitalized for > 48 hours before isolation. Polymicrobial infection was defined as the identification of more than one pathogen in the specimen of the same site within 24 hours. Comorbidities were assessed using the Charlson comorbidity index (CCI). The primary outcome was 14-day

all-cause mortality. Antibiotic treatment was considered inappropriate if the isolate was nonsusceptible to the treatment agent, based on reports from the clinical microbiology laboratory.

Clinical microbiology laboratory

During 2004 – 2013, API/ID32 GN Kits (bioMérieux, Marcy l'Etoile, France) were used for identification and the BD Phoenix™ System was used for AST. From 2014 onwards, the VITEK® MS System (bioMérieux) and the VITEK® 2 System (bioMérieux) were introduced. When the automated AST system was unable to obtain the minimum inhibitory concentrations (MICs) of specific antibiotics, a disk diffusion method was employed to determine susceptibility. All laboratory procedures followed the Clinical and Laboratory Standards Institutes (CLSI) guidelines.[18,19]

Customized broth microdilution assay and Etest for ceftazidime-avibactam

For some isolates, MICs were not available in clinical microbiology laboratory reports. Therefore, a customized broth microdilution method was used to complement the MICs, with a repeat automated AST as a correlation at the time of this study. The MICs of carbapenems (ertapenem, imipenem, meropenem, and doripenem) and other antibiotics (cefazolin, ceftriaxone, ceftazidime, cefepime, piperacillin-tazobactam, levofloxacin, ciprofloxacin, amikacin, gentamicin, trimethoprim-sulfamethoxazole, and tigecycline) were determined in all isolates using Gram Negative GN4F AST Plate (Sensititre, Trek Diagnostic Systems, Cleveland, OH, USA). MICs for ceftazidime-avibactam were obtained by Etest (bioMérieux), in which the range of ceftazidime concentration was 0.016 − 256 mg/L with a fixed avibactam concentration of 4 mg/L. After overnight culture, bacterial isolates were prepared from a single colony and adjusted to a concentration of 0.5 MacFarland for customized broth microdilution assays and Etest. Mueller-Hinton broth was used in the Sensititre plates, and Etest for ceftazidime-avibactam was performed on Mueller-Hinton agar. The MICs were read per the manufacturer's instructions after incubation for 20 – 22 hours at 37˚C, and susceptibility was determined based on CLSI M100 28th edition. Intermediate susceptibility and resistance were grouped as nonsusceptibility.

DNA extraction and detection of β-lactamase genes

After overnight subculture, DNA was extracted from included isolates using the Wizard® Genomic DNA Purification Kit (Promega, USA), followed by conventional polymerase chain reaction (PCR) or multiplex PCR assays to detect ESBL genes (encoding SHV, TEM, and CTX-M), AmpC genes (encoding CMY and DHA), and carbapenemase genes (encoding Ambler class A families KPC, NMC, IMI, SME, and GES; Ambler class B families IMP and NDM; and Ambler class D family OXA-48-type).[4,20,21] The PCR primer sequences used in this study are listed in eTable 1 in the supplement. The amplicons were sequenced, and the entire sequences were compared to the National Center for Biotechnology Information (NCBI) database at https://blast.ncbi.nlm.nih.gov/Blast.cgi.

Pulsed-field gel electrophoresis (PFGE)

The bacterial chromosomal DNA of the included isolates was digested with *Xba*I (Promega, USA). Electrophoresis was carried out for 23 hours at 14˚C with pulse times ranging from 2.16 to 54.17 s at 6 V/cm using Bio-Rad CHEF MAPPER apparatus (Bio-Rad Laboratories, Richmond, CA, USA). The acquired restriction maps of each isolate were compared using GelCompare software (version 6.0; Applied Maths, Bionumerics). Isolates with ≥ 80% similarity in their respective banding patterns were considered closely related strains.

Statistical analysis

Descriptive statistics for clinical features were calculated using the median and interquartile range or frequency count (percentage), as appropriate. The Kolmogorov-Smirnov test was used to assess whether the continuous variables had a normal distribution, and parametric or nonparametric tests were chosen accordingly. Continuous variables were analyzed using Student's t-test or Mann-Whitney U test. Categorical variables were analyzed using the Chi-square test or Fisher's exact test if the expected values were less than five in any cell. To assess the clinical features associated with the detection of carbapenemase genes and 14-day mortality, multivariate logistic regression analysis was performed, selecting variables with $p \leq 0.2$ on univariate analyses through backward elimination. The goodness-of-fit for the model was tested using the Hosmer-Lemeshow test. Two-tailed *p*-values were calculated, and a *p*-value of less than 0.05 was regarded as statistically significant. SPSS software for Windows (Release 19.0; SPSS, Chicago, IL, USA) was used for the statistical analysis.

Results

Epidemiology

The overall prevalence of CnSKP among all *K. pneumoniae* isolates was 0.96% (197/20,465) during 2004 − 2019. The annual prevalence of CnSKP increased each year (R = 0.8, $p < 0.01$) and was marked by a twofold increase in 2014 (eFigure 2 in the supplement). At least 37 of the 197 isolates remained susceptible to β-lactams of narrower spectra, and a total of 34 CnSKP isolates were included (eFigure 1 in the supplement). Table 1 shows the clinical features of the patients who carried these isolates stratified by the detection of carbapenemase genes. Most of the bacteria were hospital-acquired (64.7%) and were isolated from the urine (58.8%) and blood (23.5%). The rate of inappropriate antibiotic treatment among these patients was 91.2%. The 14-day all-cause mortality rate was 42.4%. Polymicrobial infections were observed in 52.9% of isolates. Carbapenemase genes were detected in nine of the 34 isolates (26.5%), three of which were bla_{KPC}, bla_{IMP-2}, and bla_{NDM}. Bacterial isolates which harbored bla_{IMP-2} were isolated as early as 2009 − 2010 (Fig. 1B). AmpC and ESBL β-lactamase genes were detected in 17 (50.0%) and all isolates, respectively. Table 2 shows the distribution of meropenem MICs among the 25 non-CPKP isolates stratified by the combination of β-lactamase genes. The majority of these non-CPKP isolates had meropenem MICs ranging from 2 to 4 mg/L or exhibited intermediate susceptibility to meropenem or imipenem using the disk diffusion method.

Antibiogram

Table 3 shows the results of automated AST for *K. pneumoniae* isolates stratified by carbapenemase genes. Generally, CPKP has higher MICs for most antibiotics, including carbapenems. The difference in the susceptibility rate between CPKP and non-CPKP was most significant for gentamicin ($p = 0.02$), cefepime ($p = 0.04$), and ceftazidime-avibactam ($p = 0.04$). A few isolates remained susceptible to β-lactams of narrower spectra, such as cefazolin (5.9%), ceftriaxone (5.9%), ceftazidime (5.9%), cefmetazole (11.8%), cefepime (26.5%), and piperacillin-tazobactam (20.6%). All these isolates were non-CPKPs. The overall susceptibility rate of ceftazidime-avibactam was 64.7% (22/34).

Customized microdilution assay

Nine isolates, including the isolates which remained susceptible to narrow-spectrum β-lactams, were identified as susceptible to meropenem in customized microdilution assays. Supplement eTable 2 shows the comparison of antibiograms by the automated AST system and the customized microdilution assay, treatment outcomes, and β-lactamase genes in these

Table 1. Clinical characteristics and β-lactamase mechanisms of included carbapenem-nonsusceptible Klebsiella pneumoniae isolates, stratified by detection of carbapenemase genes.

Characteristics	Carbapenemase detected (n = 9)	Carbapenemase not detected (n = 25)	All	*p*-value*
Woman, n (%)	7 (77.8)	10 (40.0)	17 (50.0)	0.12
Age (range, median)	60 − 95, 72	2 − 91, 71	2 − 95, 71	0.51
Site of isolation, n (%)				
Blood	4 (44.4)	4 (16.0)	8 (23.5)	0.17
Urine	4 (44.4)	16 (64.0)	20 (58.8)	0.44
Pleural effusion	0	2 (8.0)	2 (5.9)	1.00
Bile	1 (11.1)	3 (12.0)	4 (11.8)	1.00
Presumed source, n (%)				**0.01**
Community-acquired	0	12 (48.0)	12 (35.3)	
Hospital-acquired	9 (100)	13 (52.0)	22 (64.7)	
Polymicrobial infection, n (%)	6 (66.7)	12 (48.0)	18 (52.9)	0.45
Blood	2 (33.3)	1 (8.3)	3 (16.7)	0.25
Urine	3 (50.0)	6 (50.0)	9 (50.0)	1.00
Pleural effusion	0	2 (16.7)	2 (11.1)	0.53
Bile	1 (16.7)	3 (25.0)	4 (22.2)	1.00
Charlson comorbidity index (range, median)	2 − 9, 4	0 − 11, 6	0 − 11, 5.5	0.67
Fourteen-day all-cause mortality, n (%) (n = 33)	7 (77.8) (n = 9)	7 (29.2) (n = 24)	14 (42.4)	**0.02**
Inappropriate antibiotic treatment, n (%)	7 (77.8)	24 (96.0)	31 (91.2)	0.16
Admission, n (%)	9 (100)	20 (80.0)	29 (85.3)	0.29
Days from admission to isolation ≥ 3 days, n (%)	9 (100)	9 (45.0)	18 (62.1)	**< 0.01**
Intensive care unit (ICU) stay, n (%)	8 (88.9)	5 (25.0)	13 (44.8)	**< 0.01**
Length of hospitalization ≥ 2 weeks, n (%)	9 (100)	12 (60.0)	21 (72.4)	**0.03**
Mechanism of β-lactamase				
Carbapenemase	9 (100)	0	9 (26.5)	NA
KPC	3 (33.3)	0	3 (8.8)	NA
IMP-2	3 (33.3)	0	3 (8.8)	NA
NDM	3 (33.3)	0	3 (8.8)	NA
AmpC	3 (33.3)	14 (56.0)	17 (50.0)	0.44
CMY	0	1 (4.0)	1 (2.9)	1.00
DHA-1	3 (33.3)	14 (56.0)	17 (50.0)	0.44
ESBL	9 (100)	25 (100)	34 (100)	NA
SHV	6 (66.7)	17 (68.0)	23 (67.6)	1.00
TEM	7 (77.8)	15 (60.0)	22 (64.7)	0.44
CTX-M	3 (33.3)	5 (20.0)	8 (23.5)	0.65

* Comparison between groups of carbapenemase genes detected and carbapenemase genes not detected. *P*-values ≤ 0.05 were marked in bold.

ESBL: extended-spectrum β-lactamases; NA: not applicable.

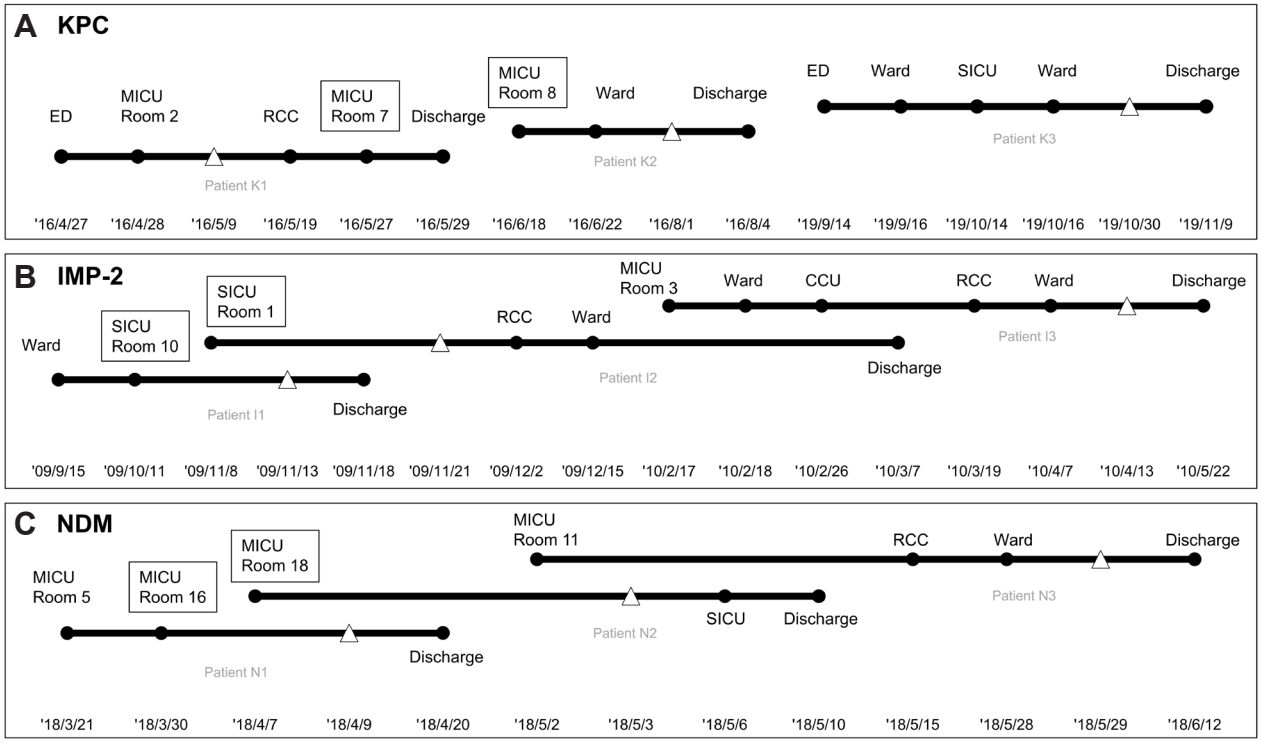


Fig. 1 Timelines of patients who had Klebsiella pneumoniae isolates harboring (A) bla$_{KPC}$, (B) bla$_{IMP-2}$, or (C) bla$_{NDM}$. Hollow triangles indicate dates of isolation. The units highlighted in the boxes are presumed routes of gene dissemination. Six of the nine bacteria were isolated before discharge. ED: emergency department; MICU: medical intensive care unit; RCC: respiratory care unit; SICU: surgical intensive care unit.

Table 2. Distribution of meropenem minimum inhibitory concentrations (MICs) among non-carbapenemase-producing carbapenem-nonsusceptible Klebsiella pneumoniae isolates (n = 25).

Mechanism of β-lactamase	No. of isolate	Meropenem MIC (mg/L)			
		2	4	8	≥ 16
Extended-spectrum β-lactamase (ESBL)					
SHV	6*	2	0	0	3
SHV + TEM	1	0	0	0	1
TEM + CTX-M	2	0	0	2	0
SHV + TEM + CTX-M	2	0	2	0	0
ESBL + AmpC					
SHV + DHA-1	4†	1	1	1	0
TEM + DHA-1	5§	1	3	0	0
SHV + TEM + DHA-1	3#	0	2	0	0
SHV + TEM + CTX-M + DHA-1	1	0	1	0	0
TEM + CMY + DHA-1	1	1	0	0	0

* One isolate had imipenem MIC > 8 mg/L.
† One isolate had intermediate susceptibility to imipenem in disk diffusion test.
§ One isolate had resistance to imipenem in disk diffusion test.
# One isolate had intermediate susceptibility to meropenem in disk diffusion test.

isolates. Eight of these nine isolates had intermediate susceptibility to meropenem or had meropenem MICs ranging from 2 to 4 mg/L. Patient survival was observed in six patients (66.7%) who received inappropriate antibiotic treatments, but the sites of isolation in those cases were mostly from the urine. There were no CPKP among the nine *K. pneumoniae* isolates. All these isolates were susceptible to ceftazidime-avibactam.

Table 3. Results of automated antimicrobial susceptibility testing for included carbapenem-nonsusceptible Klebsiella pneumoniae isolates, stratified by detection of carbapenemase genes.

Antimicrobial agent	Carbapenemase genes detected (n = 9)				Carbapenemase genes not detected (n = 25)				*p*-value*
	MIC (mg/L)			Susceptibility, n (%)	MIC (mg/L)			Susceptibility, n (%)	
	Range	50%†	90%†		Range	50%†	90%†		
Amikacin	≤ 2 − ≥ 64	≤ 2	≥ 64	5 (55.6)	≤ 2 − ≥ 64	≤ 2	≥ 64	21 (84.0)	0.17
Gentamicin	≥ 16 − ≥ 16	≥ 16	≥ 16	0	≤ 1 − ≥ 16	≥ 16	≥ 16	11 (44.0)	**0.02**
Cefazolin	≥ 64 − ≥ 64	≥ 64	≥ 64	0	≤ 4 − ≥ 64	≥ 64	≥ 64	2 (8.0)	1.00
Ceftriaxone	16 − ≥ 64	≥ 64	≥ 64	0	≤ 1 − ≥ 64	≥ 64	≥ 64	2 (8.0)	1.00
Ceftazidime	≥ 64 − ≥ 64	≥ 64	≥ 64	0	≤ 1 − ≥ 64	≥ 64	≥ 64	2 (8.0)	1.00
Cefmetazole	≥ 64 − ≥ 64	≥ 64	≥ 64	0	≤ 1 − ≥ 64	≥ 64	≥ 64	4 (16.0)	0.55
Cefepime	16 − ≥ 64	≥ 64	≥ 64	0	≤ 1 − ≥ 64	4	≥ 64	9 (36.0)	**0.04**§
Piperacillin-tazobactam	≥ 128 − ≥ 128	≥ 128	≥ 128	0	≤ 4 − ≥ 128	≥ 128	≥ 128	7 (28.0)	0.15
Ertapenem	2 − ≥ 8	≥ 8	≥ 8	0	≤ 0.5 − ≥ 8	≥ 8	≥ 8	2 (8.0)	1.00
Meropenem	≥ 16 − ≥ 16	≥ 16	≥ 16	0	2 − ≥ 16	4	≥ 16	0	NA
Levofloxacin	≤ 0.12 − ≥ 8	1	≥ 8	6 (66.7)	≤ 0.12 − ≥ 8	1	≥ 8	16 (64.0)	1.00
Trimethoprim-sulfamethoxazole	≤ 20 − ≥ 320	≥ 320	≥ 320	2 (22.2)	≤ 20 − ≥ 320	160	≥ 320	8 (32.0)	0.69
Tigecycline	1 − 4	2	4	4 (44.4)	≤ 0.5 − ≥ 8	1	4	16 (64.0)	0.44
Ceftazidime-avibactam#	2 − > 256	> 256	> 256	3 (33.3)	0.19 − > 256	2	> 256	19 (76.0)	**0.04**

* Comparison of the susceptibility rates between two groups. *P*-values ≤ 0.05 were marked in bold.
† MICs at which 50% and 90% of the isolates tested are inhibited.
§ The number in one cell (25.0%) was expected to be less than 5. *P* = 0.04 by Chi-squared test and one-sided Fisher's exact test. *P* = 0.07 by two-sided Fisher's exact test.
# MICs for ceftazidime-avibactam were obtained by Etest.
MIC: minimum inhibitory concentration; NA: not applicable.

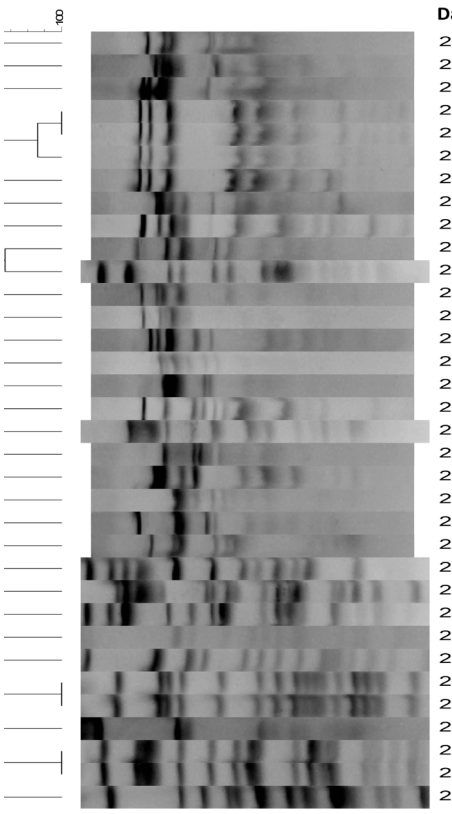
Prediction and dissemination of carbapenemase genes

As shown in Table 1 and Table 3, hospital-acquired infection ($p = 0.01$), days from admission to isolation ≥ 3 days ($p < 0.01$), ICU stay ($p < 0.01$), length of hospitalization ≥ 2 weeks ($p = 0.03$), MICs of meropenem, and nonsusceptibility to gentamicin, cefepime, and ceftazidime-avibactam were associated with the detection of carbapenemase genes. In the multivariate logistic regression, none of these factors showed significant results. In this study, the reporting of meropenem MIC ≥ 16 mg/L and gentamicin nonsusceptibility in the automated AST system had a positive predictive value of 100% for the detection of any carbapenemase gene.

On pulsed-field gel electrophoresis (Fig. 2), three groups of isolates had identical or highly similar banding patterns, using 80% similarity as the cut-off. All these isolates harbored carbapenemase genes, suggesting a phenomenon of gene dissemination. Figure 1 shows the spatial and temporal distributions of patients who carried the CPKP isolates. In most cases, ICUs were presumed to be the routes of gene dissemination. Six of the nine bacteria were isolated before discharge.

Outcome analysis

In univariate analysis, isolation from the blood ($p < 0.01$), hospital-acquired infection ($p < 0.01$), CCI ≥ 5 ($p = 0.04$), meropenem MIC



Date	Ward	No.	Site	Carbapenemase gene
2014/03/20	ER	716279	Urine	
2015/05/07	8B	865782	Pleural	
2015/06/22	OPD	119068	Urine	
2018/05/03	MICU	450700	Blood	bla_{NDM}
2018/05/29	11A	18521	Urine	bla_{NDM}
2018/05/11	11A	553768	Urine	
2018/04/09	MICU	513437	Blood	bla_{NDM}
2016/02/25	10A	116867	Bile	
2018/10/27	OPD	78964	Urine	
2015/04/13	PSI	221431	Pleural	
2019/06/06	11A	631781	Urine	
2015/06/08	6A	576648	Urine	
2013/04/09	12A	24289	Urine	
2015/07/10	6B	873468	Urine	
2012/07/12	ER	687506	Urine	
2014/08/26	12A	521577	Urine	
2018/11/14	7B	12953	Urine	
2018/12/26	6A	481733	Urine	
2014/05/17	10A	722023	Bile	
2016/06/28	ER	339997	Urine	
2013/12/24	ER	671865	Blood	
2014/11/14	ER	803893	Urine	
2016/02/03	10B	912012	Bile	
2019/09/17	OPD	973374	Urine	
2019/12/01	10A	3005816	Blood	
2019/10/30	8A	1067329	Urine	bla_{KPC}
2012/05/25	10A	653190	Blood	
2017/02/16	ER	677845	Urine	
2009/11/13	SICU	214617	Blood	bla_{IMP-2}
2009/11/21	SICU	61712	Urine	bla_{IMP-2}
2015/02/02	ER	850565	Bile	
2016/05/09	MICU	521302	Blood	bla_{KPC}
2016/08/01	13A	129913	Bile	bla_{KPC}
2010/04/13	13A	520910	Urine	bla_{IMP-2}

Fig. 2 Pulsed-field gel electrophoresis results of 34 included carbapenem-nonsusceptible Klebsiella pneumoniae isolates. Three groups of isolates have identical or highly similar banding patterns using 80% similarity as the cut-off. All these isolates were found to harbor carbapenemase genes. ER: emergency room; OPD: outpatient department; MICU: medical intensive care unit; SICU: surgical intensive care unit.

≥ 16 mg/L (p = 0.03), detection of carbapenemase genes (p = 0.02), nonsusceptibility to cefepime (p = 0.05), and nonsusceptibility to ceftazidime-avibactam (p = 0.02) significantly increased the odds of 14-day mortality (Table 4). None of these factors showed significant results in the multivariate logistic regression analysis. Inappropriate antibiotic treatment was not associated with 14-day mortality in this study (p = 0.56).

Discussion

This study highlighted the complexity of CnSKP, which encompasses one group of bacteria which remain susceptible to β-lactams of narrower spectra, as well as another group of bacteria which harbor carbapenemase genes and are generally nonsusceptible to most antibiotics. The strength of this study lies in the inclusion of clinical information and patient outcomes as a correlation between AST results and β-lactamase mechanisms. Presumed routes of carbapenemase gene dissemination may have implications for infection control policies.

In this study, the susceptibility rates of CnSKP isolates to narrow-spectrum ß-lactams were higher than those reported in other studies in Taiwan.[3-5,13,22] These results underscored the significance of non-CPKP and may reflect the complexity of developing antimicrobial resistance rather than merely an error in the automated AST system.[14,16,23] To complement the missing data in the reports from the clinical microbiology laboratory, we performed customized microdilution assays at the time of this study and incidentally found categorical disagreement of meropenem nonsusceptibility in a few isolates (eTable 2 in the supplement). This phenomenon could be attributed to the loss of plasma-mediated ß-lactams during subculture,

Table 4. Univariate and multivariate analysis of clinical features associated with 14-day all-cause mortality.

Factors	Univariate analysis			Multivariate analysis*		
	Odds ratio	95% confidence inter-val	*p*-value	Odds ratio	95% confidence interval	*p*-value
Site of isolation						
Non-blood (reference)	1.00					
Blood	18.00	1.86 − 174.21	< 0.01	3.05×10^{15}	< 0.01 − ∞	1.00
Presumed source						
Community-acquired (reference)	1.00					
Hospital-acquired	17.88	1.93 − 166.00	< 0.01	0.55	0.02 − 15.05	0.72
Charlson comorbidity index ≥ 5	5.04	1.05 − 24.19	0.04	5.98×10^{15}	< 0.01 − ∞	1.00
Inappropriate antibiotic treatment	3.00	0.24 − 36.88	0.56			
Meropenem MIC ≥ 16 mg/L	5.00	1.08 − 23.06	0.03	5.81	0.19 − 175.54	0.31
Detection of carbapenemase genes	8.50	1.40 − 51.48	0.02	1.43×10^{15}	< 0.01 − ∞	1.00
Nonsusceptibility to antibiotics						
Cefepime	9.46	1.02 − 87.80	0.05	1.40×10^{7}	< 0.01 − ∞	1.00
Ceftazidime-avibactam	7.11	1.40 − 36.12	0.02	2.22	0.17 − 28.54	0.54

* Cox & Snell R square = 0.58. *P*-value of Hosmer−Lemeshow test was 0.97.
MIC: minimum inhibitory concentration.

such as AmpC.[24] To our knowledge, no studies have investigated the clinical significance of CnSKP which remains susceptible to β-lactams of narrower spectra. In this study, most of these isolates exhibited only intermediate resistance to meropenem (MIC 2 mg/L), had a high susceptibility rate of ceftazidime-avibactam (> 90%), and had no carbapenemase genes detected. The factor determining the outcome of patients with these isolates was more likely to be the site of isolation rather than antibiotic appropriateness. However, these results should be interpreted with caution because of the scarcity of cases. The significance of these isolates may be focused more on antimicrobial stewardship and infection control measures, especially for the presence of plasma-mediated β-lactams.[5]

Another key finding was that cefepime plays an essential role in managing infections caused by non-CPKP. In this study, the susceptibility rate of cefepime (26.5%) was higher than that of piperacillin-tazobactam (20.6%), which was attributed to non-CPKP. This finding is compatible with local epidemiological reports and US data.[3,7,16] Lee et al. also found that cefepime-inclusive treatments were protective against 30-day mortality compared to other active agents in patients with carbapenem-resistant *K. pneumoniae* bacteremia.[25] They further elaborated on the impact of cefepime MICs by finding lower mortality among the cefepime-susceptible (MIC ≤ 2 mg/L) category than the susceptible dose-dependent (MIC 4 − 8 mg/L) category (16.7% vs. 27.8%). In this study, we found that cefepime nonsusceptibility and meropenem MIC values were related to the presence of carbapenemase genes (Table 3) and 14-day mortality (Table 4) in the univariate analyses. Both findings were consistent with those of previous reports.[16,25,26] In the current automated AST reporting system, the presence of meropenem MIC ≥ 16 mg/L[16] and nonsusceptibility to cefepime or gentamicin, which had 100% positive predictive value in this study, may encourage an early molecular diagnosis of carbapenemase genes. This will lead to a timely AST for ceftazidime-avibactam, which is not included in the current automated AST system in Taiwan. Satlin et al. found that a rapid test for bla_{KPC} and the use of ceftazidime-avibactam decreased mortality in patients with

carbapenem-resistant *Enterobacterales* bacteremia.[27]

Many of our results were compatible with previous epidemiological studies in Taiwan, including the increasing prevalence of CnSKP,[1,3] the site of isolation,[3] the proportion of CPKP,[3] the susceptibility rate of amikacin and tigecycline,[1,3,7,28] and the presence of polymicrobial infections.[29] The susceptibility rate of ceftazidime-avibactam in this study (64.7%) was much lower than those in other local reports,[6,7,28] which is probably attributable to sampling bias and undetected Ambler class B carbapenemases. A nonsusceptibility rate as high as 20% has been reported in carbapenem-resistant *K. pneumoniae*.[30] When managing infections caused by CnSKP, an AST for ceftazidime-avibactam is mandatory.

PFGE results revealed the clonal spread of carbapenemase genes (Fig. 2), indicating that they disseminated more readily than ESBL and AmpC. We postulated that carbapenemase gene dissemination occurred in the ICUs (Fig. 1), but we were unable to trace the index case. The inclination of isolation from patients with longer hospitalizations (Table 1) also suggests the dissemination of carbapenemase genes in healthcare settings. We speculate that the carbapenemase genes may persist in the environment because most of the bacteria with carbapenemase genes were isolated after prolonged hospitalization (Fig. 2). The fact that these isolates were isolated immediately before discharge also emphasizes the importance of terminal cleaning (Fig. 2).

This study has several limitations. First, the included isolates were selected and did not reflect the original population of CnSKP isolates. Second, we did not have enough isolates from blood to evaluate the effects of antimicrobial resistance genes and inappropriate antibiotic treatment on patient survival. Third, no isolates underwent comprehensive molecular characterization, such as ESBL subtypes and sequence typing. A more in-depth comparison of the isolates on a molecular basis could not be performed.

In summary, CnSKP which remain susceptible to β-lactams of narrower spectra exhibited only intermediate resistance to meropenem, had a high susceptibility rate of ceftazidime-avibactam, and had no carbapenemase genes detected.

Supplementary Materials

eFig. 1: Flow diagram of this study.
eFig. 2: Increasing prevalence of carbapenem nonsusceptibility among *Klebsiella pneumoniae*.
eTable 1: PCR primer sequences used for amplification of genes encoding ESBLs, AmpC β-lactamases, and carbapenemases.
eTable 2: Antibiograms, treatment outcomes and ß-lactamase genes of nine isolates which were identified as susceptible to meropenem in customized broth microdilution assays. In every column of antibiotics, the left panels are minim inhibitory concentrations (MICs) obtained by automated antimicrobial susceptibility testing at the time of isolation, and the right panels are MICs obtained by customized broth microdilution assays at the time of this study.

Author Contributions

Study Design, IFL; Data Collection, CHL and WFC; Investigation, IFL, YHH, and WFC. Statistical Analysis, IFL; Data Interpretation, IFL; Manuscript Preparation, IFL and YYW; Literature Search, IFL and YYW; Funding Acquisition, IFL and CHL. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

This study was approved by the Institutional Review Board of E-Da Hospital (EMRP-108-088).

Informed Consent Statement

The need for informed consent was waived because this retrospective study involved no more than a minimal risk of harm to patients from which bacterial isolates were collected.

Data Availability Statement

Data are available from the first author upon reasonable request.

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Conflicts of Interest

The authors declare no conflict of interest.

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