Original Article

Phenotypic and Molecular Characteristics of Carbapenem-Non-Susceptible *Enterobacteriaceae* from a Teaching Hospital in Wenzhou, Southern China

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SUMMARY: Carbapenem resistance in *Enterobacteriaceae* is increasing and has become a matter of great concern. The aim of this study was to characterize carbapenem-non-susceptible *Enterobacteriaceae* from a teaching hospital. A total of 49 carbapenem-non-susceptible *Enterobacteriaceae* clinical isolates recovered in 2007–2010 from the First Affiliated Hospital of Wenzhou Medical College were analyzed by antimicrobial susceptibility testing. The carbapenemase phenotype, outer membrane protein profiles, and clonal relatedness were investigated using the modified Hodge test, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and pulsed-field gel electrophoresis (PFGE). Multilocus sequence typing (MLST) of *Klebsiella pneumoniae* was also performed. β -Lactamase genes were examined by PCR and sequencing, and the transferability of carbapenemase genes was determined by a conjugation experiment. The rates of imipenem, meropenem, and ertapenem resistance were 59.2%, 40.8%, and 96.0%, respectively. Thirty isolates exhibited carbapenemase activity, and 32 isolates carried carbapenemase genes. Furthermore, 10 and 9 clinical isolates possessed AmpC β -lactamase and extended-spectrum β -lactamase (ESBL) genes, respectively. Eight of 32 carbapenemase-producing isolates were proved to be carried by conjugative plasmids, and there was porin loss in 34.7% (17/49) of the isolates. PFGE analysis demonstrated that 9 KPC-2-producing *Serratia marcescens* belonged to a clonal strain, suggesting the clonal dissemination of these KPC-2-bearing isolates among different wards. The MLST of *K. pneumoniae* revealed that two KPC-2 producers were ST11. This study suggests that KPC-2-type carbapenemase is the main contributor to carbapenems resistance in carbapenemase-producing *Enterobacteriaceae*, and that ESBL, AmpC β -lactamase overproduction, and porin loss contribute to the resistance level among these isolates; in carbapenemase-non-producing *Enterobacteriaceae*, ESBL, AmpC enzyme, and porin loss contribute to the carbapenems resistance of *Enterobacteriaceae*, especially the ertapenem resistance of *Enterobacter cloacae*.

INTRODUCTION

Gram-negative bacteria belonging to the family *Enterobacteriaceae* are the most frequently encountered nosocomial pathogens. Since 1950s, broad-spectrum antibiotics, including penicillins and cephalosporins, have been used to treat *Enterobacteriaceae* infections. Unfortunately, the widespread use of broad-spectrum antibiotics in clinical medicine has led to the emergence of resistance to extended-spectrum β -lactam antibiotics in *Enterobacteriaceae*, and it is mostly due to the hyperproduction of chromosomal AmpC β -lactamases and the expression of plasmid-encoded extended spectrum β -lactamases (ESBLs) (1–3). Recently, ESBL-producing *Enterobacteriaceae* have been spread worldwide, and most of them were multidrug-resistant isolates (4).

Carbapenems have a broad spectrum of antibacterial

activity and are highly resistant to β -lactamases. Therefore, carbapenems are frequently the only therapeutic options available to treat severe infections caused by *Enterobacteriaceae* organisms harboring AmpC β -lactamases and ESBLs. However, the alarm has been raised regarding the spread of resistance to carbapenems among *Enterobacteriaceae*. The main mechanism of resistance is the production of carbapenemases. Carbapenemases, including enzymes of Ambler classes A (e.g., KPC, GES, and SME types), B (metallo- β -lactamases) (e.g., IMP, VIM, and NDM types), and D (e.g., OXA-23, -24/40, -48, and -58), can hydrolyze almost all β -lactams and are not inhibited by β -lactamase inhibitors. To date, the emergence of carbapenem-resistant *Enterobacteriaceae* has been reported in many countries and become a matter of great concern (5–8). Reports on the emergence of carbapenemases in *Klebsiella pneumoniae*, *Serratia marcescens*, and *Escherichia coli* have been described in Zhejiang Province in China (9,10). In addition, the rates of carbapenems resistance among *Enterobacteriaceae* have dramatically increased recently. In this study, we investigated the characteristics of antibiotic resistance

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and the molecular characteristics of carbapenem-non-susceptible *Enterobacteriaceae* from a teaching hospital in Wenzhou, Southern China.

MATERIALS AND METHODS

Bacteria isolates: Sixty-two non-duplicated *Enterobacteriaceae* isolates (5 *K. pneumoniae*, 4 *Citrobacter freundii*, 27 *S. marcescens*, 19 *Enterobacter cloacae*, 6 *Enterobacter aerogenes*, and 1 *Enterobacter amnigenus*) with carbapenem non-susceptibility were obtained from inpatients at the First Affiliated Hospital of Wenzhou Medical College from January 2007 to October 2010. All isolates were identified by a VITEK 60 system (bioMérieux, Marcy l'Etoile, France). These isolates were recovered mainly from sputum, blood, and urine samples, and wound swabs. *E. coli* ATCC 25922 was used for quality control in antimicrobial susceptibility tests. *E. coli* EC600 (LacZ⁻ Nal^R Rif^R) was used as a recipient in conjugal transfer experiments. *Salmonella enterica* serotype Braenderup H9812 was used as the size marker for pulsed-field gel electrophoresis (PFGE).

Antimicrobial susceptibility testing: Antibiotic susceptibility testing was assayed by a VITEK 60 system and the agar dilution method according to the approved standard of Clinical and Laboratory Standards Institute (CLSI) 2011 guidelines (11). Additionally, all *Enterobacteriaceae* isolates were screened for carbapenemase production by the modified Hodge test (MHT) using 10 µg ertapenem and meropenem according to the CLSI methods.

PCR and DNA sequence analysis of *bla* genes: Total DNAs of all isolates were obtained with an AxyPrep Bacterial Genomic DNA Miniprep kit (Axygen Scientific, Union City, Calif., USA) and were used as PCR templates. The carbapenem-resistance genes (*bla*_{KPC}, *bla*_{SME}, *bla*_{IMI}/*bla*_{NMC}, *bla*_{GES}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{GIM}, *bla*_{SIM-1}, *bla*_{SPM}, *bla*_{NDM-1}, and *bla*_{OXA-48}), common ESBL genes (*bla*_{CTX-M-1}, *bla*_{CTX-M-9}, *bla*_{TEM}, and *bla*_{SHV}), and AmpC genes (*bla*_{MOX}, *bla*_{FOX}, *bla*_{DHA}, *bla*_{CIT}, and *bla*_{EBC}) were screened in all clinical strains using previously described primers (12–23). The positive PCR products were screened by electrophoresis on 1.0% agarose gel and were sequenced by Shanghai Majorbio Bio-Pharm Technology Co. (Shanghai, China). Nucleotide sequences were analyzed and compared using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>).

Resistance gene transfer experiments: Conjugation experiments were performed using rifampin-resistant *E. coli* EC600 as the recipient strain. Briefly, overnight cultures of the donor strain (500 µl) and recipient strain (500 µl) were mixed with 10 ml fresh Luria-Bertani broth and incubated for 24 h at 35°C. Then, the mixture was inoculated on Mueller-Hinton agar plates containing rifampin (600 µg/ml; Sigma, St. Louis, Mo., USA) plus imipenem (0.125 µg/ml) for 24 h at 35°C. PCR analysis was performed to screen the resistant genes with all the transconjugants.

PFGE typing: *K. pneumoniae* and *S. marcescens* were typed with PFGE. The genomic DNA of these isolates bearing the carbapenem-resistance genes was analyzed by PFGE. *K. pneumoniae* genomic DNA was digested with *Xba*I (24) while that of *S. marcescens* was digested with *Spe*I (25). The DNA fragments were separated

using a CHEF-Mapper XA PFGE system (Bio-Rad, Hercules, Calif., USA) for 22 h at 6 V/cm and 14°C, with a pulse angle of 120° and pulse duration from 5 s to 25 s. The restriction patterns were analyzed and interpreted according to the criteria proposed by Tenover et al. (26).

Multilocus sequence typing (MLST): MLST of *K. pneumoniae* was performed using seven conserved housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) according to protocols available at the MLST Pasteur website (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>).

Analysis of outer membrane proteins (OMPs): OMPs were isolated by ultrasonic treatment as described by Hernandez-Alles et al. (27). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described previously to investigate alterations in the OMPs (5,28).

RESULTS

Antimicrobial susceptibility: In this study, 62 *Enterobacteriaceae* clinical isolates were considered eligible according to hospital screening methods. However, testing of the MICs of imipenem, meropenem, and ertapenem for the 62 *Enterobacteriaceae* clinical isolates was further performed, and revealed that 49 isolates (5 *K. pneumoniae*, 4 *C. freundii*, 21 *S. marcescens*, 13 *E. cloacae*, 5 *E. aerogenes*, and 1 *E. amnigenus*) were carbapenem-non-susceptible. Six (22.2%) *S. marcescens* and 7 (31.5%) *E. cloacae* isolates exhibited false resistance to carbapenems based on the methods used in hospital laboratories. It demonstrated that hospital screening methods (VITEK 2) may produce higher MICs than agar microdilution (29). The susceptibility testing results are shown in Table 1. Almost all of the *Enterobacteriaceae* isolates exhibited resistance to penicillins and cefazolin, and approximately half of the strains were non-sensitive to third-generation cephalosporin and β-lactamase inhibitors. The susceptibility rates for gentamicin and levofloxacin were 70.2% and 75.4%, respectively, which provided antibiotic treatment options other than carbapenems for *Enterobacteriaceae* infections. The rates of imipenem, meropenem, and ertapenem resistance were 59.2%, 40.8%, and 96.0%, respectively. In this study, 49 *Enterobacteriaceae* clinical isolates that included 4 different genera exhibited different resistance profiles: 80% (4/5) of *K. pneumoniae*, 50% (2/4) of *C. freundii*, 90.4% (19/21) of *S. marcescens*, and 21.1% (4/19) of *Enterobacter* spp. were resistant to imipenem; 60% (3/5) of *K. pneumoniae*, 25% (1/4) of *C. freundii*, 66.7% (14/21) of *S. marcescens*, and 10.5% (2/19) of *Enterobacter* spp. were resistant to meropenem; and 100% (5/5) of *K. pneumoniae*, 100% (4/4) of *C. freundii*, 95.2% (20/21) of *S. marcescens*, and 94.7% (18/19) of *Enterobacter* spp. were resistant to ertapenem (Tables 2 and 3).

Determination of the presence of carbapenemase, ESBLs, AmpC enzymes, and OMPs analysis: Of the 49 *Enterobacteriaceae* isolates, 61.2% (30/49) were carbapenemase-producing isolates. However, carbapenemase genes were detected in 32 of 49 clinical isolates, which included the *bla*_{KPC-2}, *bla*_{IMP-8}, and *bla*_{IMP-4} genes. Common ESBLs and AmpC enzyme genes were

Table 1. Antimicrobial susceptibility patterns of 49 *Enterobacteriaceae* with decreased carbapenem susceptibility

Antimicrobial agent	CLSI breakpoint interpretation			MIC ($\mu\text{g/ml}$)		
	%S	%I	%R	MIC ₅₀	MIC ₉₀	Range
Ampicillin	0.0	0.0	100.0	≥ 32	≥ 32	≥ 32
Cefazolin	0.0	0.0	100.0	≥ 32	≥ 32	≥ 32
Ceftazidime	47.4	19.3	33.3	16	≥ 32	$\leq 2 - \geq 32$
Ceftriaxone	40.4	17.5	42.1	16	≥ 64	$\leq 1 - \geq 64$
Cefepime	63.2	5.3	31.6	4	≥ 64	$\leq 1 - \geq 64$
Piperacillin/tazobactam	29.8	7.0	63.2	≥ 128	≥ 128	$\leq 4 - \geq 128$
Cefoperazone/sulbactam	33.3	28.1	38.6	32	≥ 64	$2 - \geq 64$
Levofloxacin	75.4	2.0	22.6	≥ 1	≥ 16	$\leq 1 - \geq 16$
Gentamicin	70.2	2.0	27.8	≥ 0.5	≥ 16	$\leq 0.5 - \geq 16$
Imipenem	36.7	4.1	59.2	4	32	$0.25 - \geq 64$
Meropenem	42.9	16.3	40.8	1	16	$0.06 - 32$
Ertapenem	2.0	2.0	96.0	4	≥ 64	$0.015 - \geq 64$

%S, percentage susceptible; %I, percentage intermediate; %R, percentage resistant.

Table 2. Susceptibilities and resistance mechanisms of carbapenemase-producing isolates

Isolate no.	Species	MIC ($\mu\text{g/ml}$) of carbapenem			MHT	Resistance mechanism
		IPM	MEM	ETP		
F1074	<i>K. pneumoniae</i>	2	1	1	+	KPC-2
F1249	<i>K. pneumoniae</i>	4	4	32	+	KPC-2, TEM, CTX-M-14
F1260	<i>K. pneumoniae</i>	8	32	≥ 64	+	KPC-2, TEM, CTX-M-14
F1261	<i>K. pneumoniae</i>	16	32	≥ 64	+	KPC-2, TEM, CTX-M-14
NM62	<i>C. freundii</i>	16	16	16	+	KPC-2, porin loss
NM68	<i>C. freundii</i>	0.25	0.125	16	+	IMP-8
NM136	<i>C. freundii</i>	2	2	2	+	KPC-2
NM154	<i>C. freundii</i>	4	2	2	+	KPC-2, CTX-M-3
Z61	<i>S. marcescens</i>	16	1	2	+	KPC-2
Z70	<i>S. marcescens</i>	8	4	2	+	KPC-2
Z71	<i>S. marcescens</i>	16	8	2	+	KPC-2
Z72	<i>S. marcescens</i>	8	4	8	+	KPC-2, porin loss
Z73	<i>S. marcescens</i>	16	8	8	+	KPC-2
Z74	<i>S. marcescens</i>	4	1	2	–	KPC-2
Z75	<i>S. marcescens</i>	4	2	2	–	KPC-2
Z77	<i>S. marcescens</i>	8	8	4	+	KPC-2
Z78	<i>S. marcescens</i>	16	4	4	+	KPC-2, CTX-M-3
Z79	<i>S. marcescens</i>	16	8	8	+	KPC-2
Z84	<i>S. marcescens</i>	16	8	8	+	KPC-2, porin loss
Z85	<i>S. marcescens</i>	16	4	8	+	KPC-2
Z104	<i>S. marcescens</i>	16	4	1	+	KPC-2, porin loss
Z108	<i>S. marcescens</i>	8	8	4	+	KPC-2, porin loss
Z109	<i>S. marcescens</i>	16	2	2	+	KPC-2
Z122	<i>S. marcescens</i>	8	2	2	+	KPC-2
Z138	<i>S. marcescens</i>	32	16	16	+	KPC-2
Z139	<i>S. marcescens</i>	≥ 64	16	16	+	KPC-2
Z143	<i>S. marcescens</i>	32	16	8	+	KPC-2
Y265	<i>E. cloacae</i>	1	1	2	+	IMP-8, TEM, CTX-M-3
Y412	<i>E. cloacae</i>	4	4	8	+	KPC-2, TEM, CTX-M-14, EBC
Y421	<i>E. aerogenes</i>	4	2	8	+	KPC-2
Y488	<i>E. cloacae</i>	0.25	0.5	2	+	IMP-4, porin loss
Y509	<i>E. aerogenes</i>	4	2	4	+	KPC-2, porin loss

IPM, imipenem; MEM, meropenem; ETP, ertapenem; MHT, modified Hodge test.

Table 3. Susceptibilities and resistance mechanisms of non-carbapenemase-producing isolates

Isolate no.	Species	MIC ($\mu\text{g/ml}$) of carbapenem			Resistance mechanism
		IPM	MEM	ETP	
F1038	<i>K. pneumoniae</i>	8	2	32	DHA-1, porin loss
Z68	<i>S. marcescens</i>	0.25	0.06	0.5	—
Z94	<i>S. marcescens</i>	0.5	0.06	1	—
Y270	<i>E. aerogenes</i>	0.25	0.06	4	EBC, porin loss
Y294	<i>E. cloacae</i>	0.25	0.25	4	porin loss
Y335	<i>E. cloacae</i>	0.25	0.06	1	EBC
Y342	<i>E. cloacae</i>	0.25	0.125	4	EBC
Y345	<i>E. cloacae</i>	1	2	8	porin loss
Y360	<i>E. aerogenes</i>	0.25	0.06	1	—
Y364	<i>E. cloacae</i>	1	1	4	EBC, porin loss
Y367	<i>E. cloacae</i>	0.25	0.125	1	EBC, CTX-M-3
Y378	<i>E. cloacae</i>	0.5	1	16	TEM, CTX-M-14, DHA-1, EBC
Y413	<i>E. cloacae</i>	0.25	0.125	0.5	porin loss
Y443	<i>E. cloacae</i>	0.25	0.125	2	EBC
Y448	<i>E. aerogenes</i>	0.25	0.5	2	porin loss
Y454	<i>E. amnigenus</i>	0.25	0.25	2	porin loss
Y463	<i>E. cloacae</i>	0.5	1	8	DHA-1, EBC

Abbreviations are in Table 2.

identified in 9 and 10 isolates out of 49 strains, respectively. The distributions of the resistance genes in these strains are listed in Tables 2 and 3. The OMPs of all clinical isolates were analyzed by SDS-PAGE, and we determined that 17 of 49 isolates had lost porins. The distribution of *bla* genes and porin profiles varied for the different species. Therefore, each species was individually analyzed.

***K. pneumoniae*:** In this study, 80% (4/5) of the 5 *K. pneumoniae* isolates harbored *bla*_{KPC-2}. Three *K. pneumoniae* isolates possessed both the *bla*_{KPC-2} and *bla*_{CTX-M-14} genes. The carbapenems MIC values of these 3 isolates were higher ($4\text{--}\geq 64\text{ }\mu\text{g/ml}$) than the isolate that only carried the *bla*_{KPC-2} gene. Furthermore, *K. pneumoniae* F1038, possessing carbapenem resistance (MIC range, $2\text{--}32\text{ }\mu\text{g/ml}$) did not harbor any carbapenemase genes, and was negative for the MHT; only the DHA-1-type AmpC enzyme and porin loss were detected in this isolate.

***C. freundii*:** Among the 4 *C. freundii* isolates, 3 possessed the *bla*_{KPC-2} gene. One of the 3 *C. freundii* isolates that had lost porins had high-level resistance to carbapenems, and the 2 of them that had conserved their porins had intermediate or low-level carbapenem resistance. In addition, one isolate producing IMP-8-type carbapenemase was only resistant to ertapenem.

***S. marcescens*:** Among the 21 *S. marcescens* isolates, 19 harbored the *bla*_{KPC-2} gene. There was porin loss in 4 of the 19 isolates, and one isolate possessed CTX-M-3-type ESBLs. The 5 isolates producing KPC-2-type carbapenemase combined with porin loss or ESBLs had an average MIC value similar to that of the 14 isolates only producing KPC-2-type carbapenemase.

***Enterobacter* spp.:** Five of 21 *Enterobacter* spp. isolates possessed carbapenemase genes. Two of the 5 isolates bearing *bla*_{IMP-4} and *bla*_{IMP-8} combined with porin loss or ESBLs only had low-level resistance to ertapenem; however, 3 of the 5 isolates producing KPC-2-type carbapenemase were resistant to all 3 carbapenems. Furthermore, most of the remaining 14 non-carba-

penemase-producing *Enterobacter* spp. isolates had ESBLs, AmpC genes, or porin loss, and exhibited resistance to ertapenem.

Transfer of carbapenem resistance: Among the 32 carbapenemase-producing clinical isolates, carbapenemase genes (*bla*_{KPC-2} and *bla*_{IMP-8}) of 8 isolates (2 *C. freundii*, 3 *S. marcescens*, 2 *E. cloacae*, and 1 *E. aerogenes*) were successfully transferred to *E. coli* EC600; 8 transconjugants exhibited a phenotype of reduced susceptibility to carbapenems and were PCR-confirmed to carry carbapenem resistance genes similar to that of the original isolates (data not shown). Transfer of the *bla*_{IMP-4} gene of U488 isolates to *E. coli* EC600 failed. The transformants of *K. pneumoniae* that exhibited high-level resistance to carbapenems failed to detect resistance genes.

Strain typing: PFGE revealed 4 distinct patterns among the 5 *K. pneumoniae* isolates. Isolates F1260 and F1261, recovered from different patients, belonged to the same clone (data not shown). MLST showed that isolates F1260 and F1261 shared the same sequence type (ST), ST11 (allelic profile: gapA, 3; infB, 3; mdh, 1; pgi, 1; phoE, 1; rpoB, 1; and tonB, 4), and isolates F1038, F1074, and F1249 were identified as ST880, ST65, and ST23, respectively. PFGE analyses for 19 KPC-producing *S. marcescens* isolates were performed several times, and ultimately, 13 isolates were typed. The PFGE patterns of the *SpeI* DNA digests of the 13 *S. marcescens* isolates are shown in Fig. 1. Three PFGE patterns were identified, designated patterns A to C. Nine *S. marcescens* isolates, which were mainly isolated from the Neurosurgery and Cerebral Surgery Department in the First Affiliated Hospital of Wenzhou Medical College, belonged to the dominant pattern A. *S. marcescens* Z138, Z139, and Z143 belonged to pattern B. Pattern C was represented by one isolate. The 8 *S. marcescens* isolates were indistinguishable and were considered to represent the same clone. This finding suggested that clonal dissemination of *S. marcescens* might be present in these wards.

Fig. 1. Pulsed-field gel electrophoresis patterns of 13 KPC-2-producing *Serratia marcescens*.

DISCUSSION

Enterobacteriaceae isolates are important nosocomial pathogens responsible for various infections. In 2009, Yang et al. (30) found that the most frequent isolates were *K. pneumoniae*, followed by *E. coli*, *E. cloacae*, *E. aerogenes*, and *C. freundii* among the 49 *Enterobacteriaceae* isolates with decreased carbapenem susceptibility collected from 16 teaching hospitals in China. However, in the current study, we found that in the 49 *Enterobacteriaceae* isolates with carbapenem non-susceptibility, the most common isolates were *Enterobacter* spp., followed by *Serratia* spp., *K. pneumoniae*, and *C. freundii*, and almost half of the isolates were *Serratia* spp. This might be associated with the special clinical isolation rate in Wenzhou.

Antimicrobial susceptibility testing showed that although carbapenem-non-susceptible strains exhibited resistance to many antimicrobial agents, some isolates remained susceptible to gentamicin and levofloxacin, suggesting a choice for clinical therapy. The carbapenem susceptibility in *Enterobacteriaceae* was variable; the most frequent carbapenem-resistant strain was *K. pneumoniae*, followed by *S. marcescens* and *C. freundii*, and all rates of resistance of carbapenem-resistant strains were higher against ertapenem than against imipenem or meropenem.

Carbapenem resistance in *Enterobacteriaceae* is mainly caused by carbapenem-hydrolyzing β -lactamases, including IMP-, VIM-, and KPC-type enzymes (31,32). In this study, KPC-2 was the most common carbapenemase type. *S. marcescens* was the most frequently isolated KPC-2-producing species. Our results demonstrate different resistance mechanisms in different *Enterobacteriaceae* spp. Three of 5 *K. pneumoniae* isolates bearing the *bla*_{KPC-2} and ESBL genes exhibited higher-level carbapenem resistance than the isolate that only harbored *bla*_{KPC-2}. One of the 4 *C. freundii* isolates that possessed *bla*_{KPC-2} combined with lost porins also exhibited higher-level resistance to carbapenems than the other 2 KPC-2 producing *C. freundii* isolates that conserved their porins. However, *S. marcescens* isolates producing KPC-2-type carbapenemase combined with porin loss or ESBLs had an average MIC value similar to that of other isolates only bearing KPC-2-type car-

bapenemase. Therefore, carbapenem resistance in *S. marcescens* must be mediated by KPC-2-type carbapenemase, but in *K. pneumoniae* and *C. freundii*, resistance is mainly mediated by KPC-2-type carbapenemase, and ESBL production or porin loss may contribute to the resistance level among these isolates. Imipenem and meropenem resistance in *Enterobacter* spp. is rarer, and more likely to be due to acquired carbapenemase. In this study, the resistance to 3 carbapenems in *Enterobacter* spp. was mediated by KPC-2-type carbapenemase.

In this study, 34.7% (17/49) of carbapenem-resistant *Enterobacteriaceae* isolates was not mediated by true carbapenemase. Instead, porin loss, or ESBL or AmpC-type enzymes mediated resistance. One *K. pneumoniae* isolate only harbored the DHA-1 AmpC enzyme and porin loss, but exhibited carbapenem resistance. Shin et al. found that DHA-1 AmpC enzyme and OmpK36 and/or OmpK35 loss mediated carbapenem resistance in 16 *K. pneumoniae* isolates (33). Although the dissemination of KPC-producing *K. pneumoniae* has been repeatedly reported in many parts of the world, AmpC enzymes such as DHA-1 and CMY-2, and OmpK36 and/or OmpK35 loss may play a crucial role in the acquisition of carbapenem resistance (34). Furthermore, most of the 14 non-carbapenemase-producing *Enterobacter* spp. had ESBLs or AmpC enzyme genes, or porin loss, and exhibited resistance to ertapenem. Moreover, 2 *E. cloacae* isolates bearing *bla*_{IMP-4} and *bla*_{IMP-8} combined with porin loss or ESBLs only exhibited low-level ertapenem resistance. Therefore, ertapenem resistance in *Enterobacter* spp. is not uncommon and is mostly mediated by a combination of ESBLs or AmpC enzyme production and porin loss (35); in this study, IMP may have played a partial role in ertapenem resistance. In addition, 2 non-carbapenemase-producing *S. marcescens* and 1 *E. cloacae* isolate exhibited intermediate or total resistance to ertapenem, but we did not detect β -lactamase genes or porin loss in these isolates, thus they may contain other mechanisms that require further investigation.

The PFGE data indicated that all *S. marcescens* isolates in this study belonged to 3 subgroups. The dominant subgroup included 9 strains, mostly isolated from the Neurosurgery and Cerebral Surgery Depart-

ment, suggesting the clonal dissemination of these KPC-2-bearing isolates among different wards. This finding also demonstrated the potential epidemic threat of KPC-2-bearing *S. marcescens* isolates.

In this study, we did not detect the international KPC clone *K. pneumoniae* ST258, but 2 KPC-producing *K. pneumoniae* isolates shared the same ST (ST11). In Korea, Ko et al. (36) detected 4 ST11 isolates among 82 ESBL-producing *K. pneumoniae* isolates, and Shin et al. (33) detected 15 ST11 isolates among 16 carbapenem-resistant *K. pneumoniae* isolates. This merits careful monitoring.

Carbapenem resistance among *Enterobacteriaceae* is a serious clinical problem, and resistance genes can be spread among isolates by plasmids. The spread of such resistant strains may become a serious problem in Wenzhou. Effective measures for early identification and control should be adopted to prevent the potential continuous dissemination of these carbapenem-resistant pathogens.

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Conflict of interest None to declare.

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