Spreading of extended-spectrum β-lactamase-producing *Escherichia coli* ST131 and *Klebsiella pneumoniae* ST11 in patients with pneumonia: a molecular epidemiological study

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Abstract

Background: Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are the important pathogens causing pneumonia. This study aimed to investigate the clinical characteristics and molecular epidemiology of ESBL-producing *E. coli* and *K. pneumoniae* causing pneumonia at a large teaching hospital in China.

Methods: We collected patient's clinical data and ESBL-producing *E. coli* and *K. pneumoniae* strains causing pneumonia (from December 2015 to June 2016) at a hospital in Wuhan. The susceptibilities, multi-locus sequence typing, homologous analysis, ESBL genes by polymerase chain reaction and sequencing were determined.

Results: A total of 59 ESBL-producing strains (31 *E. coli* and 28 *K. pneumoniae*) isolated from patients with pneumonia were analyzed. The majority of strains were isolated from patients were with hospital-acquired pneumonia (37/59, 62.7%), followed by community-acquired pneumonia (13/59, 22.0%), and ventilator-related pneumonia (9/59, 15.3%). The *E. coli* ST131 (9 isolates, 29.0%) and *K. pneumoniae* ST11 (5 isolates, 17.9%) were the predominant sub-types. The most prevalent ESBL gene was *CTX-M-14*, followed by *SHV-77*, *CTX-M-3*, *SHV-11*, and *CTX-M-27*. At least 33 (55.9%) of the ESBL-producing strains carried two or more ESBL genes. The *ISEcp1* and *IS26* were found upstream of all blaCTX-M (CTX-Ms) and of most blaSHV (SHVs) (57.6%), respectively. Moreover, three ESBL-producing *K. pneumoniae* ST11 strains which were resistant to carbapenems carried the $bla_{\text{NDM-1}}$ and $bla_{\text{KPC-2}}$, two of which also bearing $bla_{\text{OXA-48}}$ were resistant to all antibiotics (including Tigecycline).

Conclusions: Hospital-acquired pneumonia is more likely correlated with ESBL-producing *E. coli* and *K. pneumoniae*. ESBL-producing *E. coli* ST131 and multi-drug resistance ESBL-producing, as well as New Delhi metallo-β-lactamase-1 (NDM-1) and Klebsiella pneumoniae carbapenemases-2 (KPC-2) bearing *K. pneumoniae* ST11 are spreading in patients with pneumonia in hospital.

Keywords: *Escherichia coli*; *Klebsiella pneumoniae*; β-lactamase; Carbapenem resistance; New Delhi metallo-β-lactamase

Introduction

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, especially *Escherichia coli (E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), which are the important pathogens causing pneumonia,[1-3] pose distinct clinical challenges.[4,5] The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* is high and varied from different geographical areas in China and type of infections.[6-9] It was reported that 67.7% of *E. coli* and 27.5% of *K. pneumoniae* isolated from patients in Shanghai with blood stream infections were found to be ESBL producers[9]; however, the percentage was 58.4% in *E. coli* and 43.0% in *K. pneumoniae* reported by a multi-center epidemiological study from other regions of China.[10] Of note, the prevalence of ESBL production in *E. coli* and *K. pneumonia* was caused by clonal transmission of predominant sub-types such as *E. coli* ST131[11-14] or *K. pneumonia* ST11.[15-18] So continuous surveillance on the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in different areas is of great significance. Therefore, the aim of this study was to investigate the clinical and molecular epidemiology characteristics of ESBL-producing *E. coli* and *K. pneumoniae* causing pneumonia at a large teaching hospital with 5000 beds in Hubei province, China.

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Methods

Ethical approval

The study was approved by Tongji Medical College Ethics Committee, Huazhong University of Science and Technology (2018-S356).

Clinical strains, anti-microbial susceptibility testing, and confirmation test of ESBL production

All ESBL-producing *E. coli* and *K. pneumoniae* strains causing pneumonia and the clinical data were collected from December 2015 to June 2016 at a teaching hospital in Hubei province. Community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), and ventilator-related pneumonia (VAP) were defined according to the guidelines.[19,20] Samples were inoculated using blood agar medium, and cultured at 37°C for 18 to 24 h. BD Phoenix™ 100 Automated Microbiology System (BD Ltd., Franklin Lakes, NJ, USA) was used to identify strains and anti-microbial susceptibility. Confirmation of ESBL production was performed by using double-disc synergy test. The modified Hodge test was applied to confirm carbapenemases-producing strains. All results were interpreted based on the Clinical and Laboratory Standards Institute guidelines.[21] The disks used for confirmation test were obtained from Beijing Tiantan Biological Products Corporation (China). *E. coli* (ATCC25922) and *K. pneumoniae* (ATCC700603) were used as quality control strains.

Microbiological studies

Clonal relationships were analyzed by multi-locus sequence typing (MLST) and enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR). Bacterial DNA was prepared according to the manufacturer instructions of bacteria DNA extraction kit (TIANamp Bacteria DNA kit, China). MLST was performed using the standard seven housekeeping loci for *E. coli* and *K. pneumoniae* according to protocols at http://bigsdb.pasteur.fr. The ERIC-PCR was performed as follows: PCR amplifications were performed in a volume of 25.0 μL of reaction mixtures containing 12.5 μL Go Taq (Dalian TaKaRa Corporation, China); 1.0 μL of 10 ρmol ERIC1R (5′-ATG TAA GCT CCT GGG GAT-3′) and 1.0 μL of 10 ρmol ERIC2 (5′-AAG TAA GTG ACT GGG GGT GAGC-3′)[22]; 1.0 μL of DNA template; 9.5 μL of PCR grade water for ERIC-PCR; the ERIC-PCR thermal cycler (Eastwin Scientific equipments limited, China) program for this method followed Wei *et al*[22] Amplified PCR products stained with ethidium bromide were separated by electrophoresis on 1.5% (w/v) agarose (1st base) at 100 V for 30 to 40 min, the molecular size of fragments generated by electrophoresis was determined by comparison to 2-kb DNA ladders (Dalian TaKaRa Corporation), and band patterns were captured under an ultraviolet illuminator. The GelCompar software package (version 7.6; Applied Maths, Bionumerics, Belgium) was used to compare the band patterns of aggregated data. The pattern analysis was calculated using the dice correlation coefficient at 1.0% band position tolerance and unweighted pair group method using arithmetic average.

Identification of ESBL and carbapenemase genes and ESBL genetic environment

All ESBL producers were screened for the presence of plasmid carrying bla_{SHV}, $bla_{CTX\text{-}M}$, and bla_{TEM}. Three ESBL-producing carbapenem-resistant strains were screened for the detection of carbapenemases: bla_{KPC}, bla_{IMI}, bla_{AIM}, bla_{SME}, bla_{GES}, bla_{GIM}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and $bla_{OXA\text{-}48\text{-}like}$ genes by PCR. The oligonucleotide primers specific for the ESBL and carbapenemase genes in the PCR assays were designed by Doyle and Wang.[7,23-25] We used previously described primers to investigate the surrounding regions of the bla_{SHV}, $bla_{CTX\text{-}M}$, and bla_{TEM} genes.[7] All positive PCR products were sent to the Invitrogen Corporation (Shanghai, China) for sequencing. The nucleotide sequences were analyzed with the basic local alignment search tool online.

Statistical analysis

Percentages and frequencies were used to analyze categorical variables. A Chi-square test or Fisher exact test was used for categorical variables (invasive procedures). A P value <0.05 was considered to be statistically significant. Analyses were performed using SPSS 23.0 for MacIntosh (SPSS Inc., Chicago, IL, USA).

Results

Prevalence of E. coli and K. pneumoniae and patient characteristics

A total of 976 non-reduplicate *E. coli* and *K. pneumoniae* were collected from December 1st, 2015 to June 30th, 2016. Among these isolates, 28.7% (280/976) were confirmed as ESBL producers, and 59 strains (31 *E. coli* and 28 *K. pneumoniae*) isolated from non-reduplicate patients with pneumonia enrolled in this study. The flowchart of patient's enrollment is shown in Figure 1. Most of the strains ($n = 54$) were detected from sputum samples followed by blood samples ($n = 5$), and no strains were isolated from pleural effusion and alveolar lavage fluid. ESBL-producing *E. coli* and *K. pneumoniae* isolated from patients with pneumonia were mostly located in the neurosurgery department, followed by the cardiovascular department and the intensive care department. The mean age of the 59 adult patients was (59.0 ± 14.7) years and 35.6% (21/59) were elderly (>65years). Compared to patients with CAP, those with HAP and VAP had higher frequency of previous hospitalization history and invasive procedures, including invasive catheterization ($\chi^2 = 10.404$, $P = 0.005$), urinary catheterization ($\chi^2 = 6.767$, $p = 0.025$), indwelling gastric tube ($\chi^2 = 6.427$, $P = 0.037$) and intubation ($\chi^2 = 14.888$, $P = 0.001$), and the 28-day mortality in patients with HAP and VAP were also higher than those with CAP ($\chi^2 = 2.368$, $P = 0.281$). There were no statistical differences in the comorbidities, including diabetes mellitus, cardiovascular disease, heart failure, malignancies, and chronic renal failure among the three groups. Patients with HAP or VAP were more likely to have complications with respiratory failure, acute respiratory distress syndrome, sepsis, or plural effusion [Table 1].

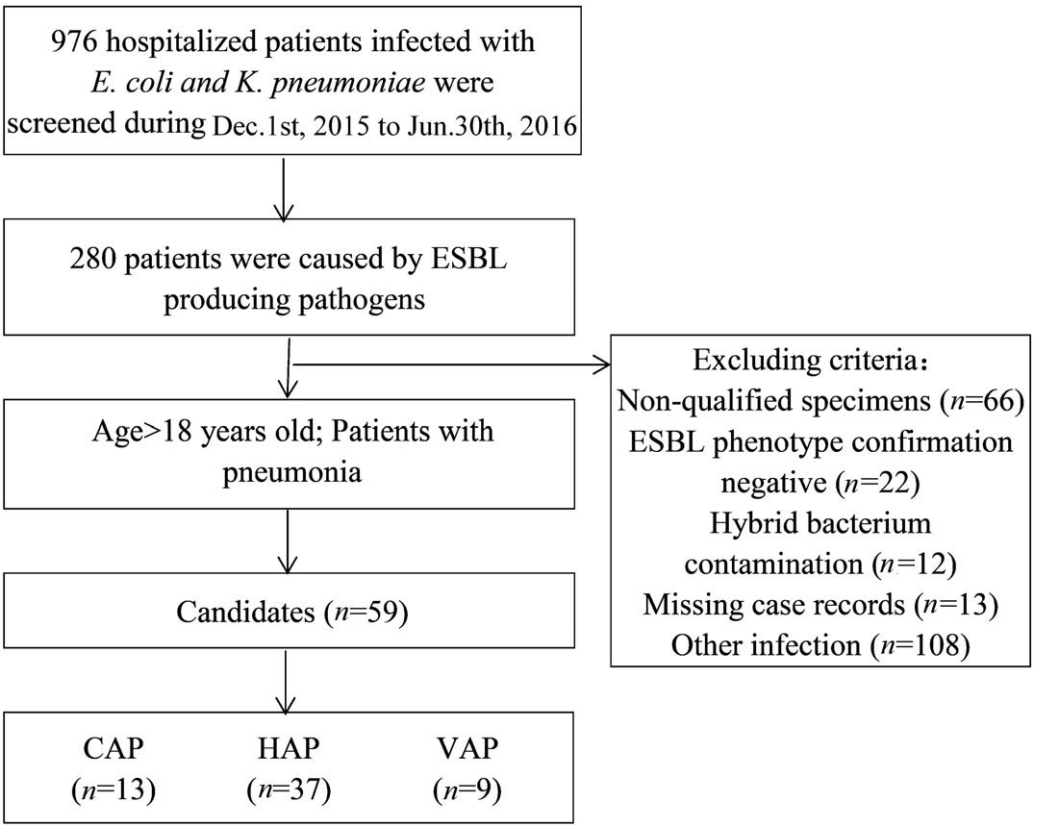
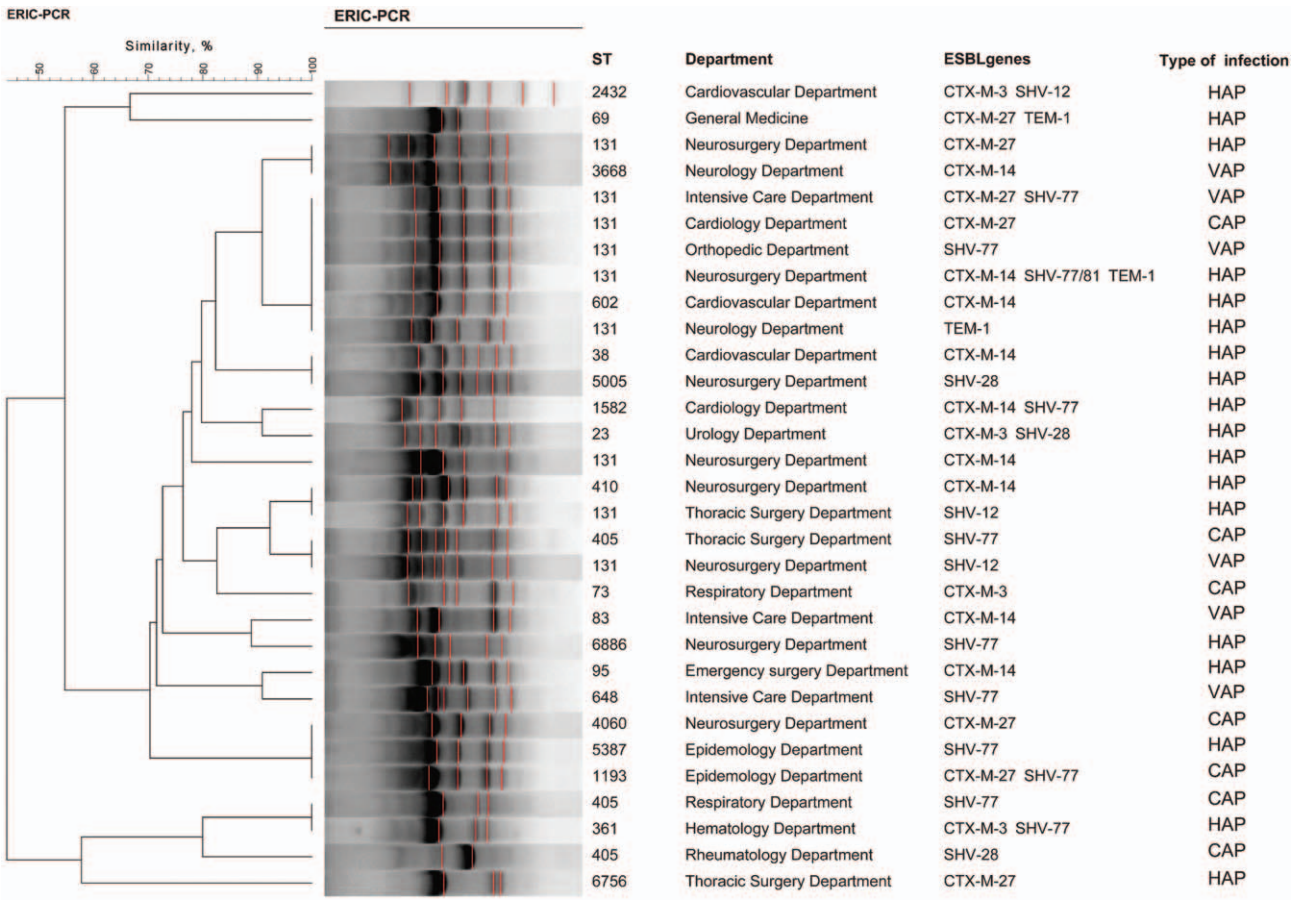


Figure 1: Flowchart of CAP, HAP, and VAP patients enrollment in this study. CAP: Community-acquired pneumonia; HAP: Hospital-acquired pneumonia; VAP: Ventilator-related pneumonia.

Table 1: Demographic and clinical characteristics of 59 patients with pneumonia.

Variables	CAP ($n = 13$)	HAP ($n = 37$)	VAP ($n = 9$)	Statistics	*P* values
Age (years)	67 ± 11	57 ± 14	55 ± 18	2.709*	0.075
Male/female	10/3	24/13	7/2	0.873†	0.659
Prior antibiotic exposure	10 (76.9)	32 (86.5)	9 (100)	2.073†	0.345
Previous hospitalization history	2 (15.4)	23 (62.2)	7 (77.8)	10.706†	0.004
Previous intubation	3 (23.1)	14 (37.8)	9 (100)	14.888†	0.001
Ventilation >5 days	1 (7.7)	5 (13.5)	8 (88.9)	20.390†	0.000
Invasive catheterization	3 (23.1)	13 (35.1)	8 (88.9)	10.404†	0.005
Urinary catheterization	5 (38.5)	27 (73.0)	8 (88.9)	6.767†	0.025
Indwelling gastric tube	2 (15.4)	10 (27.0)	6 (66.7)	6.427†	0.037
Comorbidities					
Diabetes mellitus	1 (7.7)	3 (8.1)	1 (11.1)	0.554†	1.000
Cardiovascular disease	3 (23.1)	9 (24.3)	0 (0)	2.566†	0.293
Heart failure	4 (30.8)	6 (16.2)	0 (0)	3.215†	0.213
Malignancies	2 (15.4)	7 (18.9)	2 (22.2)	0.341†	1.000
Chronic renal failure	1 (7.7)	1 (2.7)	1 (11.1)	2.123†	0.310
No-comorbidities	6 (46.2)	18 (48.6)	5 (55.6)	0.280†	1.000
Complications					
Respiratory failure	5 (38.5)	16 (43.2)	9 (100)	11.162†	0.004
ARDS	0 (0)	2 (5.4)	1 (11.1)	1.472†	0.495
Sepsis	0 (0)	1 (2.7)	1 (11.1)	2.140†	0.330
Plural effusion	5 (38.5)	10 (27.0)	1 (11.1)	1.877†	0.370
28-day mortality	1 (7.7)	6 (16.2)	3 (33.3)	2.368†	0.281

Data are presented as mean ± SD or number of patients (%). *F value; †χ^2 values. CAP: Community-acquired pneumonia; HAP: Hospital-acquired pneumonia; VAP: Ventilator-acquired pneumonia; ARDS: Acute respiratory distress syndrome.



ST	Department	ESBLgenes	Type of infection
2432	Cardiovascular Department	CTX-M-3 SHV-12	HAP
69	General Medicine	CTX-M-27 TEM-1	HAP
131	Neurosurgery Department	CTX-M-27	HAP
3668	Neurology Department	CTX-M-14	VAP
131	Intensive Care Department	CTX-M-27 SHV-77	VAP
131	Cardiology Department	CTX-M-27	CAP
131	Orthopedic Department	SHV-77	VAP
131	Neurosurgery Department	CTX-M-14 SHV-77/81 TEM-1	HAP
602	Cardiovascular Department	CTX-M-14	HAP
131	Neurology Department	TEM-1	HAP
38	Cardiovascular Department	CTX-M-14	HAP
5005	Neurosurgery Department	SHV-28	HAP
1582	Cardiology Department	CTX-M-14 SHV-77	HAP
23	Urology Department	CTX-M-3 SHV-28	HAP
131	Neurosurgery Department	CTX-M-14	HAP
410	Neurosurgery Department	CTX-M-14	HAP
131	Thoracic Surgery Department	SHV-12	HAP
405	Thoracic Surgery Department	SHV-77	CAP
131	Neurosurgery Department	SHV-12	VAP
73	Respiratory Department	CTX-M-3	CAP
83	Intensive Care Department	CTX-M-14	VAP
6886	Neurosurgery Department	SHV-77	HAP
95	Emergency surgery Department	CTX-M-14	HAP
648	Intensive Care Department	SHV-77	VAP
4060	Neurosurgery Department	CTX-M-27	CAP
5387	Epidemology Department	SHV-77	HAP
1193	Epidemology Department	CTX-M-27 SHV-77	CAP
405	Respiratory Department	SHV-77	CAP
361	Hematology Department	CTX-M-3 SHV-77	HAP
405	Rheumatology Department	SHV-28	CAP
6756	Thoracic Surgery Department	CTX-M-27	HAP

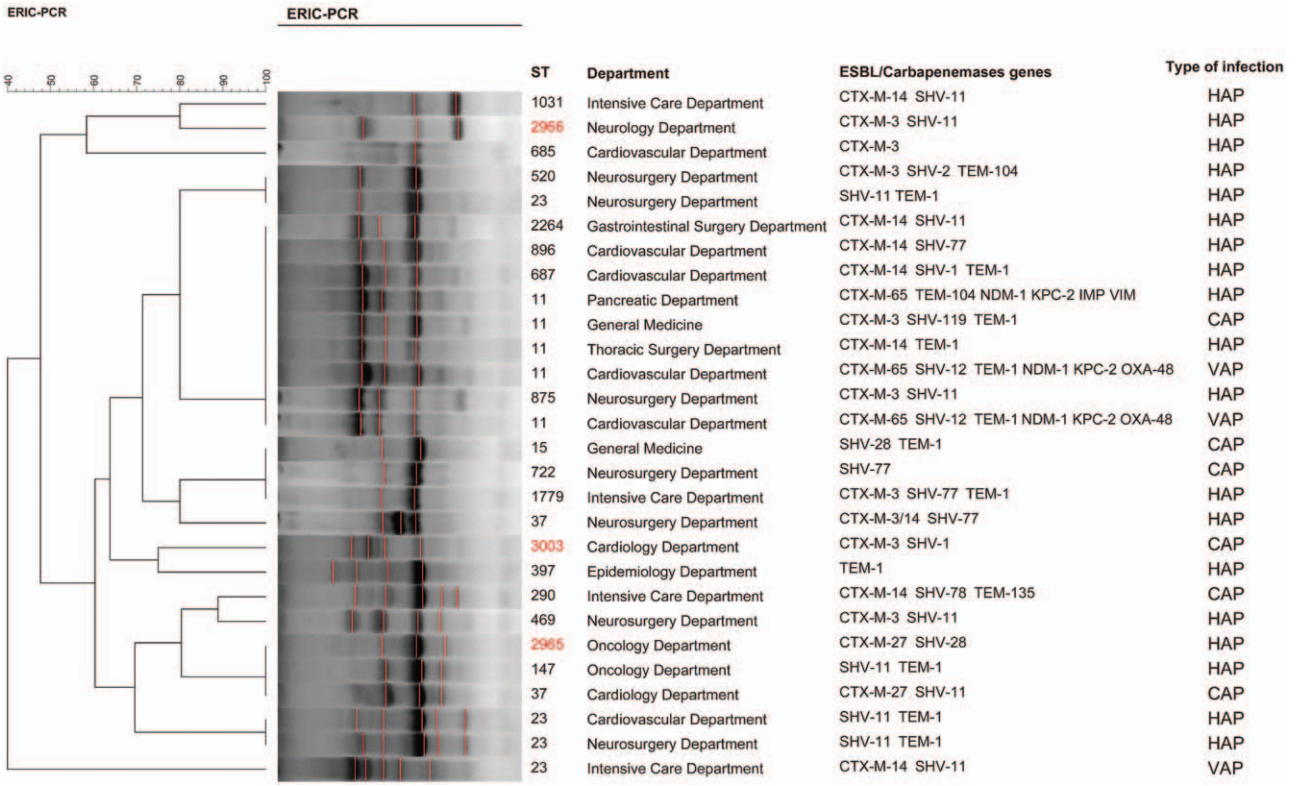
Figure 2: Cluster analysis of *E. coli.* isolates are grouped according to their XaI restriction patterns by BioNumerics version 7.6 software. CAP: Community-acquired pneumonia; HAP: Hospital-acquired pneumonia; VAP: Ventilator-related pneumonia.

Susceptibility of E. coli and K. pneumoniae to anti-microbial agents

Among the 59 isolates, 31 *E. coli* and 28 *K. pneumoniae* strains were confirmed as ESBL producers. All ESBL-producing *E. coli* strains were multi-resistant to most of β-lactam antibiotics and fluoroquinolones, whereas they were sensitive to carbapenems, amikacin, and piperacillin-tazobactam. Unlike ESBL-producing *E. coli*, ESBL-producing *K. pneumoniae* strains showed poor susceptibility to piperacillin-tazobactam, but most strains showed higher sensitivity to carbapenems and amikacin. It is worth noting that there were three ESBL producing *K. pneumoniae* strains resistant to carbapenems, which were confirmed as carbapenemases producers by modified Hodge test [Supplementary Table 1, http://links.lww.com/CM9/A74].

MLST and ERIC-PCR

MLST experiments identified 21 unique sequence typings (STs) in 31 *E. coli* strains. The most prevalent ST was ST131 ($n = 9$, 29.0%) which was mainly located in the neurosurgery department ($n = 4$), followed by ST405 ($n = 3$, 9.7%) and the rest of 19 isolates belonged to different ST types [Figure 2]. *E. coli* ST131 isolates were predominately isolated from patients with HAP ($n = 5$), followed by VAP ($n = 3$), and the remaining one was isolated from a patient with CAP. The *E. coli* ST131 isolates showed lower susceptibility to β-lactam antibiotics and fluoroquinolones than non-ST131 isolates [Supplementary Table 1, http://links.lww.com/CM9/A74], furthermore, patients with pneumonia caused by *E. coli* ST131 isolates had higher proportion of respiratory failure and poor outcomes than those caused by non-ST131 isolates (data not shown). There were 20 STs identified among 28 *K. pneumoniae* strains, the most prevalent ST type was ST11 ($n = 5$, 17.9%), followed by ST23 ($n = 4$, 14.3%), ST37 ($n = 2$, 7.1%), and the remaining 17 strains corresponded to different ST types [Figure 3]. The *K. pneumoniae* ST11 isolates were detected from patients with VAP ($n = 2$) and HAP ($n = 2$), the remaining one was isolated from a patient with CAP. Among the five *K. pneumoniae* ST11, two located in the cardiovascular department were found to be the pan-drug resistance isolates; one *K. pneumoniae* ST11 strain was a multi-drug resistance (MDR) isolate, which was only susceptible to tigecycline; the remaining two *K. pneumoniae* ST11 strains were resistant to most antibiotics, but susceptible to carbapenems, tigecycline, and amikacin. We also discovered three new *K. pneumoniae* STs: ST2965, ST2966, and ST3003 [Figure 3]. Of note, the housekeeping gene (*Phoe313*) sequence in *K. pneumoniae* ST3003 was first reported. The ERIC-PCR results showed that 31 *E. coli* isolates were separated into 19 groups [Figure 2], and 28



ST	Department	ESBL/Carbapenemases genes	Type of infection
1031	Intensive Care Department	CTX-M-14 SHV-11	HAP
2956	Neurology Department	CTX-M-3 SHV-11	HAP
685	Cardiovascular Department	CTX-M-3	HAP
520	Neurosurgery Department	CTX-M-3 SHV-2 TEM-104	HAP
23	Neurosurgery Department	SHV-11 TEM-1	HAP
2264	Gastrointestinal Surgery Department	CTX-M-14 SHV-11	HAP
896	Cardiovascular Department	CTX-M-14 SHV-77	HAP
687	Cardiovascular Department	CTX-M-14 SHV-1 TEM-1	HAP
11	Pancreatic Department	CTX-M-65 TEM-104 NDM-1 KPC-2 IMP VIM	HAP
11	General Medicine	CTX-M-3 SHV-119 TEM-1	CAP
11	Thoracic Surgery Department	CTX-M-14 TEM-1	HAP
11	Cardiovascular Department	CTX-M-65 SHV-12 TEM-1 NDM-1 KPC-2 OXA-48	VAP
875	Neurosurgery Department	CTX-M-3 SHV-11	HAP
11	Cardiovascular Department	CTX-M-65 SHV-12 TEM-1 NDM-1 KPC-2 OXA-48	VAP
15	General Medicine	SHV-28 TEM-1	CAP
722	Neurosurgery Department	SHV-77	CAP
1779	Intensive Care Department	CTX-M-3 SHV-77 TEM-1	HAP
37	Neurosurgery Department	CTX-M-3/14 SHV-77	HAP
3003	Cardiology Department	CTX-M-3 SHV-1	CAP
397	Epidemiology Department	TEM-1	HAP
290	Intensive Care Department	CTX-M-14 SHV-78 TEM-135	CAP
469	Neurosurgery Department	CTX-M-3 SHV-11	HAP
2965	Oncology Department	CTX-M-27 SHV-28	HAP
147	Oncology Department	SHV-11 TEM-1	HAP
37	Cardiology Department	CTX-M-27 SHV-11	CAP
23	Cardiovascular Department	SHV-11 TEM-1	HAP
23	Neurosurgery Department	SHV-11 TEM-1	HAP
23	Intensive Care Department	CTX-M-14 SHV-11	VAP

Figure 3: Cluster analysis of *K. pneumoniae*. Isolates are grouped according to their XaI restriction patterns by BioNumerics version 7.6 software. Three new *K. pneumoniae* ST numbers are marked in red. ST: Sequence typing. CAP: Community-acquired pneumonia; HAP: Hospital-acquired pneumonia; VAP: Ventilator-related pneumonia.

K. pneumoniae isolates were separated into 14 groups [Figure 3]. There were several pulsotypes in clonal groups among *E. coli* ST131 isolates, and one predominant pulsotype included four isolates. The five *K. pneumoniae* ST11 isolates belonged to one single pulsotype.

β-Lactamase genes characterization

All 59 strains were confirmed to carry plasmid-mediated β-lactamase genes by PCR. Sequence analysis revealed that $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM} genes were present in 41, 40, and 19 isolates, respectively. A total of 55.9% isolates (33/59) harbored two or more ESBL genes. The main type of $bla_{\text{CTX-M}}$ was $bla_{\text{CTX-M-14}}$ (17 isolates), followed by $bla_{\text{CTX-M-3}}$ (12 isolates), $bla_{\text{CTX-M-27}}$ (9 isolates), and $bla_{\text{CTX-M-65}}$ (3 isolates). Group II, III, or V $bla_{\text{CTX-M}}$ were not detected. Sequencing results indicated that the most prevalent ESBL bla_{SHV} was SHV-77 ($n = 14$, 23.7%), which mutated on the 637 base compared with non-ESBL enzymes SHV-77 (data not shown), followed by SHV-11 ($n = 11$, 18.6%), $bla_{\text{SHV-28}}$ ($n = 5$), $bla_{\text{SHV-12}}$ ($n = 5$), $bla_{\text{SHV-2}}$ ($n = 1$), $bla_{\text{SHV-78}}$ ($n = 1$), $bla_{\text{SHV-81}}$ ($n = 1$), $bla_{\text{SHV-1}}$ ($n = 1$), and $bla_{\text{SHV-119}}$ ($n = 1$). Twenty-five (42.4%) isolates carried two ESBL genes, and eight (13.6%) isolates had three ESBL genes [Table 2]. Three ESBL producing *K. pneumoniae* ST 11 strains, which were resistant to carbapenems, were detected to carry $bla_{\text{NDM-1}}$ (three isolates), $bla_{\text{KPC-2}}$ (three isolates), $bla_{\text{OXA-48}}$ (two isolates), bla_{IMP} (one isolates), and bla_{VIM} (one isolates) [Figure 3].

Genetic environment of $bla_{\text{CTX-M}}$ and bla_{SHV}

ISEcp1 was found upstream of the start codon of all CTX-Ms. The region between the end of ISEcp1 and the start codon of CTX-Ms was an identical 42 base pairs (bp) length sequence, the ISEcp1 was disrupted by IS1X2 768 bp from the 3′non-coding region of the ISEcp1 in all CTX-Ms bearing strains (data not shown). A non-coding region which belonged to truncated *IS26* element provided the promoter to the bla_{SHV} gene, the truncated *IS26* element has also been found further from the start codon of $bla_{\text{SHV-77}}$, $bla_{\text{SHV-12}}$, $bla_{\text{SHV-28}}$, and $bla_{\text{SHV-11}}$ genes (data not shown).

Discussion

This study analyzed the clinical and molecular characteristics of ESBL-producing *E. coli* and *K. pneumoniae* strains isolated from patients with pneumonia at the large teaching hospital, and the results demonstrated that the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was lower than that reported in other regions of China; however, the worldwide spreading *E. coli* ST131 and *K. pneumoniae* ST11 were prevalent in patients with pneumonia in the hospital.

In this study, we found the prevalence of ESBL-producing *E. coli* and *K. pneumoniae* was 33.5% and 19.4%, respectively (average 28.7%), which was lower than that reported by Yu *et al* (35.7%) and Hawser *et al* (48.2%).[26,27] In accordance to other studies,[28,29] our

Table 2: Genotypes of ESBL-producing *E. coli* and *K. pneumoniae* isolates, *n* (%).

Genotype	*E. coli* ($n = 31$)	*K. Pneumonia* ($n = 28$)	Total ($n = 59$)
CTX-M-3	4 (12.9)	8 (28.6)	12 (20.3)
CTX-M-14	9 (29.0)	8 (28.6)	17 (28.8)
CTX-M-27	7 (22.6)	2 (7.1)	9 (15.3)
CTX-M-65	0 (0)	3 (10.7)	3 (5.1)
SHV-1	0 (0)	1 (3.6)	1 (1.7)
SHV-2	0 (0)	1 (3.6)	1 (1.7)
SHV-11	0 (0)	11 (39.3)	11 (18.6)
SHV-12	3 (9.7)	2 (7.1)	5 (8.5)
SHV-28	3 (9.7)	2 (7.1)	5 (8.5)
SHV-77	10 (32.3)	4 (14.3)	14 (23.7)
SHV-78	0 (0)	1 (3.6)	1 (1.7)
SHV-81	1 (3.2)	0 (0)	1 (1.7)
SHV-119	0 (0)	1 (3.6)	1 (1.7)
TEM-1	3 (9.7)	13 (46.4)	16 (27.1)
TEM-104	0 (0)	2 (7.1)	2 (3.4)
TEM-135	0 (0)	1 (3.6)	1 (1.7)
CTX-M + SHV	6 (19.4)	11 (39.3)	17 (28.8)
CTX-M + TEM	1 (3.2)	2 (7.1)	3 (5.1)
SHV + TEM	0 (0)	5 (17.9)	5 (8.5)
CTX-M + SHV + TEM	1 (3.2)	7 (25.0)	8 (13.6)

results also showed that the prevalence rates of ESBL-producing *E. coli* and *K. pneumoniae* in HAP were higher than that in CAP, which suggested that HAP was more likely correlated with ESBL producers than CAP. Additionally, patients with HAP or VAP caused by ESBL producers had a higher 28-day mortality than those with CAP. High proportion of co-existence of ESBL genes, multidrug resistant and excessive complications had been put forward to poor outcomes in patients with HAP or VAP. However, more than one-fifths of the patients were CAP, which was much higher than that reported by other studies,[30,31] this indicated that there was a trend of ESBL-producing strains being community outbreak.

The most predominant ESBL genes was *CTX-M-14*, which was consistent with other reports in Asia-Pacific region.[32-34] For all $bla_{\text{CTX-M}}$, the *ISEcp1* insertion sequence had been detected. The close relationship between $bla_{\text{CTX-M}}$ and *ISEcpl* had been frequently reported,[35,36] these results demonstrated the important role of *ISEcpl* in the worldwide spread of $bla_{\text{CTX-M}}$. In contrast to $bla_{\text{CTX-M}}$, SHV-11 was the main bla_{SHV} in our study, followed by SHV-77. It is worth noting that SHV-77 detected in our study was an encoding ESBL enzyme, which was different from the study in which it was reported as a non-ESBL enzyme.[37] Sequencing analysis demonstrated that it may be attributed to the mutation of the coding region of $bla_{\text{SHV-77}}$. Furthermore, *IS26* was detected upstream of most of bla_{SHV}, the bla_{SHV} genes associated with *IS26* were regulated by the strong promoter, which was consistent with previous studies.[38,39] These findings suggested that *IS26* could play an important role in expression of bla_{SHV} and contribute to transmission of bla_{SHV}.

MLST analysis demonstrated that *E. coli* ST131 was the most prevalent strain of ESBL-producing *E. coli* causing pneumonia in our hospital. *E. coli* ST131 strain originally had risen to prominence as early as 2003, receiving increasing attention due to its rapid global dissemination and frequent multi-drug-resistant phenotype.[40] Previous studies reported that *E. coli* ST131 strain was prevalent in patients with urinary tract infections[41] and blood stream infections[42]; however, there were few studies about the prevalence of *E. coli* ST131 in patients with pneumonia. Although Cha *et al*[43] reported that CTX-M-15-producing *E. coli* ST131 has emerged and disseminated among patients with HAP in South Korea, Thailand and the Philippines, limited information existed about its clinical impacts on patients with pneumonia from China. To our knowledge, this is the first study to identify *E. coli* clone ST131 strain prevalent in patients with pneumonia in the mainland of China. Consistent with previous study,[43] our study also demonstrated that the *E. coli* ST131 was the most prevalent strain causing HAP. However, more importantly, our study showed *E. coli* ST131 strains were higher anti-microbial resistant than non-ST131 strains, and patients with pneumonia caused by *E. coli* ST131 strains had poor prognosis owing to acute respiratory failure, these results indicated that the ST131 strains were more virulent than non-ST131 isolates. The high resistant and virulent ST131 strain may also be associated with the widespread nature of this strain, due to the more adaptive characteristic than non-ST131 in the hospital environment.[44] Additionally, previous studies indicated that *E. coli* ST131 which was from phylogenetic group B2 has a fitness advantage owing to their group B2 genomic backbone and this may attribute to the remarkable success prevalence of ST131 in hospital worldwide.[45-47] However, the mechanism about the genomic backbone contributing to the fitness advantage was still unclear, which deserved further exploration.

In contrast to *E. coli*, our study demonstrated that *K. pneumoniae* ST11 was the major type of ESBL-producing

K. pneumoniae strains. It was reported that *K. pneumoniae* ST11, which was multi-drug resistant and had highly transmissible characteristics, was the predominant clone of KPC-producing *K. pneumoniae* in China.[48,49] In our study, most (60.0%) of the *K. pneumoniae* ST11 strains resistant to carbapenems carried bla_{NDM-1} and bla_{KPC-2}, this was different from previous studies that bla_{KPC-2} was reported as the prevalent gene in China,[50-52] and bla_{NDM-1} or bla_{OXA-48} was the predominant gene type in other countries.[53-55] Previous studies reported that *K. pneumoniae* ST11 was the dominant strain causing UTI and bacteremia, but the CTX-M-24 and KPC-2 producing *K. pneumoniae* ST11 in patients with VAP was hospital outbreak and dissemination in other regions of China.[15,52,56] However, in our study, *K. pneumoniae* ST11 strains, which mainly carried CTX-M-65, were isolated from patients with HAP, VAP, or CAP.

Previous studies reported that dissemination of *K. pneumoniae* ST11 related to mobile genetic elements (MGEs).[57,58] MGEs including transposons, integrons, prophages, integrative and conjugative elements, and genomic islands, which carried anti-microbial resistance and adaptability associated genes, made *K. pneumoniae* ST11 formidable adaptive and caused drug-resistant infections in hospitals.[59,60] Our results indicated epidemic trends of multidrug or pandrug resistant *K. pneumoniae* ST11 harboring bla_{NDM-1} and bla_{KPC-2} in patients with pneumonia in the hospital, which is a serious threat to the public health. Thus, continuous surveillance on the prevalence of *K. pneumoniae* ST 11 was of great significance to control the outbreak of the resistant strain.

In summary, we documented the emergence and dissemination of multidrug resistant *E. coli* ST131 and *K. pneumoniae* ST11 causing pneumonia at a Chinese teaching hospital, which is a major threat for hospitalized patients. Meanwhile, our study revealed that the bla_{CTX-M} and bla_{SHV} genes were mobilized by *ISEcpl* and *IS26*. These results underline the paramount significance of the consequent surveillance of MDR ESBL-producing *E. coli* and *K. pneumoniae* strains in hospital.

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Conflicts of interest

None.

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