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ORIGINAL ARTICLE

Molecular surveillance and clinical outcomes of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* infections

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Escherichia coli;
Klebsiella pneumoniae

Background/Purpose: The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) is a cause for great concern. The aim of this study was to evaluate antimicrobial susceptibility, mechanisms of carbapenem-resistance in two members of the *Enterobacteriaceae* family (*Escherichia coli* and *Klebsiella pneumoniae*), and clinical outcomes of their infections.
Methods: The susceptibility tests of 16 *E. coli* and 60 *K. pneumoniae* isolates, collected from 2010 to 2011, were assessed. The minimal inhibitory concentrations of eight antimicrobial agents were assessed by the broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute. The detection of beta-lactamase genes was performed by polymerase chain reaction. The genetic relatedness of these isolates was determined by pulsed-field gel electrophoresis (PFGE) fingerprinting.
Results: The carbapenemase genes bla_{KPC-2} and bla_{OxA} were detected in one and five *K. pneumoniae* isolates, respectively. The genetic combinations bla_{SHV-5}–bla_{DHA} and bla_{SHV-5}–$bla_{CTx-M-G9}$ were prevalent in 45% and 26.7% of 60 *K. pneumoniae* isolates, respectively. The susceptibility rates of 60 *K. pneumoniae* isolates to colistin and tigecycline were 58.3% and 50.0%, respectively. The 30-day mortality rates of the patients treated with carbapenem, colistin, or tigecycline were as high as 60.6%. Nine clusters of *K. pneumoniae* isolates were identified by PFGE fingerprinting.

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Conclusion: The findings of carbapenemase genes in a few isolates and small clusters of CRE indicated the emerging problems in the hospital. The high mortality rates were observed in the patients treated by colistin and tigecycline, although they were the only alternative treatment options for CRE infections. Active surveillance and an effective infection control strategy should be implemented to control the spread of CRE infections.

Introduction

The global spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is an alarming crisis. According to the Study for Monitoring Antimicrobial Resistance Trends (SMART) program (2009—2010), the ertapenem resistance rate of *Escherichia coli* isolates from urinary tract infections was less than 2%.[1] However, the prevalence of CRE was as high as 31.6% in long-term acute-care facilities in south-east Michigan, USA.[2] This finding suggested that CRE is more prevalent in high-risk groups than in the healthy population.

Mechanisms of carbapenem resistance included carbapenemase production, a combination of AmpC hyperproduction and/or extended spectrum beta-lactamase (ESBL) production, and porin mutation[3] (such as ESBLs of SHV- and CTX-M types combined with a deficiency of OmpK35 or OmpK36 in *Klebsiella pneumoniae*,[4,5] AmpC overexpression and OmpC or OmpF in *Enterobacter cloacae*,[5] and CTX-M-type ESBL and loss of OmpC in *E. coli*[6]). Various carbapenemases have been classified, including class A beta-lactamases (NMC, IMI, SME, KPC, GES), class B beta-lactamases (IMP, VIM, GIM, SPM, NDM), and class D beta-lactamase (OXA).[7] The carbapenemases KPC-2[8,9] and NDM-1[9,10] have been reported to have been spreading around the world since late 2000s. ST11 was reported to be the dominant clone of KPC-2-producing *K. pneumoniae* in China.[11] OXA-48-producing *Enterobacteriaceae* have been reported in Europe, East-Central Asia, and Africa.[9,12]

In Taiwan, the rate of ertapenem resistance in *E. coli* isolates increased from 0.1% in 1999 to 1.7% in 2007.[13] The rate of ertapenem resistance (shown by disk diffusion tests) among 2421 *K. pneumoniae* isolates from a Taiwanese hospital in 2008 was 13.5%.[14] Carbapenemases have been detected in *Enterobacteriaceae* in Taiwan, such as IMP-8 in *K. pneumoniae*[14] and *E. cloacae*,[15] VIM-2 in *Citrobacter freundii*,[15] and KPC-2 in *K. pneumoniae*.[16] However, OXA-producing *Enterobacteriaceae* have not yet been reported in Taiwan. The production of ESBL and/or AmpC combined with a loss of porins has been reported in Taiwan, for example the production of CMY-2 combined with a decreased expression of OmpF and/or OmpC in *E. coli*[13]; and the loss of OmpK36 combined with a production of both ESBL and AmpC in *K. pneumoniae*[17] (such as CTX-M-14, SHV-12, and DHA-1; SHV-5 and DHA-1; SHV-12 and DHA-1; and CTX-M-14 and CMY-2). Few studies have investigated the prevalence rate of CRE, the types of carbapenemase, and the outcomes of CRE infections in Taiwan. We therefore conducted this study to evaluate these CRE-related issues.

Materials and methods

Bacterial strains

During the period January 2010 to December 2011, 8904 *E. coli* and 5820 *K. pneumoniae* isolates were identified at the Microbiology Laboratory of Taichung Veterans General Hospital (TCVGH). The disk diffusion test for *Enterobacteriaceae* was read according to the 2009 recommendations of the Clinical and Laboratory Standards Institute (CLSI).[18] In order to increase the possibility of identifying the carbapenemase, ertapenem-resistant *Enterobacteriaceae* with a positive confirmatory test for ESBL were not included in this study. Sixteen *E. coli* isolates and 60 *K. pneumoniae* isolates, which were resistant to ertapenem or intermediately resistant to ertapenem on disk diffusion tests and had negative confirmatory tests for ESBL using the double disk diffusion method, were collected for this study. The sources of 16 *E. coli* isolates included urine (25.0%), blood (12.5%), bile (6.3%), and others (including pus, discharge, ascites, and pleural effusion) (56.3%). The sources of 60 *K. pneumoniae* isolates included urine (21.7%), blood (18.3%), bile (18.3%), sputum (16.7%), and others (including pus, discharge, ascites, and pleural effusion) (25.0%).

Susceptibility test

The minimal inhibitory concentrations (MICs) of eight antimicrobial agents — aztreonam, amikacin, and cefepime (Bristol-Myers Squibb, NY, USA), imipenem (MSD, Rahway, NJ, USA), ceftazidime (GlaxoSmithKline, Greenford, UK), colistin (Sigma, St. Louis, MO, USA), ertapenem (MSD, Rahway, NJ, USA) and tigecycline (Wyeth, Monza, Italy) — were assessed using the broth microdilution method according to the CLSI's recommendations.[19] The MICs of colistin and tigecycline were interpreted according to the breakpoints recommended by the 2012 European Committee on Antimicrobial Susceptibility Testing (EUCAST),[20] while those of the other six antimicrobial agents were read according the breakpoints recommended by the 2012 CLSI guidelines.[21] All MIC tests were performed twice to avoid technical error. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the quality control strains.

Modified Hodge test

The modified Hodge test (MHT; using ertapenem disks) was performed for all 76 isolates and interpreted according to the 2012 recommendations of the CLSI.[21] Positive and negative control testing of the MHT was performed using *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-1706.

Molecular techniques

To carry out the polymerase chain reaction (PCR) to detect beta-lactamase genes, bacterial DNA extraction was performed using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). All isolates were screened for the resistance genes encoding KPC, GES, IMP-1, IMP-2, VIM-1, AmpC, SHV, CTX-M-Group 9 (CTX-M-G9), and OXA by PCR assay using previously described primers (Table 1).[22–27] PCR products were sequenced for KPC-2, SHV-5, and CMY-2.

Pulsed-field gel electrophoresis (PFGE) was performed with a contour-clamped homogeneous electric field DRII apparatus from Bio-Rad Laboratories (Richmond, CA, USA) as previously described.[28] The chromosomal DNA was digested overnight with XbaI (Promega, Madison, WI, USA). DNA was electrophoresed in 1.2% SeaKem GTG agarose (FMC, Rockland, ME, USA) at 6 V/cm for 22 hours; the pulse time was increased from 2.2 to 54.2 seconds. The PFGE patterns were also analyzed using the computer software Gelcompar for Windows version 3.1b (Applied Math, Kortrijk, Belgium). The PFGE patterns were compared using the unweighted pair group method with arithmetic averages clustering method by using the Dice coefficient, according to the instructions of the Gelcompar manufacturer. A tolerance of 1.2% in the band position was applied during the comparison of PFGE fingerprinting patterns.

Multilocus sequence typing was carried out with seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*) for *K. pneumoniae*. Allele sequences and sequence

Table 1 Primers used for detection of beta-lactamase genes

Enzyme	Primer name	Sequence(5′ to 3′)	Amplicon size (bp)	Reference
Class A carbapenemases				
KPC	KPC forward	ATGTCACTGTATCGCCGTCT	893	[22]
	KPC reverse	TTTTCAGAGCCTTACTGCCC		
GES	GES-C	GTTTTGCAATGTGCTCAACG	371	[23]
	GES-D	TGCCATAGCAATAGGCGTAG		
Class B metalloenzymes				
IMP-1	IMP-1 forward	TGAGCAAGTTATCTGTATTC	740	
	IMP-1 reverse	TTAGTTGCTTGGTTTTGATG		
IMP-2	IMP-2 forward	GGCAGTCGCCCTAAAACAAA	737	
	IMP-2 reverse	TAGTTACTTGGCTGTGATGG		
VIM-1	VIM-1 forward	TTATGGAGCAGCAACCGATGT	920	
	VIM-1 reverse	CAAAAGTCCCGCTCCAACGA		
Class D oxacillinases				
OXA	OXA forward	ACACAATACATATCAACTTCGC	813	[24]
	OXA reverse	AGTGTGTTTAGAATGGTGATC		
AmpC beta-lactamases				
MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11	MOXMF	GCTGCTCAAGGAGCACAGGAT	520	[25]
	MOXMR	CACATTGACATAGGTGTGGTGC		
LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1	CITMF	TGGCCAGAACTGACAGGCAAA	462	[25]
	CITMR	TTTCTCCTGAACGTGGCTGGC		
DHA-1, DHA-2	DHAMF	AACTTTCACAGGTGTGCTGGGT	405	[25]
	DHAMR	CCGTACGCATACTGGCTTTGC		
ACC	ACCMF	AACAGCCTCAGCAGCCGGTTA	346	[25]
	ACCMR	TTCGCCGCAATCATCCCTAGC		
MIR-1T ACT-1	EBCMF	TCGGTAAAGCCGATGTTGCGG	302	[25]
	EBCMR	CTTCCACTGCGGCTGCCAGTT		
FOX-1 to FOX-5b	FOXMF	AACATGGGGTATCAGGGAGATG	190	[25]
	FOXMR	CAAAGCGCGTAACCGGATTGG		
ESBL				
SHV-5	SHV-5F	TGTTAGCCACCCTGCCGCT	825	[26]
	SHV-5R	GTTGCCAGTGCTCGATCAG		
9 variants of CTX-M group	CTX-M-G9 (F)	ATGGTGACAAAGAGAGTGCAAC	876	[27]
	CTX-M-G9 (R)	TTACAGCCCTTCGGCGATG		

types (STs) were identified at <http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>. A different allele number was given to each distinct sequence within a locus, and a distinct ST number was assigned to each distinct combination of alleles.

Survival and bacteriologic assessments

The clinical outcomes of the 76 patients with acquired ertapenem-resistant *Enterobacteriaceae* isolates were analyzed by chart review. The clinical conditions (such as intensive care stay, ventilator use, previous stay in a nursing home, and prior antibiotic treatment) associated with the acquisition of ertapenem-resistant *Enterobacteriaceae* isolates were reviewed. The 30-day mortality rates among different groups of the 76 patients were analyzed by Yates' correction of continuity and Fisher's exact test, using the Statistical Package for the Social Science (version 15.1; SPSS Inc, Chicago, IL, USA). If the antibiotics (imipenem, meropenem, colistin, and tigecycline) were reported as active against these isolates and were administered to patients with ertapenem-resistant *Enterobacteriaceae* infection, these patients were classified into the observation group. None of the patients was treated with any combination of imipenem, colistin, or tigecycline. The mortality rates of patients treated with tigecycline, colistin, meropenem, and imipenem were also compared. Bacteriologic eradication was defined as a lack of growth of CRE from culture, or a lack of culture report due to removal of the drainage tube on the 14th day of antibiotic treatment.

Results

The beta-lactamase genes detected in each ertapenem-resistant *E. coli* and *K. pneumoniae* isolate are listed in Table 2. Twenty-five isolates carried a single gene, including bla_{CMY-2} in 11 *E. coli* and 1 *K. pneumoniae* isolates, bla_{SHV-5} in 6 *K. pneumoniae* isolates, bla_{DHA} in 6 *K. pneumoniae* isolates, and bla_{ACC} in 1 *E. coli* isolate. The two-gene combination bla_{SHV-5} and bla_{DHA} was found in 19 *K. pneumoniae* isolates. The combination of three genes, bla_{SHV-5}, bla_{DHA}, and $bla_{CTx-M-G9}$, was found in 6 *K. pneumoniae* isolates. The class D beta-lactamase gene, bla_{OXA}, was found in five *K. pneumoniae* isolates. Only one *K. pneumoniae* isolate was found to carry bla_{KPC-2} and was classified as ST11. A positive MHT result was found in 15 isolates (4 *E. coli* and 11 *K. pneumoniae*).

The most prevalent genes in ertapenem-resistant *E. coli* and *K. pneumoniae* isolates are listed in Table 3. The bla_{CMY-2} gene (alone or in combination with other genes) was found in 93.8% of 16 *E. coli* isolates. The bla_{SHV-5} gene (alone or in combination) was found in 75.0% of 60 *K. pneumoniae* isolates. The bla_{DHA} gene (alone or in combination with other genes) was found in 58.3% of 60 *K. pneumoniae* isolates. The combination bla_{SHV-5}—bla_{DHA} (including further combination with other genes) was found in 45% of 60 *K. pneumoniae* isolates. The combination,

Table 2 The beta-lactamase genes detected in each of the ertapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates

Genes encoding beta-lactamases	*Escherichia coli* (*n*)	*Klebsiella pneumoniae* (*n*)	Positive modified Hodge test
None	0	6	1 (*K. pneumoniae*)
Single gene			
CMY-2	11	1	2 (*E. coli*), 1 (*K. pneumoniae*)
SHV-5	0	6	3 (*K. pneumoniae*)
DHA	0	6	2 (*K. pneumoniae*)
ACC	1	0	
Two genes			
DHA, SHV-5	0	19	2 (*K. pneumoniae*)
SHV-5, CTX-M-G9	0	4	
CTX-M-G9, CMY-2	2	0	2 (*E. coli*)
SHV-5, CMY-2	1	0	
DHA, CTX-M-G9	0	1	
SHV-5, OXA	0	1	
Three genes			
DHA, SHV-5, CTX-M-G9	0	6	
SHV-5, CMY-2, OXA	0	3	
DHA, SHV-5, CTX-M-G9	0	2	
DHA, CTX-M-G9, CMY-2	0	1	1 (*K. pneumoniae*)
KPC-2, SHV-5, CTX-M-G9	0	1	1 (*K. pneumoniae*)
SHV-5, CTX-M-G9, ACC	0	1	
SHV-5, CTX-M-G9, OXA	0	1	
SHV-5, CTX-M-G9, CMY-2	0	1	
DHA, SHV-5, CMY-2	1	0	
Total	16	60	15

Table 3 Prevalent gene of ertapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates

Genes encoding beta-lactamases	*Escherichia coli* (%)	*Klebsiella pneumoniae* (%)	*p*
Prevalent gene, alone or in combination with other genes			
CMY-2	15/16 (93.8)	6/60 (10.0)	<0.001[a]
SHV-5	2/16 (12.5)	45/60 (75.0)	<0.001[b]
DHA	1/16 (6.3)	35/60 (58.3)	0.001[b]
Prevalent genetic combination (including combination with other genes)			
SHV-5, DHA	1/16 (6.3)	27/60 (45.0)	0.010[b]
SHV-5, CTX-M-G9	0	16/60 (26.7)	0.017[a]

[a] Fisher's exact test.
[b] Yates' correction of continuity.

bla_{SHV-5}—$bla_{CTx-M-G9}$ (including further combination with other genes) was found in 26.7% of 60 *K. pneumoniae* isolates.

The antimicrobial susceptibilities of ertapenem-resistant *E. coli* and *K. pneumoniae* isolates are listed in Table 4. These 76 isolates showed moderate to high resistance to aztreonam, cefepime, and ceftazidime. All 16 *E. coli* isolates were susceptible to amikacin, but only 40% of the 60 *K. pneumoniae* isolates were susceptible to amikacin. Eight (50%) of 16 *E. coli* isolates were susceptible to imipenem. Fourteen (23.3%) of the 60 ertapenem-resistant *K. pneumoniae* were susceptible to imipenem. The susceptibility rates of 16 *E. coli* isolates to colistin and tigecycline were 62.5% and 75.0%, respectively. The susceptibility rates of 60 ertapenem-resistant *K. pneumoniae* to colistin and tigecycline were 58.3% and 50.0%, respectively.

The clinical conditions associated with the acquisition of ertapenem-resistant *Enterobacteriaceae* were treatment with antibiotics (93.4%), intensive care stay (60.5%), ventilator use (60.5%), and previous stay in a nursing home (26.3%). A comparison of 30-day mortality among the different groups of 76 patients who acquired ertapenem-resistant *Enterobacteriaceae* is shown in Table 5. The 30-day mortality rate of all the 76 patients was 40.8% (31/76). The 7-, 14-, 30-, and 90-day mortality rates of 33 patients in the observation group were 51.5%, 51.5%, 60.6%, 72.7%, respectively ($p = 0.247$). There was no significant difference in the 30-day mortality rate between the 15 patients treated with tigecycline (73.3%) and the other 18 patients treated with colistin, imipenem, or meropenem (73.3% vs. 50.0%; $p = 0.313$). Among 33 patients in the observation group, 20 patients had an abscess at the infection site. Among these 20 patients, 13 patients who underwent adequate drainage or surgical debridement had a higher survival rate than the other seven patients who did not (100% vs. 14.3%; $p < 0.001$). There was no significant difference in the 30-day mortality rate between the group with an imipenem MIC $\leq$ 1 μg/mL and the group with an imipenem MIC $\geq$ 4 μg/mL (40.9% vs. 35.0%; $p = 0.852$). The bacteriologic eradication rates of tigecycline, imipenem, and colistin on the 14th day of treatment were 66.7%, 50%, and 25%, respectively.

The dendrograms of PFGE fingerprinting of 16 *E. coli* isolates and 60 *K. pneumoniae* isolates are shown in Figs. 1 and 2, respectively. Nine clusters (A—I) of 60 ertapenem-resistant *K. pneumoniae* isolates were identified. Each of clusters A and B included six isolates. Each of clusters C and D included three isolates, and each of clusters E—I included two isolates. Two clusters (A and B) of ertapenem-resistant *E. coli* isolates were identified, each cluster containing two *E. coli* isolates.

Discussion

The prevalence rates of ertapenem-resistant *E. coli* and *K. pneumoniae* isolated at TCVGH (tested by the disk diffusion method; CLSI 2009)[18] from 2010 to 2011 were 0.52% and 2.97%, respectively. The prevalence rates of CRE were lower than those of ESBL-producing *Enterobacteriaceae*. However, we were concerned that the major source of CRE isolates was the gastrointestinal tract. Colonization of CRE of the gastrointestinal tract could be observed for a median of 87 days (range 60—450 days).[29] CRE can spread from infected patients or hospital staff to families and communities by household or social contact, in contrast to the multidrug-resistant healthcare-associated, glucose-nonfermentive pathogens harbored mainly in the

Table 4 Antibiotic susceptibility of ertapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates

Antibiotics	*Escherichia coli* ($n = 16$)			*Klebsiella pneumoniae* ($n = 60$)		
	MIC range (μg/mL)	MIC_{50} /MIC_{90} (μg/mL)	Susceptibility (%)	MIC range (μg/mL)	MIC_{50} /MIC_{90} (μg/mL)	Susceptibility (%)
Amikacin	0.25—8	2/8	100	0.25 to >256	>256/>256	40.0
Aztreonam	2—256	32/256	12.5	1—256	32/256	6.7
Cefepime	0.5—128	16/128	43.8	1 to > 256	128/> 256	16.7
Ceftazidime	64—256	256/256	0	16—256	256/256	0
Colistin	1—16	2/4	62.5[a]	1—64	2/4	58.3[a]
Ertapenem	0.25—64	8/32	6.3	0.5—256	16/128	0
Imipenem	0.25—16	2/4	50.0	0.25—256	4/16	23.3
Tigecycline	0.5—4	1/2	75.0[a]	0.5—8	1/4	50.0[a]

[a] According to the breakpoints recommended by EUCAST, 2012.[20]
MIC = minimal inhibitory concentration.

Table 5 Comparison of 30-day mortality rates among different groups of 76 patients who acquired ertapenem-resistant *Enterobacteriaceae*

	Mortality (%)	*p*
Source of culture[c]		
Blood	8/13 (61.5)	0.173[a]
Sputum	4/10 (40.0)	1.000[b]
Urine	4/17 (23.5)	0.173[a]
Bile	2/12 (16.7)	0.108[b]
Others	13/24 (54.2)	0.173[a]
MIC of imipenem		
≤1 μg/mL	9/22 (40.9)	0.852[a,d]
2 μg/mL	8/14 (57.1)	
≥ 4 μg/mL	14/40 (35.0)	
Comparison among observation group[e]		
Colistin	2/4 (50.0)	1.000[b]
Tigecycline	11/15 (73.3)	0.313[a]
Imipenem	6/11 (54.5)	0.714[b]
Meropenem	1/3 (33.3)	0.547[b]

[a] Yates' correction of continuity.
[b] Fisher's exact test.
[c] The 30-day mortality rates of infections from each single source were compared with those from all the other sources.
[d] The difference in the 30-day mortality rates between the two groups, imipenem MIC ≤ 1 μg/mL and ≥ 4 μg/mL.
[e] The 30-day mortality rate of infections treated with each antibiotic of the observation group was compared with those treated by all the other antibiotics of the observation group.
MIC = minimal inhibitory concentration.

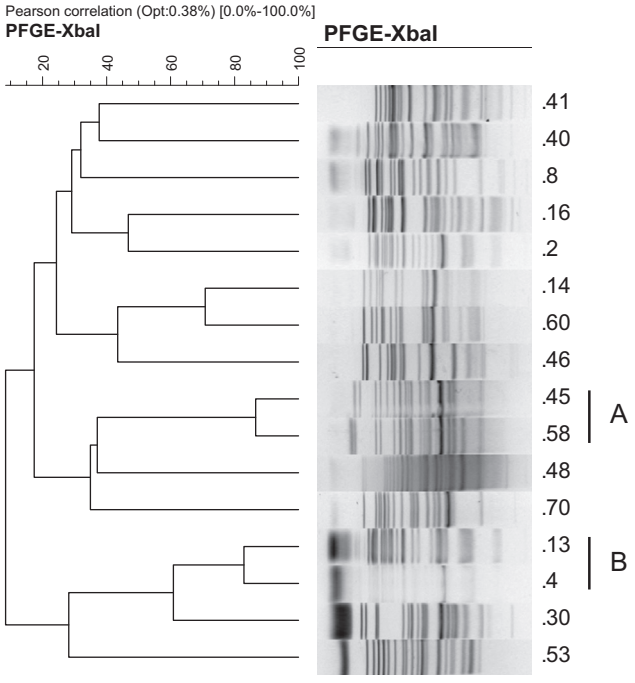

Figure 1. A dendrogram of pulsed-field gel electrophoresis (PFGE) fingerprinting of 16 *Escherichia coli* isolates after digestion with XbaI.

hospital environments. The example of ESBL-producing *Enterobacteriaceae* in the past 10 years indicates that CRE may rapidly reach a high rate worldwide[6] and result in a crisis of treatment failure.

The zone diameters of 12 isolates (5 *E. coli* and 7 *K. pneumoniae*) were interpreted as intermediately resistant to ertapenem by the microbiology laboratory at TCVGH according to breakpoints (susceptible, ≥19 mm; intermediately resistant, 16–18 mm; resistant, ≤15 mm) recommended by the CLSI (2009).[18] However, ertapenem MICs for all these 12 isolates were ≥ 1 μg/m (range 1–32 μg/mL) and were read as resistant according to breakpoints recommended by the CLSI (2012).[21] If the breakpoints of disk diffusion were updated according to the 2012 CLSI recommendations[21] (susceptible, ≥ 23 mm; intermediately resistant, 20–22 mm; resistant, ≤ 19 mm), the results of disk diffusion tests for these isolates would be read as resistant. Therefore, the susceptibility measurements would be consistent for the disk diffusion test and broth microdilution method. The ertapenem MICs for two additional isolates were 0.25 and 0.5 μg/mL, respectively, and were interpreted as susceptible according to breakpoints recommended by the CLSI (2012).[21] The beta-lactamase gene(s), MHT, and treatment outcomes for these two isolates were: CMY-2, positive MHT, treatment failure for the first one; and SHV-5, CTX-M-G9, and ACC, negative MHT, treatment failure for the second one.

Due to the complexity of the resistance mechanisms, CRE may exhibit a wide range of MICs for carbapenem, including some with only low levels of carbapenem resistance.[3] Intermediate susceptibility and even susceptibility to carbapenems have been observed for producers of all types of carbapenemase.[30] There is no consensus on the cut-off value of MICs of carbapenems that should be applied for research into carbapenemase activity.[30] The criteria for ertapenem resistance had not been clearly established by microbiologic (mechanisms and breakpoints) and clinical data, so these two isolates were included in this study.

The most prevalent genes were bla_{CMY-2} in 93.8% of 16 *E. coli* isolates, and bla_{SHV-5} in 75% of 60 *K. pneumoniae* isolates. Most isolates harbored combination genes, such as bla_{SHV-5} and bla_{DHA} in 45% of 60 *K. pneumoniae* isolates, and bla_{SHV-5} and $bla_{CTx-M-G9}$ in 26.7% of 60 *K. pneumoniae* isolates in this study. The *bla* genes associated with carbapenem resistance are complex. The *bla* genes of 16 bla_{KPC-2}-producing *K. pneumoniae* strains that have spread worldwide were analyzed.[8] These harbored a natural chromosome-encoded *bla* gene [bla_{SHV-1} ([12.5%), bla_{SHV-11} (68.7%), or $bla_{OKP-A/B}$ (18.8%)] and several acquired and plasmid-encoded genes [bla_{TEM-1} (81.3%), $bla_{CTx-M-2}$ (31.3%), $bla_{CTx-M-12}$ (12.5%), $bla_{CTx-M-15}$ (18.7%), and bla_{OxA-9} (37.5%)]. The bla_{KPC-2} gene was always associated with one of the Tn*4401* isoforms (a, b, or c). The complexity of beta-lactamase genes harbored in carbapenem-resistant isolates makes the phenotype-based identification of carbapenem resistance more difficult.

One *K. pneumoniae* isolate that carried the bla_{KPC-2} gene was classified as ST11. It was isolated from a patient who had been hospitalized in China. The dominant clone (ST11) of KPC-producing *K. pneumoniae* isolates was identified as originating from Zhejiang province (4 hospitals in Hangzhou

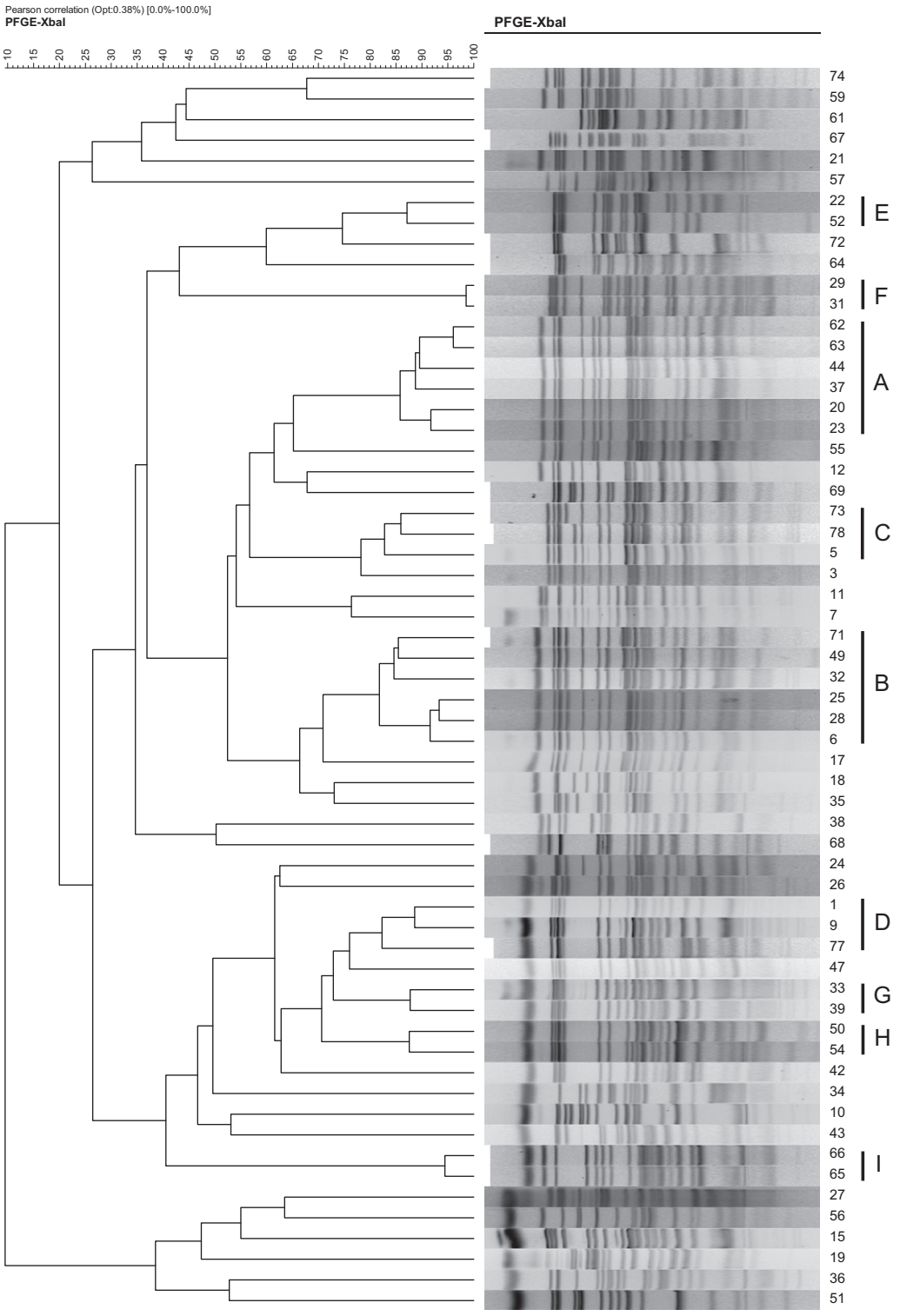

Figure 2. A dendrogram of pulsed-field gel electrophoresis (PFGE) fingerprinting of 60 *Klebsiella pneumoniae* isolates after digestion with XbaI.

and 1 hospital in Ningbo), Jiangsu province (1 hospital in Nanjing) and Anhui province (1 hospital in Hefei) in China.[11] Early identification of the KPC-producing *K. pneumoniae* isolates from patients linked epidemiologically with these endemic hospitals in Chinese cities is mandatory to control the spread.

Mechanisms of carbapenem resistance include carbapenemase production,[7–10] combination of AmpC-hyperproduction and/or extended spectrum beta-lactamase (ESBL)-production, and porin mutation.[3–6] *Enterobacteriaceae* with ESBL production and porin mutation have been identified in Taiwan.[13,17] According to the

report from the microbiology laboratory at TCVGH from 2010 to 2011, 12 (26%) of 46 ertapenem-resistant *E. coli* isolates had a positive confirmatory test for ESBL; 34 (20%) of 173 ertapenem-resistant *K. pneumoniae* isolates had a positive confirmatory test for ESBL.

To increase the possibility of identifying the carbapenemase, ertapenem-resistant *Enterobacteriaceae* with a positive confirmatory test for ESBL were not included in this study. The selection of *Enterobacteriaceae* with a positive screening test and negative confirmatory test for ESBL did not indicate that *Enterobacteriaceae* with ESBL genes and porin mutation were excluded. In this study, 56.7% of 60 *K. pneumoniae* isolates with a positive screening test and negative confirmatory test for ESBL harbored ESBL genes as well as *ampC* genes. Similarly, results from the SENTRY Asia-Pacific Surveillance Program showed that 57% of 51 *K. pneumoniae* with a positive screening test and negative confirmatory test for ESBL harbored a combination of ESBL and plasmid-borne AmpC enzyme of the CIT or DHA type.[31] For the *Enterobacteriaceae* isolates carrying both the ESBL and AmpC enzymes, the phenotype appeared to be a positive screening test and a negative confirmatory test. The AmpC enzyme can hydrolyze clavulanic acid, and thus make the confirmatory test negative.[32]

Among the 15 isolates with a positive MHT result, only one isolate harbored a carbapenemase gene, the bla_{KPC-2} gene. One isolate was negative on PCR. The other 13 isolates harbored the bla_{ESBL} gene(s), bla_{AmpC} gene(s), or both types of gene. It is important to be aware of false-positive results when interpreting the MHT.[33,34] The sensitivity and specificity of the MHT for detecting carbapenemase were 77.4% and 38.9% in 54 carbapenemase and/or ESBL/AmpC-producing *Enterobacteriaceae* isolates, respectively, in one previous study.[33] False-positive MHT results could result from the production of ESBL or ampC.[33,34] Five isolates were found to contain the bla_{OxA} gene. Since OXA-48 was first detected in *K. pneumoniae* isolates in Turkey in 2003, OXA-48-producing *Enterobacteriaceae* have spread worldwide.[9,12] *Acinetobacter baumannii* isolates with OXA-23, OXA-24, OXA-51, and OXA-58 have been reported in Taiwan.[35] However, *Enterobacteriaceae* with a bla_{OxA} gene have never been reported in Taiwan. Therefore, sequencing of the bla_{OxA} gene is needed to identify the types of bla_{OxA} gene present.

The susceptibility rates of 60 ertapenem-resistant *K. pneumoniae* to colistin and tigecycline were 58.3% and 50.0%, respectively, in this study. The susceptibility patterns of CRE to colistin and tigecycline varied in different strains.[36] Susceptibility tests on 81 CRE isolates from the UK were performed using the agar dilution method recommended by the CLSI.[36] Chloramphenicol, ciprofloxacin, and nitrofurantoin inhibited less than 25% of isolates, whereas colistin was active against 75/81 isolates (92.6%), and tigecycline was active against 38/81 isolates (46.9%).[36] The MICs of ertapenem and imipenem against *K. pneumoniae* isolates showed a range from 0.5 to 256 μg/mL and 0.25 to 256 μg/mL, respectively, in this study. The range of carbapenem MIC for 49 CRE strains from China with different resistance mechanisms has been reported.[37] Moderate- to high-level carbapenem resistance in most isolates is more closely associated with a loss or decreased expression of both major porins combined with the production of AmpC or ESBL, while KPC-2, IMP-4, and IMP-8 carbapenemase production may lead to a low to moderate level of carbapenem resistance in *Enterobacteriaceae* in China.[37]

There was no significant difference between the mortality rates of the group who acquired CRE susceptible to imipenem and those who acquired CRE resistant to imipenem ($p = 0.852$). Patients in the observation group showed poor survival. In contrast, removal of the focus of infection (by debridement or drainage of the abscess) was associated with good survival. In a recent case-control study on the outcomes of carbapenem-resistant *K. pneumoniae* infection, removal of the focus of infection focus by an adjunctive procedure (i.e., debridement, drainage, or removal of the catheter) was associated with patient survival [odds ratio (OR) 0.14, 95% confidence interval (CI) 0.04–0.49; $p = 0.002$].[38] The administration of antibiotics with *in vitro* activity against carbapenem-resistant *K. pneumoniae* was not associated with patient survival (OR 2.3, 95% CI 0.73–7.24; $p = 0.15$).[38] Although the patients treated with tigecycline had higher bacteriologic eradication rates, there was no significant difference in the 30-day mortality rate between these patients and the other patients in the observation group. The causes of mortality could be attributed to inadequate debridement or drainage, severity, and patient co-morbidity. If patients were stratified according to infection site, severity, and co-morbidity, the number in each group would be too small to provide data of adequate sample size for the statistical analysis in this study. However, this study focused on the overview of clinical outcome rather than an analysis of risk factors for mortality.

The PFGE patterns of 60 *K. pneumoniae* isolates were relatively diverse, since some strains could have originated from different ancestors. This indicated that the *Enterobacteriaceae* could acquire a resistance gene from horizontal transfer of mobile genetic elements carrying a resistance gene (such as a plasmid or transposon). The fact that several clusters of ertapenem-resistant *Enterobacteriaceae* isolates were identified indicated that they had spread in the hospital via contacts. Appropriate strategies should be implemented to limit the spread of these pathogens, including active surveillance of patients with epidemiologic links to persons from whom CRE have been recovered, especially for high-risk groups (those with prior antibiotic treatment, an intensive care stay, ventilator use, or a previous stay in a nursing home). However, the cost-effectiveness of active surveillance remains to be explored.

In conclusion, two carbapenemase genes, bla_{KPC-2} and bla_{OxA}, were identified in carbapenem-resistant *K. pneumoniae* isolates. Other carbapenemase genes such as bla_{GES}, bla_{IMP}, and bla_{VIM} were not detected. The genetic combinations bla_{SHV-5}–bla_{DHA} and bla_{SHV-5}–$bla_{CTx-M-G9}$ were prevalent in *K. pneumoniae* isolates. The mortality rates of patient-acquired ertapenem-resistant *Enterobacteriaceae* infection were high. Removal of the focus of infection (by debridement or drainage of the abscess) was associated with good survival. The findings of carbapenemase genes in a few isolates and small clusters of CRE indicated emerging problems at the hospital. Appropriate strategies should be implemented to control the spread of CRE.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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