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ORIGINAL ARTICLE

Laboratory identification, risk factors, and clinical outcomes of patients with bacteremia due to *Escherichia coli* and *Klebsiella pneumoniae* Producing Extended-Spectrum and AmpC type β-Lactamases

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KEYWORDS
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K pneumoniae

Background: Extended-spectrum β-lactamase (ESBL)-producing bacteria coexpressing AmpC type β-lactamase (ACBL) are associated with the laboratory issue of false susceptibility to third-generation cephalosporins. This study was to evaluate laboratory tests and clinical significance of bacteremic isolates of *Escherichia coli* and *Klebsiella pneumoniae* with both ESBL and ACBL [dual-type lactamases (DTL)].
Methods: From 2006 to 2009, 78 *E coli* and 12 *pneumoniae* bacteremic isolates with reduced susceptibility to cefotaxime (CTX) or ceftazidime (CAZ) were identified and relevant patients' data were collected for analysis. Phenotypic and genotypic characterizations of these selected isolates were determined by inhibitor-based assays and polymerase chain reaction–based genetic analyses, respectively.
Results: Among the 90 isolates, 47 had DTL production. There was an increasing annual prevalence from 29% in 2006 to 56% in 2009 ($p = 0.02$). Phenotypic assays had a sensitivity and specificity of 57% (43/76) and 93% (13/14) for ESBL detection and 95% (58/61) and 34% (10/29) for ACBL, respectively. Among the DTL-producing isolates, phenotypic assays yielded a higher false negative rate of ESBL detection than that of ACBL detection (70% versus 6%), while all false negative ESBL results were associated with ESBL genes other than $bla_{CTX\text{-}M}$.

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The majority of the DTL-producing isolates were in the category of resistance to CTX (47/47, 100%) and CAZ (44/47, 94%) by the Clinical and Laboratory Standards Institute (CLSI) 2010 interpretive criteria, of which many were considered intermediate or fully susceptible to CTX (25/47, 53%) and CAZ (15/47, 32%) by the previous ones (CLSI-2009). The DTL-producing isolates exhibited a lower susceptibility rate to fluoroquinolones, aztreonam, and β-lactam/lactamase inhibitors than those with either ESBL or ACBL alone. The use of indwelling catheters or nasogastric tubes was associated with bacteremia due to the DTL isolates, but the mortality rates were not different among those due to isolates with ESBL, ACBL, or both. By multivariate analysis, Pittsburg bacteremia score and Charlson comorbidity index were the significant predictors for all-cause mortalities.

Conclusion: Bacteremic episodes due to DTL-producing *E coli* and *K pneumoniae* became increasingly prevalent and were often associated with coresistance to antibiotics other than β-lactams, but they were not associated with a worse prognosis than those due to ESBL- or ACBL-producing bacteria.

Introduction

Extended-spectrum β-lactamase (ESBL) and AmpC type β-lactamase (ACBL) are class A and class B β-lactamases, respectively, both conferring resistance to third-generation cephalosporins (3GC-R) in *Escherichia coli* and *Klebsiella pneumoniae*.[1] These strains normally colonize and spread among hospitalized patients, complicating the treatment of nosocomial infections. However, community-acquired ESBL producing—*E coli*, especially of CTX-M-type ESBL, has now become a growing problem worldwide and has changed the epidemiology of infections related to antibiotic-resistant bacteria.[2,3,4,5] Similarly, plasmid-mediated ACBL-producing bacteria also serve as community-based reservoirs for 3GC-R strains.[3,6] Effective treatments for infections caused by ESBL-producing *E coli* or *K pneumoniae* are limited to only a few antibiotics, including carbapenems and non—β-lactam antibiotics.[7] Antibiotics for ACBL-producing bacteria, on the other hand, are chosen based on the susceptibility testing results because there are no established guidelines for ACBL identification; thus, most laboratories do not routinely test for the presence of ACBL.

Laboratory confirmation of bacteria with ESBL activity relies on the evidence of increased susceptibility to extended-spectrum cephalosporins with an aid of class A β-lactamase inhibitor, clavulanic acid. The inhibitor-based assay is subjected to negative interference by concurrent expression of other β-lactamases, including ACBL, because different β-lactamases have variable susceptibility to clavulanic acid.[8] Since the coexistence of ESBL and ACBL is not uncommon among *Enterobacteriaceae*, infections by ESBL-producing bacteria are potentially underdiagnosed and are thus inappropriately treated.[9,10,11] Moreover, clinical significance of infections by bacteria with dual types of β-lactamases (DTL) was less addressed in the literature than that by ESBL-producing bacteria. In this study we first evaluated the efficacy of two inhibitor-based tests for the prediction of ESBL or ACBL production in bacteremic *E coli* and *K pneumoniae* isolates with reduced susceptibility to 3-GC. Subsequently, the derived data were combined with clinical information to elucidate risk factors and clinical outcomes associated with different types of β-lactamases.

Material and methods

Study population and definitions

Cathay General Hospital, an 800-bed tertiary care teaching hospital in northern Taiwan, has an average of 27,000 admissions per year. From January 2006 through December 2009, all *E coli* and *K pneumoniae* isolates obtained from blood cultures with reduced susceptibility to cefotaxime (CTX) or ceftazidime (CAZ) were selected for this study. Bacteria isolates from a patient in less than 1 month apart were considered to be of the same isolate, and only the first isolate was included for analysis unless they had different antibiograms. Clinical information, including demographic data, concurrent infection foci, comorbidity, severity of sepsis at presentation, antimicrobial therapy, and 7-, 14- and 30-day mortality rates were obtained from medical records. The study was approved by the internal review board of Cathay General Hospital.

Phenotypic assays for ESBL and ACBL, and antibiotic susceptibility determination

The strains preserved at −80 °C were recovered by resuspension of frozen stocks in brain-heart infusion broth and plated on a 5% sheep blood agar, which were then incubated for 24 hours before tests. Phenotypic methods to detect ESBL or ACBL were inhibitor-based assays. The presence of ESBL activity was indicated by the Clinical and Laboratory Standards Institute (CLSI) disc diffusion tests, in which a ≥5-mm increase in a zone diameter for either CTX or CAZ in combination with ESBL inhibitor clavulanic acid (CLA) was demonstrated to compare with either drug alone

(CLSI M100-S20).[12] ACBL activity was evaluated by the double-disc synergy test (DDST) using an AmpC β-lactamase inhibitor, 3-aminophenylboronic acid (APBA).[13] The isolates with ACBL-producing phenotype demonstrated a dumbbell-like diffusion zone extending from either CTX or CAZ disc toward an APBA-impregnated disc. Susceptibility to CTX and CAZ was evaluated by the disc diffusion method, whereas susceptibility to other antibiotics was determined by the automatic Phoenix ID/AST instrument (Becton Dickinson, Sparks, MD, USA).

Molecular characterization of ESBL and ACBL

Relevant β-lactamase genes(*bla*) were detected by PCRs and gene specific primers. Primers for $bla_{TEM}bla_{SHV}$ and $bla_{CTx\text{-}M}$ families were used for the detection of ESBL genes as described,[14,15] and multiplex PCR was applied for plasmid-borne AmpC genes (bla_{MOx}, bla_{CIT}, bla_{DHA}, bla_{ACC}, bla_{EBC}, bla_{FOx}).[16] Other lactamases known to confer CTX/CAZ resistance, such as class A carbapenemase (bla_{KPC}, bla_{PER}) and class B metallo-β-lactamases (bla_{VIM}, bla_{IMP}, bla_{SPM}), were subjected to genetic analysis as described.[17,18,19]

Statistical analysis

Data were collected and analyzed using Windows Office Excel and SPSS software version 17 (SPSS Inc., Chicago, IL USA). Changes in incidence over time were analyzed by a Chi-square test for trend. Correlations between nominal variables were assessed for significance by the Pearson Chi-squared test or Fisher's exact test. Multiple logistic regression analysis was conducted to determine the independent risk factors for mortality. A p value of 0.05 or less was considered statistically significant.

Results

From January 2005 to December 2009, 90 episodes of bacteremia caused by CTX and/or CAZ nonsusceptible *E coli* (78; 85%) and *K pneumoniae* (12; 15%) were identified. There was no bacteremia caused by CTX/CAZ-resistant *K oxytoca* or *Proteus mirabilis* during the study period. Forty-four and 77 isolates demonstrated ESBL- or ACBL-producing phenotypes, respectively, including 31 isolates manifesting DTL-phenotypes.

Molecular characterization of the β-lactamases revealed different results from those of the phenotypic assays. ESBL and ACBL genes were found in 76 and 61 isolates, respectively, of which 47 had both ESBL and ACBL genes. The major type of ESBL genes was bla_{TEM} (62), followed by $bla_{CTx\text{-}M}$ (35) and bla_{SHV} (14). One-third (28; 37%) of the 76 isolates with ESBL genes contained more than one type of ESBL gene. Of note, seven isolates with all three types of ESBL genes were *K pneumoniae*. The major ACBL genes found were bla_{CIT} (54, 89%), followed by bla_{DHA} (7, 11%).

Among the isolates with DTL genes, more negative results by the phenotypic assay for the ESBL activity (33/47; 70%) were observed than those for the ACBL activity (3/47; 6%) (Table 1). Subsequent analysis revealed the absence of ESBL-producing phenotype was only associated with ESBL genes other than $bla_{CTx\text{-}M}$. On the other hand, almost all cases (18/19; 95%) of false-positive ACBL-producing phenotype were observed in ESBL-producing isolates with $bla_{CTx\text{-}M}$, suggesting some variants of $bla_{CTx\text{-}M}$ were susceptible to class A and class C β-lactamase inhibitors. Overall, the phenotypic assays for ESBL or ACBL activities had a sensitivity and specificity of 57% (43/76) and 93% (13/14), respectively, for the detection of isolates with genes encoding ESBL and 95% (58/61) and 34% (10/29) for those with genes encoding ACBL.

Table 1 Phenotype-genotype correlation among isolates with ESBL, ACBL, or both lactamases (DTL)

Phenotype	Genotype (%)		
	ESBL, $n = 29$	ACBL, $n = 14$	DTL, $n = 47$
ESBL	10 (34.5)	0	3[a] (6.4)
ACBL	0	13 (92.9)	33[b] (70.2)
DTL	19[c] (65.5)	1[d] (7.1)	11 (23.4)

[a] false-negative ACBL phenotype.
[b] false-negative ESBL phenotype.
[c] false-positive ACBL phenotype.
[d] false-positive ESBL phenotype.
ACBL = AmpC type β-lactamase; DTL = dual-type lactamases; ESBL = extended-spectrum β-lactamase.

There was an increasing isolation rate over time of DTL-producing bacteria among CTX- or CAZ-nonsusceptible isolates, from 29% in 2006 to 56% in 2009 [$p = 0.02$, Chi-square for trend analysis (Fig. 1)]. These bacteria had higher rates of coresistance to many antibiotics, such as fluoroquinolones, monobactam, and β-lactam /β-lactamase inhibitors (Fig. 2). More importantly, a higher rate of resistance to piperacillin-tazobactam was seen in DTL-producing isolates than ESBL- or ACBL-producing isolates. Eighty-eight of the 90 isolates were susceptible to carbapenems. Neither class A carbapenems (bla_{KPC}, bla_{PER}) nor class B metallo-β-lactamases (bla_{VIM}, bla_{IMP}, bla_{SPM}) were identified in the carbapenem nonsusceptible isolates, suggesting other mechanisms, including porin deficiency, might mediate the carbapenem resistance. Finally, nearly all DTL-producing bacteria were classified in the category of resistance to CTX (47/47; 100%) and CAZ (44/47; 94%) by the CLSI-2010 susceptibility breakpoints, although many of them were intermediate or fully susceptible to CTX (25/47; 53%) and CAZ (15/47; 32%) if interpreted based on previous (CLSI-2009) criteria (Table 2).

Univariate analysis of clinical features among cases of bacteremia due to isolates with different β-lactamases revealed an association of bacteremia due to DTL-producing bacteria with indwelling catheters and endotracheal/nasogastric tubes [$p < 0.01$ and 0.03, respectively (Table 3)]. However, clinical outcomes, as assessed by crude mortality rates at 7, 14, and 30 days, did not differ significantly among ESBL-, ACBL- and DTL-producing bacteremia. After adjusted for age, types of infection, bacterial species, and acute bacteremia or comorbidity indices, mortality rates remained similar among the three groups (Table 4). However, a higher Pittsburgh bacteremia score, which was not associated with a specific type of β-lactamases, was a significant predictor for mortalities at 7, 14, and 30 days. In addition, Charlson comorbidity index was associated only with 30-days morbidity.

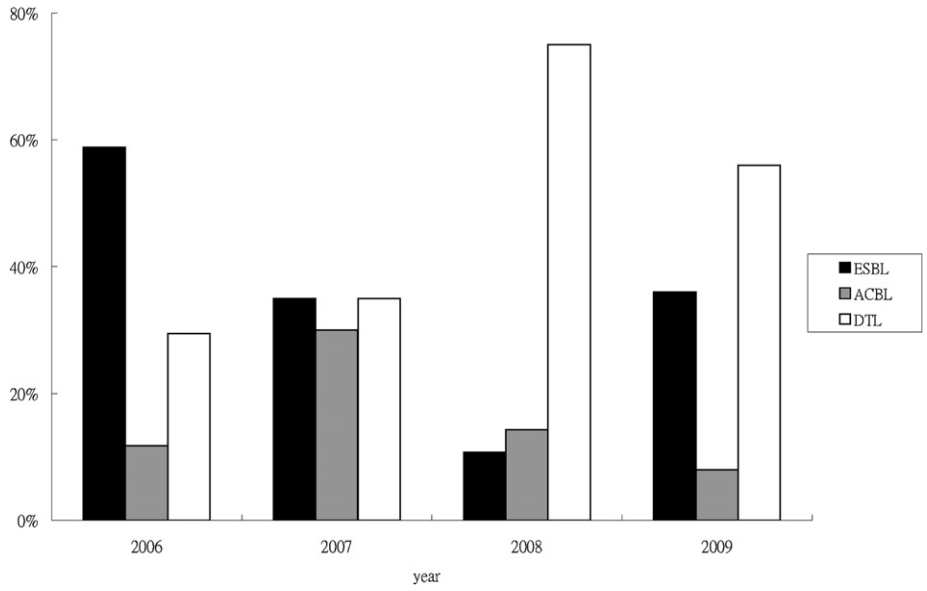

Figure 1. Annual distribution of ESBL-, ACBL-, and DTL-producing *Escherichia coli* and *Klebsiella pneumoniae* causing bacteremia at a tertiary care hospital in north Taiwan from 2006 to 2009. ACBL = AmpC type β-lactamase; DTL = dual-type lactamases; ESBL = extended-spectrum β-lactamase.

Discussion

In this retrospective study of bacteremia caused by *K pneumoniae* or *E coli* with reduced susceptibility to extended-spectrum cephalosporins, more than one-half of the episodes (47/90) were caused by DTL-producing bacteria. DTL-producing isolates were associated with higher rates of coresistance to fluoroquinolones, monobactam, and piperacillin-tazobactam than ESBL-or ACBL-producing isolates. Patients acquiring DTL-producing bacteremia more frequently had indwelling catheters, endotracheal, or nasogastric tubes, but the mortality rate was comparable with that caused by either ESBL- or ACBL-producing isolates, suggesting coexistence of ESBL and ACBL in bacteria did not pose additional risks for mortality as compared with either type of β-lactamases alone.

The phenotypic assay for ESBLs is known to be interfered by ACBL,[20,21] as the latter is not inhibited by CLA. Thus, failure to demonstrate ESBL phenotype in DTL-producing bacteria may occur if the hydrolytic activity of ACBL

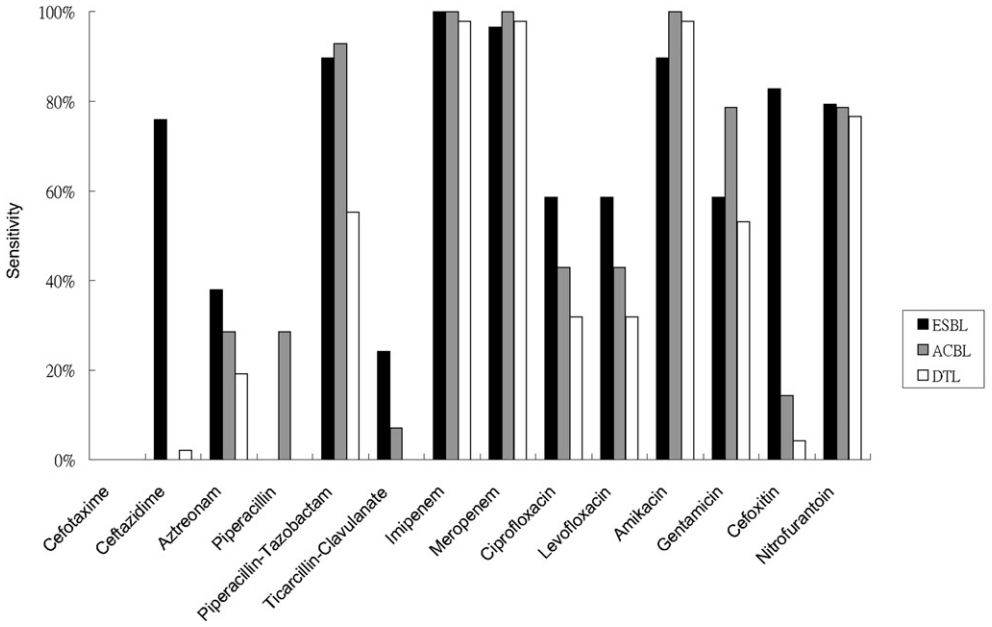

Figure 2. Antimicrobial susceptibilities of bacteremic isolates of ESBL-, ACBL-, and DTL-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care hospital in north Taiwan from 2006 to 2009. ACBL = AmpC type β-lactamase; DTL = dual-type lactamases; ESBL = extended-spectrum β-lactamase.

Table 2 Third-generation cephalosporin susceptibilities of 90 isolates with reduced susceptibility to cefotaxime and/or ceftazidime and producing ESBL, ACBL or both lactamases (DTL), as defined by 2009 and 2010 CLSI interpretive breakpoints

Antibiotic		Isolate number (%)											
		ESBL, *n* = *29*				ACBL, *n* = *14*				DTL, *n* = *47*			
		CLSI-2010		CLSI-2009		CLSI-2010		CLSI-2009		CLSI-2010		CLSI-2009	
Cefotaxime	S	0	(0)	2	(6.9)	0	(0)	2	(14.3)	0	(0)	1	(2.1)
	I	0	(0)	2	(6.9)	0	(0)	9	(64.3)	0	(0)	24	(51.1)
	R	29	(100)	25	(86.2)	14	(100)	3	(21.4)	47	(100)	22	(46.8)
Ceftazidime	S	22	(75.9)	24	(82.8)	0	(0)	1	(7.1)	1	(2.1)	3	(6.4)
	I	2	(6.9)	1	(3.4)	1	(7.1)	5	(35.7)	2	(4.3)	12	(25.5)
	R	5	(17.2)	4	(13.8)	13	(92.9)	8	(57.1)	44	(93.6)	32	(68.1)

ACBL = AmpC type β-lactamase; CLSI = Clinical and Laboratory Standards Institute; DTL = dual-type lactamases; ESBL = extended-spectrum β-lactamase.

exceeds the level of CLA-mediated inhibition of ESBL activity. In our study the interference only occurred when ESBL genes belonged to families other than the $bla_{CTX\text{-}M}$ type. The lack of interference by ACBL was in part due to unequal hydrolytic activities toward different 3-GCs between CTX-M_{ESBL} and ACBL. Since CTX-M_{ESBL} preferentially hydrolyze cefotaxime, CLA inhibitory effect is adequate to warrant ESBL confirmation despite the coexistence of ACBL activity. On the other hand, susceptibility to ceftazidime in these isolates followed the patterns of ACBL-producing isolates with minimal inhibition by CLA, indicating that ceftazidime is a poorer substrate for CTX-M_{ESBL} than for ACBL. Interestingly, almost all (18/19; 95%) ESBL-producing isolates concurrently exhibiting ACBL phenotype had $bla_{CTX\text{-}M}$, in which ACBL activity was demonstrated only with ceftazidime but not with cefotaxime. Whether the substrate-specific effect is due to CTX-M variants or is caused by occult ACBLs remains to be known.

Notwithstanding comparable clinical outcomes, coresistance to other antibiotics, such as fluoroquinolone, monobactam, and β-lactam/lactamase inhibitors, was more frequently observed in DTL-producing isolates than in

Table 3 Demographics, predisposing factors, and clinical outcomes of patients acquiring bacteremia due to *Escherichia coli* and *Klebsiella pneumoniae* with ESBL, ACBL, or both lactamases (DTL)

Variables	ESBL (*n* = *29*)		ACBL (*n* = *14*)		DTL (*n* = *47*)		*p* values
Sex, male	11	(37.9)	8	(57.1)	24	(51.1)	0.4
Age (yr) >65	18	(62.1)	10	(71.4)	29	(61.7)	0.79
Diabetes	12	(41.4)	4	(28.6)	16	(34)	0.68
Chronic pulmonary disease	2	(6.9)	1	(7.1)	7	(14.9)	0.49
Heart failure	5	(17.2)	0	(0)	7	(14.9)	0.27
Renal insufficiency	5	(17.2)	2	(14.3)	9	(19.1)	0.72
Malignancy	15	(51.7)	4	(28.6)	18	(38.3)	0.30
Use of immunosuppressive drugs	5	(17.2)	0	(0)	3	(6.4)	0.12
Pittsburg bacteremia score >2	10	(34.5)	6	(42.9)	21	(44.7)	0.67
Charlson co-morbidity index >2	19	(65.5)	7	(50)	33	(70.2)	0.38
Severe sepsis or septic shock	16	(44.8)	8	(57.1)	28	(59.6)	0.93
Mode of infection							0.29
Hospital-acquired	10	(34.5)	4	(28.6)	23	(48.9)	
Healthcare-associated	6	(20.7)	3	(21.4)	13	(27.7)	
Community-acquired	13	(44.8)	7	(50)	11	(23.4)	
Clinical syndromes							0.30
Urinary tract infection	17	(58.6)	4	(28.6)	22	(46.8)	
Primary bloodstream infection	5	(15.6)	3	(21.4)	10	(21.3)	
Biliary/gastrointestinal tract infection	7	(28.1)	7	(50)	12	(25.5)	
Pneumonia	0	(0)	0	(0)	3	(6.4)	
Mortality within							
7 d	5	(17.2)	1	(7.1)	9	(19.1)	0.57
14 d	5	(17.2)	1	(7.1)	11	(23.4)	0.38
30 d	6	(20.7)	4	(28.6)	18	(38.3)	0.27

Note: Data are presented as case number (%).
ACBL = AmpC type β-lactamase; DTL = dual-type lactamases; ESBL = extended-spectrum β-lactamase.

Table 4 Risk factors for mortality at three time points in patients acquiring bacteremia due to *Escherichia coli* and *Klebsiella pneumoniae* with ESBL, ACBL, or both lactamases (DTL)

	Adjusted odds ratio (95% confidence interval)		
	7-d mortality	14-d mortality	30-d mortality
Age (yr), >65 vs ≤ 65	0.674 (0.124–3.672)	0.330 (0.066–1.651)	0.278 (0.070–1.102)
β-lactamase			
ESBL	Ref	Ref	Ref
ACBL	0.183 (0.012–2.748)	0.277 (0.020–3.767)	2.757 (0.404–18.821)
DTL	0.529 (0.106–2.626)	0.972 (0.230–4.111)	2.298 (0.601–8.779)
Source of bacteremia			
No source	Ref	Ref	Ref
Urinary tract	0.253 (0.036–1.766)	0.650 (0.119–3.535)	0.555 (0.131–2.343)
Biliary/gastrointestinal tract	0.790 (0.148–4.210)	0.757 (0.144–3.986)	0.476 (0.104–2.181)
Lung	14.921 (0.227–979)	12.995 (0.279–604)	∞
K pneumoniae vs *E coli*	0.630 (0.069–5.727)	0.585 (0.065–5.281)	3.684 (0.646–21.013)
Pitt bacteremia score >2 vs ≤ 2	14.557 (2.623–80.792)*	11.781 (2.659–52.198)*	9.806 (2.895–33.218)*
Charlson index >2 vs ≤ 2	4.274 (0.569–32.090)	5.273 (0.805–34.535)	7.380 (1.506–36.171)*

*$p < 0.05$.
∞ = All pneumonia cases were fatal.
ACBL = AmpC type β-lactamase; DTL = dual-type lactamases; ESBL = extended-spectrum β-lactamase.

either ESBL- or ACBL-producing bacteria. Profiles of aminoglycoside susceptibility, on the other hand, were similar among three types of isolates. Our results are in line with the observation by Lee and colleagues,[22] in which higher MIC_{50} and MIC_{90} of carbapenems, ciprofloxacin, and piperacillin-tazobactam were demonstrated in DTL-producing *K pneumoniae* than in ESBL-producing *K pneumoniae*. Importantly, the authors found patients exposed to either aminoglycosides or piperacillin-tazobactam were at lower risk for ESBL-producing *K pneumoniae* bacteremia, whereas exposure to aminoglycoside but not piperacillin-tazobactam was associated with a lower risk for DTL-producing *K pneumoniae* bacteremia. Thus, the risk of acquiring DTL-producing bacteremia may be determined by differences in the profiles of antibiotic coresistance among 3-GC resistant bacteria. For example, piperacillin-tazobactam, to which most ESBL- and ACBL-producing isolates remained susceptible, lost activity toward many DTL-producing isolates in our study. Therefore, use of piperacillin-tazobactam may pose selective pressure against ESBL- and ACBL-producing isolates, allowing DTL-producing bacteria to become the dominant colonizers among residential microbiota. Further, large-scale analyses of antibiotic use in temporal relation to the evolving epidemiology of DTL-producing bacteria among 3-GC–resistant *Enterobacteriaceae* would provide deep insight to this emerging issue.

In summary, our study revealed DTL-producing isolates were increasingly prevalent among CAZ- or CTX- nonsusceptible *E coli* and *K pneumoniae*, and, not uncommonly, had a false-negative ESBL phenotype, according to current CLSI guidelines for ESBL confirmation. The new CLSI susceptibility breakpoints (CLSI-2010) for third-generation cephalosporins were shown to be more sensitive than previous criteria (CLSI-2009) in detecting ESBL-, ACBL-, or DTL-producing *E coli* or *K pneumoniae*. However, since our study was conducted retrospectively without a standardized dosing schedule and sufficient patient numbers, further generalization of our clinical data to patients acquiring DTL-producing bacteremia is not possible. In addition, the use of genotypes as the surrogate for actual β-lactamase activity might underestimate case-mortality, as a reduction in the gene expression or enzyme activity would lead to an erroneous inflation of case numbers and an increase in the survival rate. Nevertheless, our data are in agreement with CLSI-2010 regulations by which routine ESBL testing is not necessary before reporting results of susceptibility to cephalosporins in *E coli* and *K pneumoniae* because the revised criteria are more inclusive for the detection of antimicrobial resistance mediated by plasmid-borne AmpC β-lactamase and/or ESBL. This has been shown in our study as well as others.[23,24]

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