Antimicrobial resistance and epidemiology of extended spectrum-β-lactamases (ESBL)-producing *Escherichia coli* and *Enterobacter cloacae* isolates from intensive care units at obstetrics & gynaecology departments: a retrospective analysis

Kun Chen[1], Guo-Liang Yang[2], Wen-Ping Li[3], Ming-Cheng Li[4,*,†], Xue-Ying Bao[5,†]

[1] *Department of Clinical Nursing, School of Nursing, Army Medical University, 400038 Chongqing, China*

[2] *Department of Ultrasound, the Second Affiliated Hospital, Army Medical University, 400037 Chongqing, China*

[3] *Department of Obstetrics and Gynecology, DaPing Hospital, Army Medical Univerity, 400042 Chongqing, China*

[4] *Department of Laboratory Diagnosis, School of Laboratory Medicine, Beihua University, 132013 Jilin, Jilin, China*

[5] *Department of Laboratory Diagnosis, Associated Hospital, Beihua University, 132013 Jilin, Jilin, China*

*Correspondence: limingcheng1964@163.com; limingcheng@beihua.edu.cn (Ming-Cheng Li)
† These authors contributed equally.

Background: There are increasing concerns towards the transmission of extended spectrum-β-lactamases (ESBL)-producing *Enterobacteriaceae* in hospital intensive care units (ICUs) at obstetrics & gynaecology departments. The aim of this study was to determine the clinical characteristics and prevalence of ESBL-producing *Escherichia coli* (*E. coli*) and *Enterobacter cloacae* (*E. cloacae*) isolates collected from ICUs at obstetrics & gynaecology departments in a tertiary care hospital, China. This study also explored the treatment options for *E. coli* and *E. cloacae* infections. *Methods*: *E. coli* and *E. cloacae* isolates from ICU samples were identified by using the Vitek 2 Compact System with the GN and ASTGN13 cards. Antimicrobial susceptibility profiles were determined by using the broth microdilution method. Double-disk synergy test (DDST) was performed to screen for ESBLs and combined with the EDTA-disc synergy to detect the production of carbapenemase. Enterobacterial repetitive intergenic consensus (ERIC)-PCR was applied to investigate the clonality of the isolates. *Results*: A total of 223 strains isolated from 283 hospitalized patients in the ICU with nosocomial infections between 2017 and 2019 were analyzed. Of these, 104 isolates were classified as *E. coli* and 103 isolates as *E. cloacae* by the VITEK GNI system. Of the 207 isolates, 131 (63.3%) were separated from sputum or tracheal secretions. ESBL-screen positive was 45.2% (47/104) for *E. coli*, and 44.7% (46/103) for *E. cloacae*. Resistance rates of ESBL-producing *E. coli* and *E. cloacae* isolates were 95.5% and 91.3% for ampicillin, respectively; 80.6% and 76.1% for ampicillin/tazobactam; 88.1% and 28.3% for ciprofloxacin; 89.6% and 15.2% for levofloxacin; 34.3% and 45.7% for netilmicin; 82.1% and 41.3% for compound sulfamethoxazole; 20.9% and 43.5% for amikacin; 58.2% and 37.0% for gentamicin; 20.9% and 69.6% for piperacillin/tazobactam. Additionally, all ESBL-producing isolates were fully resistant to cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, and aztreonam. On the other hand, isolates were fully susceptible to imipenem and meropenem. Results of ERIC-PCR in all of ESBL-producing *E. coli* isolates exhibited 11 distinct patterns with a similarity coefficient of 0.8. Only one distinct ERIC pattern was observed amongst the 46 strains of ESBL-producing *E. cloacae*. Analysis of ERIC patterns demonstrated that there was an outbreak of nosocomial infection of ESBL-producing *E. coli* and *E. cloacae* in obstetrics & gynecology ICU of this hospital. *Discussion*: Our data indicate that the ESBL-producing *E. coli* and *E. cloacae* are circulating in the ICU and constitute a major source infection spread. It is necessary to increase surveillance of infections in the ICU and develop adequate infection prevention strategies.

Keywords

Enterobacteriaceae; Outbreak; ESBLs; Infection control; Intensive care units

1. Introduction

Enterobacteriaceae resistance to extended-spectrum β-lactams (ESBL) is a major public health problem worldwide [1]. Both *Escheridhia coli* (*E. coli*) *and Enterobacter cloacae* (*E. cloacae*) are ESBL-producing Gram-negative *Enerobacteriaceae*, which are the most common cause of healthcare-associated infections (HAIs)-that comprise of hospital and community infections-admitted to intensive-care settings [2]. HAIs in intensive care units (ICUs) from ESBL-producing pathogens have become particularly problematic since they arise from the treatment and rehabilitation procedures of critically-ill patients [3]. Risk factors for infection of drug-resistant bacteria in the ICU include the following: widespread abuse, misuse, or overuse of antibiotics; the rapid renewal of antibiotics; increasing quantity and variety of pathogens present in the ICU; the diagnosis and progression of critical ill-

nesses; undergoing surgery; the use of invasive medical devices for treatment or surgery; and prolonged stay in hospital ICUs [4, 5]. Although some research has reported a significant increase in the proportion of ESBL-positive *Enterobacteriaceae* including *Klebsiella pneumoniae* hospital infections worldwide, ICUs in China have reported only a limited number of ESBL-positive *E. coli* and *E. cloacae* isolates, especially in ICUs at obstetrics & gynaecology departments [6]. Effective and safe antimicrobial treatment is essential for treating infections in ICUs. Organizational-wide surveillance of infection-derived bacteria isolates and analysis of their susceptibility to different antimicrobial agents provides crucial information for the most effective antimicrobial therapy. Furthermore, a comprehensive analysis of epidemiology of ICU infection, and evaluating disease prognosis are essential for reducing infection mortality and morbidity in ICU [7].

This study focused on the bacterial distribution and drug resistance characteristics of ICU infections in obstetrics & gynecology departments. Furthermore, this study aimed to explore the epidemiology of pathogenic bacteria transmission and diffusion resistance by examining infectious cases at the ICU of a third-level hospital in Jilin, China between 2017 and 2019.

2. Materials and methods

2.1 Study setting and specimen collection

This study was conducted at a university-affiliated hospital, which is one of the largest hospitals in North-East China with approximately 2000 beds. This study conducted an analysis of retrospective data of *Enterobacteriaceae*-infected patients in three ICUs belonging to obstetrics & gynaecology settings. Two hundred and eighty-three patients who received treatment for cancer or tumor between June 2017 and June 2019 at the three ICUs were enrolled in this study. Patients with concurrent HAIs were classified according to the respiratory tract infections: pneumoniae, bloodstream infections such as catheter-related infections, urinary tract infections, surgical site infections, and other infections. For the most part, the pathogenic bacteria associated with an HAI were collected within 48 hours of hospitalization according to the local hospital criterion. Some samples were collected after 48 hours post-hospitalization. The specimens were mainly obtained from sputum or tracheal secretions, pus, blood, ascites, catheters, and drainage tubes. Multiple isolates from a single patient were excluded. The isolates from different infected sites of the same patient were also excluded. Ethical approval for collecting clinical samples was received by the institutional ethics committees of the participating hospital. Informed consent forms were reviewed and signed by all participants before sample collection.

2.2 Identification and detection of resistance to 17 antibiotics agents

E. coli and *E. cloacae* isolates were identified by using the Vitek 2 Compact System with GN and ASTGN13 cards (bioMérieux, Marcy l'Etoile, France). Susceptibility to a panel of 17 antimicrobial agents was assessed by using the broth microdilution method according to the recommendations by the Clinical and Laboratory Standards Institute (CLSI, 2012) [8]. The following antibiotics (AB Biodisk, Solna, Sweden) were tested: ampicillin, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, levofloxacin, netilmicin, aztreonam, ciprofloxacin, amikacin, gentamicin, imipenem, meropenem, ampicillin/tazobactam, piperacillin/tazobactam and compound sulfamethoxazole.

E. coli ATTCC 25922 was the susceptible control strain, and *K. pneumoniae* 700603 and *E. cloacae* ATCC 13047 were the ESBL-positive control strains. *K. pneumoniae* A1500 was the carbapenemase-positive control strains.

2.3 Determination of ESBL-producing and carbapenemase-producing strains

Double-disk synergy test (DDST) was performed to screen for ESBLs and combined with the EDTA-disc synergy to detect carbapenemase. Suspected ESBL-producing strains were screened using cefotaxime (Oxoid, Basingstoke, UK) (30 μg) and ceftazidime (Oxoid) (30 μg) in combination with clavulanic acid (Oxoid) (10 μg) according to the CLSI recommended disk diffusion method [8]. The screening test was considered positive if the inhibitory zone diameter to cefotaxime/clavulanic acid or ceftazidime/clavulanic acid exhibited an increase of more than 5 mm. For EDTA-disc synergy to detect production of carbapenemase, a 10 μg imipenem disc (AB Biodisk, Solna, Sweden) and a blank filter disc containing 10 μL of 0.5 M EDTA solution (Oxoid) were placed 10 mm between the disc edges on a Mueller-Hinton agar plate whose surface was inoculated with the test strains. The presence of an enlarged inhibition zone was considered to be positive test result.

2.4 Clonality analysis of E. coli and E. cloacae isolates by ERIC
2.4.1 Extraction of total DNA from E. coli and E. cloacae isolates

A single colony was selected from the passage medium and incubated overnight at 37 $^\circ$C after being added to a test tube containing 2 mL of Luria-Bertani liquid. The next day, 2 mL of bacterial liquid was centrifuged at 12,000 r/min for five minutes. The supernatant was discarded and added to 400 μL ddH$_2$O and boiled for 10 minutes after mixing. The mixture was then cooled and centrifuged at 12,000 r/min for five minutes, and the supernatant was absorbed and stored at -20 $^\circ$C.

2.4.2 ERIC-PCR amplification

The primer sequences were P1-ATGTAAGCTCCTGGGGATTCAC and P2-AAGTAAGTGACTGGGGTGAGCG. The system contained 2 μL of P1 and P2 primers, and the DNA template solution at 3 μg/L. ddH$_2$O was added to the total reaction system of 50 μL. PCRs were conducted in a GeneAmp PCR system 9600 (Perkin-Elmer, Waltham, MA, USA) under the following reaction conditions: denaturation at 94 $^\circ$C for five min, denaturation at 94 $^\circ$C for 30 s, annealing at 56~58 $^\circ$C for 45 s, extension at 72 $^\circ$C for 30 s, and 32 cycles at 72 $^\circ$C

after five min. PCR products were analyzed by 2% agarose gel electrophoresis at 60 V for 40 min. A molecular weight DNA marker from 100~600 bp was used as a reference and a gel imaging analysis system was used to observe and analyze the results. Band comparisons were carried out by clustering analysis with the unweighted pair group method using Quantity One (Version 4.6.2) (Bio-Rad Laboratories, Hercules, CA, USA). Isolates were considered as the same origins if their similarity coefficients were equal to or more over 0.8, whereas similarity coefficients below 0.8 meant that isolate origins were dissimilar [9].

2.5 Statistical analysis

Differences in drug resistance rates of non-ESBL-producing and ESBL-producing strains were tested by Chi-square test. All drug resistant data were analyzed using SPSS version 13.0 (IBM Corp., Armonk, NY, USA). Analyses with a value of $P < 0.05$ were considered to be statistically significant.

3. Results

3.1 Epidemiological characteristics of specimens and isolates

From June 2017 to June 2019, a total of 283 patients from three ICUs were enrolled in this study to estimate the quantity and types of infections present in this population. These patients received treatment or surgery for cancer or tumor at a median age of 63 years. Of the 283 samples, 104 were classified as *E. coli* and 103 as *E. cloacae* by the VITEK GNI system. The remaining samples were classified as other *Enterobacteriaceae* and non-*Enterobacteriaceae*, which were not included in this study (data not shown).

The clinical distribution of the specimens was mainly composed of sputum or tracheal secretions (n = 179, 63.3%), followed by skin and purulent infections (n = 33, 11.5%), blood (n = 43, 15.2%), ascites (n = 20, 7.1%), and catheters and drainage tubes (n = 8, 2.8%).

3.2 Antimicrobial susceptibility analysis

Resistance frequencies of ESBL-producing *E. coli* and *E. cloacae* isolates based on CLSI microdilution demonstrated that 67 (64.4%) strains of *E. coli* and 46 (44.7%) strains of *E. cloacae* isolates were ESBL-positive. No isolates were resistant to carbapenem.

Resistance rates of ESBL-producing *E. coli* and *E. cloacae* isolates were 95.5% and 91.3% for ampicillin, respectively; 80.6% and 76.1% for ampicillin/tazobactam; 88.1% and 28.3% for ciprofloxacin; 89.6% and 15.2% for levofloxacin; 34.3% and 45.7% for netilmicin; 82.1% and 41.3% for compound sulfamethoxazole; 20.9% and 43.5% for amikacin; 58.2% and 37.0% for gentamicin; and 20.9% and 69.6% for piperacillin/tazobactam. All of ESBL-producing isolates were 100% resistant to cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, and aztreonam. The susceptibilities of isolates to imipenem and meropenem were 100%.

The susceptibilities of non-ESBL-positive *E. coli* and *E. cloacae* isolates were 89.2% and 91.3% to ampicillin, respec-

tively; 97.3% and 96.5% to ampicillin/tazobactam; 94.6% and 94.7% to cefazolin; 89.2% and 96.5% to cefuroxime; 97.3% and 98.3% to ceftazidime; 97.3% and 98.3% to ceftriaxone; 91.9% and 92.3% to cefepime; 89.2% and 100.0% to aztreonam; 32.4% and 68.4% to ciprofloxacin; 37.8% and 91.2% to levofloxacin; 70.3% and 56.1% to netilmicin; 43.2% and 61.4% to compound sulfamethoxazole; 62.2% and 71.9% to amikacin; 43.2% and 68.4% to gentamicin; and 97.3% and 93.0% to piperacillin/tazobactam. The susceptibilities to imipenem and meropenem were the only ones at 100%. The resistance rates to different antibiotics between ESBL-positive and non-ESBL-positive isolates are summarized in Tables 1 and 2.

Next, this study investigated a local difference of antibiotic resistance between ESBLs and non-EBSL strains. This study found a significant difference between non-ESBL-producing strains and ESBL-producing strains ($P < 0.05$) for the exception of susceptibility to ciprofloxacin ($P > 0.05$).

3.3 Isolate clonality analysis by ERIC-PCR

Isolate clonality analysis of all ESBL-producing strains was conducted by ERIC-PCR typing. One distinct ERIC profile was observed amongst 46 strains of ESBL-producing *E. cloacae*, showing that these isolates were similar to clones (Fig. 1). A representative of the same band was selected in 67 ESBL-producing *E. coli* isolates from different samples for dendrogram cluster analysis. This analysis revealed 11 distinct patterns with a similarity coefficient of 0.8, indicating that these isolates have similar origins. A clonal association was found between these strains: 54 (80.6%) originated from identical clones (Figs. 2 and 3), indicating that there is an outbreak at the study setting. Furthermore, the medical history of ICU patients with the same drug-resistant strains was similar. The majority of drug-resistant strains with identical clones infected the lower respiratory tract. Approximately 76% of ICU patients had a history of mechanical ventilation.

4. Discussion

Antibacterial drugs have been administered extensively in health care for the treatment of disease, especially for critically ill patients in the ICU. However, the incidence of infections resistant to ESBL-producing *Enterobacteriaceae* has rapidly increased in recent years [10]. Research has documented many instances of outbreaks of HAIs in ICUs caused by ESBL-producing *E. coli* and *E. cloacae* [11].

This study collected samples from ICU patients and found that the prevalence of *E. coli* and *E. cloacae* isolates among ESBL-producing *Enterobacteriaceae* was estimated at 64.4% and 44.7%, respectively. These isolates were most common in respiratory tract and skin infections. The results of this study indicated that female patients with a tumor or cancer were likely to be infected by ESBL-producing *Enterobacteriaceae* due to their immunological status or the excessive use of β-lactams. This finding agrees with other research that isolates from ICUs in China contain *E. coli* and *Klebsiella pneumoniae* in addition to *E. cloacae* [12, 13]. *E. coli* and *E. cloacae* belong to a group of conditional pathogens that are part of the

Table 1. Susceptibility to common antimicrobials of 104 *E. coli* isolates.

Antimicrobiol agents	ESBL positive (n = 67)		ESBL negative (n = 37)		X^2	P value
	R (%)*	S (%)	R (%)	S (%)		
Ampicillin	64 (95.5)	3 (4.5)	4 (10.8)	33 (89.2)	4.42	< 0.005
Ampicillin/azobactam	54 (80.6)	13 (19.4)	1 (2.7)	36 (97.3)	5.01	< 0.005
Cefazolin	67 (100.0)	0 (0.0)	2 (5.4)	35 (94.6)	130.67	< 0.005
Cefuroxime	67 (100.0)	0 (0.0)	4 (10.8)	33 (89.2)	169.60	< 0.005
Ceftazidime	67 (100.0)	0 (0.0)	1 (2.7)	36 (97.3)	24.34	< 0.005
Ceftriaxone	67 (100.0)	0 (0.0)	3 (8.1)	34 (91.9)	83.79	< 0.005
Cefepime	67 (100.0)	0 (0.0)	3 (8.1)	34 (91.9)	19.51	< 0.005
Aztreonam	67 (100.0)	0 (0.0)	4 (10.8)	33 (89.2)	88.48	< 0.005
Ciprofloxacin	59 (88.1)	8 (11.9)	25 (67.6)	12 (32.4)	3.31	> 0.005
Gentamicin	39 (58.2)	28 (41.8)	21 (56.8)	16 (43.2)	3.80	< 0.005
Imipenem	0 (0.0)	67 (100.0)	0 (0.0)	37 (100.0)	2.01	> 0.005
Meropenem	0 (0.0)	67 (100.0)	0 (0.0)	37 (100.0)	0	0
Levofloxacin	60 (89.6)	7 (10.5)	23 (62.2)	14 (37.8)	4.69	< 0.005
Netilmicin	23 (34.3)	44 (65.7)	11 (29.7)	26 (70.3)	4.91	< 0.005
Compound sulfamethoxazole	55 (82.1)	12 (17.9)	21 (56.8)	16 (43.2)	61.68	< 0.005
Piperacillin/tazobactam	14 (20.9)	53 (79.1)	1 (2.7)	36 (97.3)	4.54	< 0.005
Amikacin	20 (29.9)	47 (70.2)	14 (37.8)	23 (62.2)	4.74	< 0.005

Note: *P < 0.05 compared with the ESBL negative group.
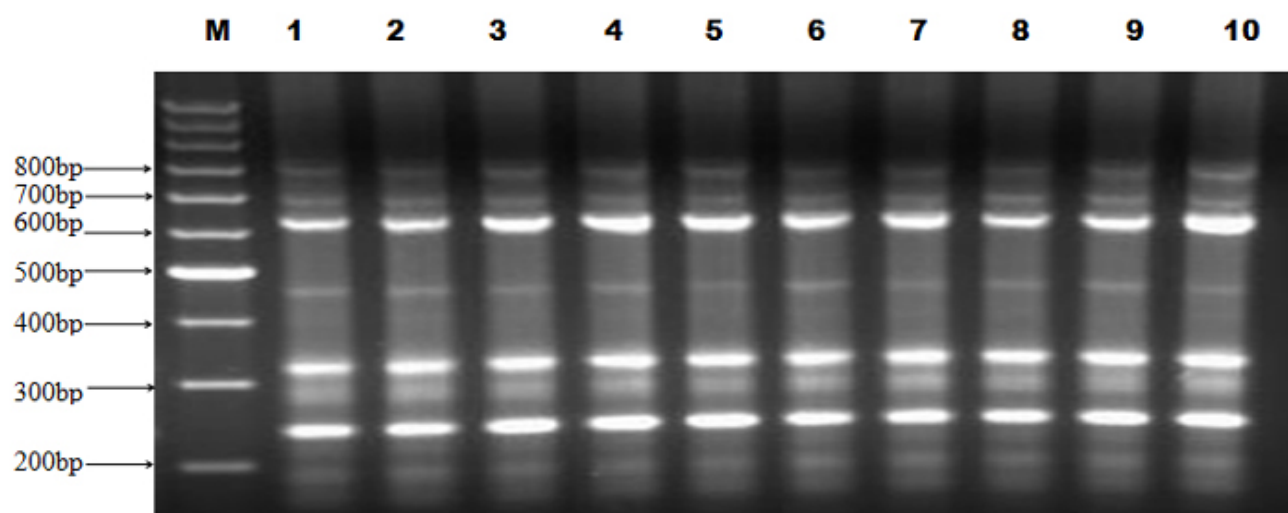
Fig. 1. Representative gel showing banding profiles by ERIC-PCR analysis in ESBLs-producing *E. cloacae* isolates.

Note:

M: DNA molecular weight; 1~13: ESBLs-producing *E. cloacae* isolates from different samples in ICU.

1-2: strains isolated from pus; 3-4: strains isolated from urine; 5-6: strains isolated from blood; 7: strains isolated from ascite; 8-9: strains isolated from cathers and drainage tube; 11-13: strains isolated from sputum or tracheal secretions.

ERIC, Enterobacterial repetitive intergenic consensus.

normal intestinal flora, but can cause infections of the respiratory and urinary tracts [14, 15]. An epidemiological study reported high rates of *E. coli* and *E. cloacae* producing ESBLs in Singapore (44%) and China (37%) [16]. Another research indicates that both ESBL-producing *E. coli* and *E. cloacae* are closely related to antibacterial drug resistance. The same bacterium is likely to carry a variety of ESBLs, which may cause multi-drug resistance [17, 18].

ESBL-producing strains of *E. coli* and *E. cloacae* isolates showed statistically higher resistance rates to cephalosporins than non-ESBL-producing strains. In contrast, there was no obvious difference in the resistance to ciprofloxacin between ESBL-producing and non-ESBL-producing strains. Comparing the sensitivity of cephalosporin antibiotics with sulbactam and tazobactam, drug resistance in ESBL-producing strains was much lower than cephalosporins alone. The find-

Table 2. Susceptibility to common antimicrobials of 103 *E. cloacae* isolates.

Antimicrobiol agents	ESBL positive (n = 46)		ESBL negative (n = 57)		X^2	P value
	R (%)*	S (%)	R (%)	S (%)		
Ampicillin	42 (91.3)	4 (8.7)	4 (7.0)	53 (93.0)	4.42	< 0.005
Ampicillin/azobactam	35 (76.1)	11 (23.9)	2 (3.5)	55 (96.5)	4.52	< 0.005
Cefazolin	46 (100.0)	0 (0.0)	3 (5.3)	54 (94.7)	130.67	< 0.005
Cefuroxime	46 (100.0)	0 (0.0)	2 (3.5)	55 (96.5)	169.60	< 0.005
Ceftazidime	46 (100.0)	0 (0.0)	1 (1.8)	56 (98.3)	24.34	< 0.005
Ceftriaxone	46 (100.0)	0 (0.0)	4 (7.0)	53 (93.0)	83.79	< 0.005
Cefepime	46 (100.0)	0 (0.0)	2 (3.5)	55 (96.5)	19.51	< 0.005
Aztreonam	46 (100.0)	0 (0.0)	0 (0.0)	57 (100.0)	88.48	< 0.005
Ciprofloxacin	13 (28.3)	33 (71.7)	18 (31.6)	39 (68.4)	3.31	> 0.005
Gentamicin	17 (37)	29 (63.0)	20 (35.1)	37 (64.9)	3.80	< 0.005
Imipenem	0 (0.0)	46 (100.0)	0 (0.0)	57 (100.0)	2.01	> 0.005
Meropenem	0 (0.0)	46 (100.0)	0 (0.0)	57 (100.0)	0	0
Levofloxacin	7 (15.2)	39 (84.8)	5 (8.8)	52 (91.2)	4.69	< 0.005
Netilmicin	21 (45.7)	25 (54.4)	25 (43.9)	32 (56.1)	4.91	< 0.005
Compound sulfamethoxazole	19 (41.3)	27 (50.7)	22 (38.6)	35 (61.4)	61.68	< 0.005
Piperacillin/tazobactam	32 (69.6)	14 (30.4)	4 (7.0)	53 (93.0)	4.21	< 0.005
Amikacin	20 (43.5)	26 (56.5)	16 (28.1)	41 (71.9)	4.27	< 0.005

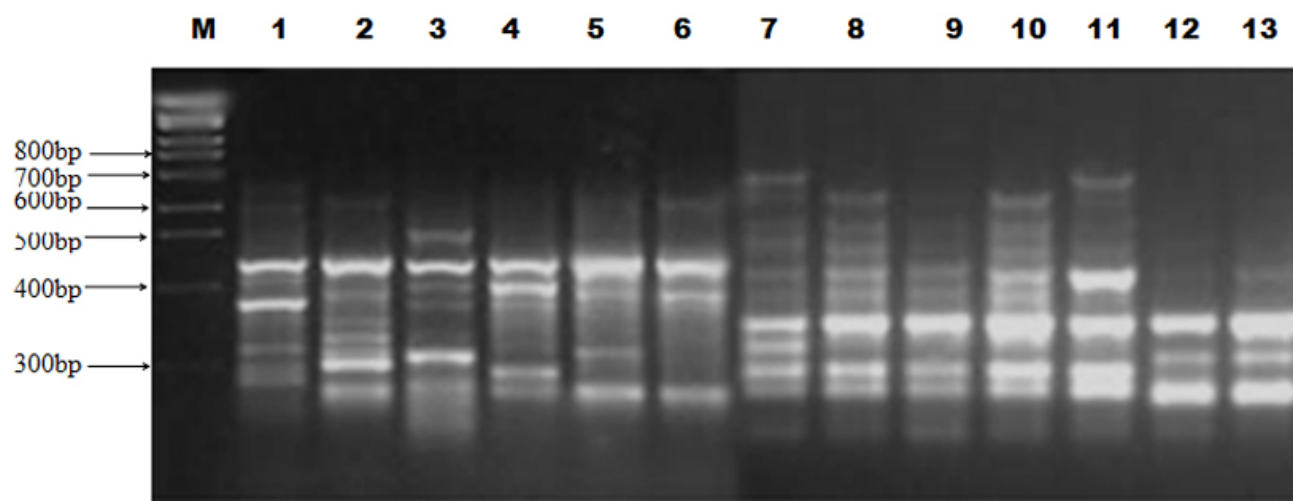**Note:** *$P < 0.05$ compared with the ESBL negative group.

Fig. 2. Representative gel showing banding profiles by ERIC-PCR analysis in ESBLs-producing *E. coli* isolates.

Note:

M: DNA molecular weight; 1~13: ESBLs-producing *E. coli* isolates from different samples in ICU.

1-2: strains isolated from pus; 3-4: strains isolated from urine; 5-6: strains isolated from blood; 7: strains isolated from ascite; 8-9: strains isolated from cathers and drainage tube; 11-13: strains isolated from sputum or tracheal secretions; ERIC, Enterobacterial repetitive intergenic consensus.

ings of this study demonstrate that antibiotic resistance in *E. coli* and *E. cloacae* can be reduced if ESBL production is addressed. All ESBL-producing strains exhibited high resistance to cephalosporins, while at the same time showed high sensitivity to carbapenems. Because the northeast part of China is the coldest region in the country with the highest incidence of respiratory system diseases, overuse of antimicrobial drugs with the exception of carbapenems might explain why this study found a difference in antimicrobial agent susceptibility. Additionally, the findings showed that

ESBL-producing bacteria were more resistant to aminoglycosides and levofloxacin than those with high resistance to cephalosporin antibiotics. Resistance to synthetic antibiotics such as sulfonamides was also lower than resistance to ampicillin.

The initial use of advanced cephalosporin antibiotics, especially the unreasonable use of third generation cephalosporins, marks the beginning of habitual therapy. Habitual therapy may cause health care providers to ignore the role of aminoglycosides and other antibacterial
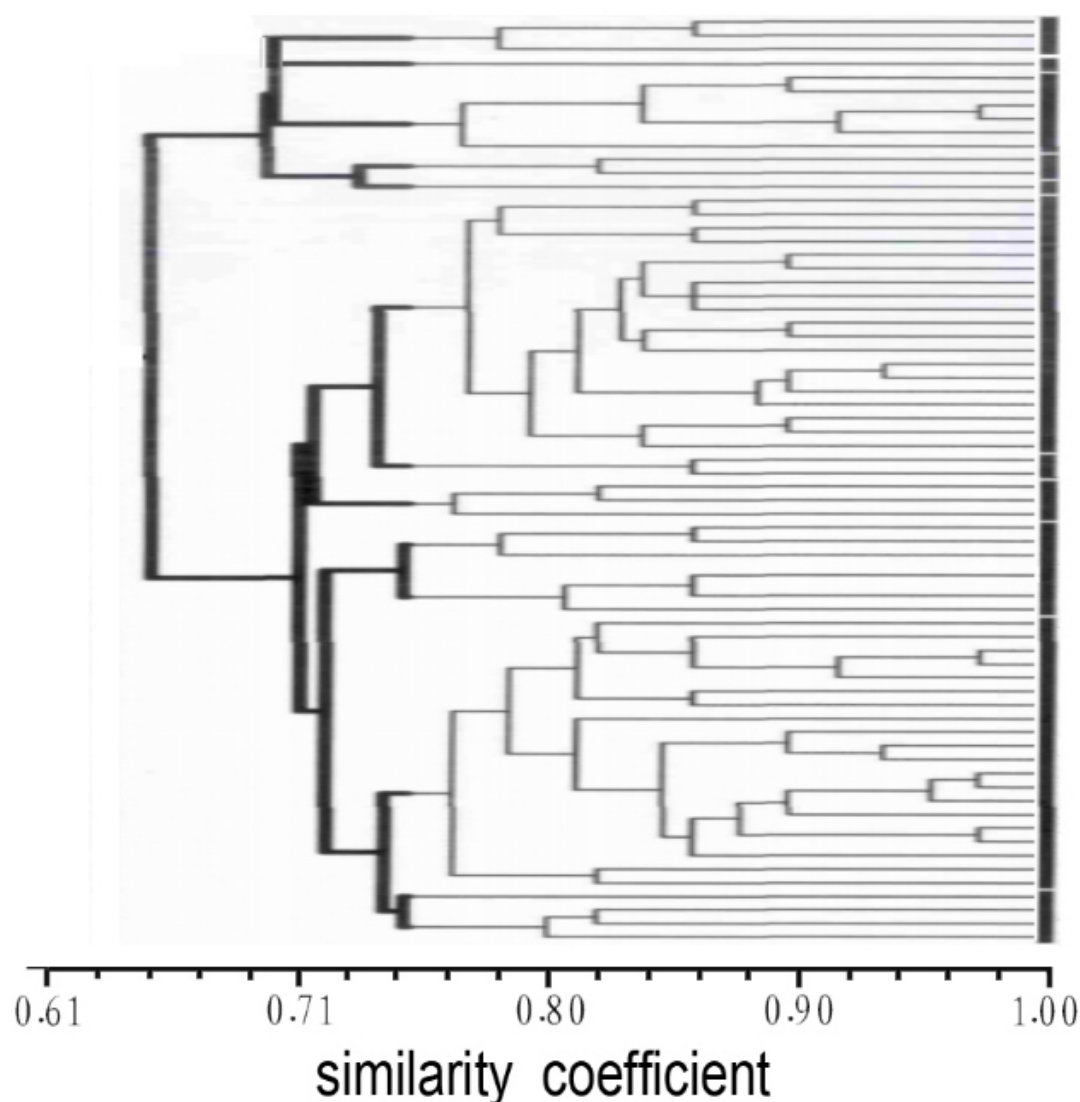
Fig. 3. Dendrogram from ERIC-PCR analysis in ESBLs-producing *E. coli* isolates.

Note:

The scale bar showed the similarity values. Isolates were considered as the same origins if their similarity coefficients were equal to or more over 0.8, whereas, lower 0.8 is different origins.

ERIC, Enterobacterial repetitive intergenic consensus.

drugs that may contribute to resistance to drugs. ESBL-producing *E. coli* and *E. cloacae* isolates transiently colonize on the hands of hospital staff, increasing the likelihood of horizontal transmission between patients. ESBL-producing *Enterobacteriaceae* were frequently accompanied by resistance to an array of antibiotics. Studies have shown that multiple drug resistance mechanisms exist for *E. coli and E. cloacae* including mutations in Amp C enzymes and porin loss [19, 20]. However, the molecular mechanism of resistance was outside the scope of this study and future research may consider paying attention to this research priority.

Clinically, the common utilization of high-grade cephalosporin antibiotics for the treatment of ICU patients with nosocomial infections alongside the unreasonable use of third-generation cephalosporins may have caused the large number of drug-resistant strains that were found in the current study [21, 22]. As a result, patients who are ineffectively treated with advanced cephalosporins or β-lactamase inhibitors due to antimicrobial resistance may only be treated by carbapenems [23].

In the present investigation, both *E. coli* and *E. cloacae* were highly sensitive to carbapenems. No significant difference in resistance was found between ESBL-producing and non-ESBL-producing strains, indicating that carbapenems could be used to treat ESBL-resistant strains. Carbapenems, which are atypical β-lactam antimicrobial agents, have the strongest antimicrobial activity and the broadest antimicrobial spectrum. The low toxicity and high stability of β-lactamase makes them an important target for the treatment of severe infections, especially when other antimicrobial agents

are ineffective [24, 25]. High sensitivity of ESBL-producing *E. coli* isolates and ESBL-producing *E. cloacae* isolates to carbapenems might be explained by the fact that carbapenem has been seldom administered in China compared to other drugs. However, in contrast to data collected from health service organizations in East China, reduced susceptibilities of *E. coli* and *E. cloacae* indicate a local resistance to carbapenem [26]. Taken together, carbapenem overuse might explain why there might be a difference between areas with patients from a higher socioeconomic status than those areas with lower socioeconomic status [27].

In the epidemiological analysis of nosocomial infections in the ICU, ERIC-PCR was used to identify isolates based on ERIC-PCR fingerprints. Since the introduction of the ERIC-PCR technique by Versalovic *et al.* in 1991, the technique has been critical for the epidemiological investigation of Gram-negative bacteria [28]. Results of ERIC-PCR performed in this study found 11 distinct profiles across ESBL-producing *E. coli* isolates, and one distinct profile across 46 strains of ESBL-producing *E. cloacae*. Overall, ERIC profiles demonstrated that there is an outbreak of nosocomial infection (ESBL-producing *E. coli* and *E. cloacae*) in the ICUs of the hospital.

Interestingly, this study found that all 46 ESBL-producing *E. cloacae* strains belonged to the same clonal type. However, there was variation in the antimicrobial profile for netilmicin, sulphamethaxazole, piperacillin/tazobactam, and amikacin. These four antibiotics were seldom used in the study hospital. It is possible that the encoding resistant genes for these antibiotics were primarily carried by mobile genetic elements, such as transposons and introns on a plasmid. Evidence from this study illustrated that plasmid mediating ESBLs might produce other resistant gene expressions, which may be associated with an increased use of antibiotics. Moreover, the *E. cloacae* complex encompasses several species comprising of 12 genetic clusters [29]. This study found significant variation in the four antimicrobial profiles among these isolates from ICU across different years of this study. This may be due to shifting or point mutations in these mobile genetic structures that may exert a pressure for selection [30, 31].

In order to control the production and spread of ESBLs, health care providers may consider careful and reasonable use of antibacterial drugs. For example, health care providers might consider limiting the empirical routine application of high-level antibiotics. Education and awareness surrounding the appropriate use of antibacterial drugs amongst health care workers and patients should be strengthened alongside improving and clarifying antibiotic use guidelines in pharmacies and hospital ICUs [32]. These measures could effectively mitigate or reduce the spread of infection of ESBLs bacteria. Overall, this study emphasizes the need for medical staff to use antimicrobial agents rationally [33].

This study has some limitations. First, genotypic or molecular data of all strains were not documented. Second, the molecular epidemiology of isolates was not included. Future research may consider focusing on genetic types and the mechanism of transmission.

5. Conclusions

The findings of this study indicate that the ESBL-producing *E. coli* and *E. cloacae* clones are circulating in the ICU at obstetrics & gynaecology departments and constitute a major source of infection at a large hospital in China. This study also found that carbapenems may be a reasonable choice in the treatment of ESBL-producing bacteria. In summary, there is a strong need for increased hospital-wide surveillance and the development of adequate infection prevention strategies.

Abbreviations

CLSI, the Clinical and Laboratory Standards Institute; DDST, Double-disk synergy test; *E. cloacae*, *Enterobacter cloacae*; *E. coli*, *Escherichia coli*; ERIC, Enterobacterial repetitive intergenic consensus; ESBL, extended-spectrum β-lactams; HAIs, healthcare-associated infections; ICU, intensive care unit.

Author contributions

KC conceived, designed the experiments and wrote a draft manuscript. MCL and XYB analyzed, interpreted the results of the experiments and revised the manuscript. GLY and WPL performed the experiments. XYB collected the clinical data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval for collecting clinical samples was received by the institutional ethics committees of the participating hospital. Informed consent forms were reviewed and signed by all participants before samples collection (Ethical approval number: Protocol Number 2019-01-01).

Conflict of interest

The authors declare no conflict of interest.

References

[1] Singh N, Yu VL. Rational empiric antibiotic prescription in the ICU. Chest. 2000; 117: 1496-1499.

[2] Ripabelli G, Tamburro M, Guerrizio G, Fanelli I, Flocco R, Scutellà M, *et al.* Tracking multidrug-resistant klebsiella pneumoniae from an italian hospital: molecular epidemiology and surveillance by PFGE, RAPD and PCR-based resistance genes prevalence. Current Microbiology. 2018; 75: 977-987.

[3] Yoon EJ, Hong JS, Yang JW, Lee KJ, Lee H, Jeong SH. Detection of mcr-1 plasmids in *Enterobacteriaceae* isolates from human specimens: comparison with those in *Escherichia coli* isolates from livestock in Korea. Annals of Laboratory Medicine. 2018; 38: 555-562.

[4] Bradford PA. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clinical Microbiology Reviews. 2001; 14: 933-951.

[5] Mulvey MR, Bryce E, Boyd D, Ofner-Agostini M, Christianson S, Simor AE, *et al.* Ambler class A extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella spp.* in Canadian hospitals. Antimicrobial Agents and Chemotherapy. 2004; 48: 1204-1214.

[6] Quinteros M, Radice M, Gardella N, Rodriguez MM, Costa N, Korbenfeld D, *et al.* Extended-spectrum beta-lactamases in *Enterobacteriaceae* in Buenos Aires, Argentina, public hospitals. Antimicrobial Agents and Chemotherapy. 2003; 47: 2864-2867.

[7] Ripabelli G, Sammarco ML, Scutellà M, Felice V, Tamburro M. Carbapenem-resistant KPC- and TEM-producing *Escherichia coli* ST131 isolated from a hospitalized patient with urinary tract infection: first isolation in Molise region, central Italy, July 2018. Microbial Drug Resistance. 2019; 26: 38-45.

[8] Clincal and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testings; nineteeth informational supplement (M100-S19). Wayne, PA, USA: CLSI. 2012.

[9] Su DH, Lu JC, Zuo C, Li HY, Chen DM, Wang M, *et al.* Antibiotic resistance of ESBLs-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates with CTX-M genotypes in guangzhou: analysis of their genotyping. Chinese Journal of Nosocomiology. 2009; 19: 3004-3006. (In Chinese)

[10] Tian SF, Chen BY, Chu YZ, Wang S. Prevalence of rectal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* among elderly people in community settings in China. Canadian Journal of Microbiology. 2008; 54: 781-785.

[11] Rordorf G, Koroshetz W, Efird JT, Cramer SC. Predictors of mortality in stroke patients admitted to an intensive care unit. Critical Care Medicine. 2000; 28: 1301-1305.

[12] Cohen J. Confronting the threat of multidrug-resistant Gram-negative bacteria in critically ill patients. The Journal of Antimicrobial Chemotherapy. 2013; 68: 490-491.

[13] Vincent J. Nosocomial infections in adult intensive-care units. The Lancet. 2003; 361: 2068-2077.

[14] Gniadkowski M. Evolution and epidemiology of extended-spectrum beta-lactamases (ESBLs) and ESBL-producing microorganisms. Clinical Microbiology and Infection. 2001; 7: 597-608.

[15] Nathisuwan S, Burgess DS, Lewis JS. Extended-spectrum beta-lactamases: epidemiology, detection, and treatment. Pharmacotherapy. 2001; 21: 920-928.

[16] Vinué L, Sáenz Y, Martínez S, Somalo S, Moreno MA, Torres C, *et al.* Prevalence and diversity of extended-spectrum beta-lactamases in faecal *Escherichia coli* isolates from healthy humans in Spain. Clinical Microbiology and Infection. 2009; 15: 954-957.

[17] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, *et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012; 18: 268-281.

[18] Leistner R, Schröder C, Geffers C, Breier AC, Gastmeier P, Behnke M. Regional distribution of nosocomial infections due to ESBL-positive *Enterobacteriaceae* in Germany: data from the German National Reference Center for the Surveillance of Nosocomial Infections (KISS). Clinical Microbiology and Infection. 2015; 21: 255.e1-255.e5.

[19] Zhang H, Yang Q, Liao K, Ni Y, Yu Y, Hu B, *et al.* Update of incidence and antimicrobial susceptibility trends of *Escherichia coli* and *Klebsiella pneumoniae* isolates from Chinese intra-abdominal infection patients. BMC Infectious Diseases. 2017; 17: 776.

[20] Castanheira M, Farrell SE, Deshpande LM, Mendes RE, Jones RN. Prevalence of β-lactamase-encoding genes among *Enterobacteriaceae* bacteremia isolates collected in 26 U.S. hospitals: report from the SENTRY antimicrobial surveillance program (2010). Antimicrobial Agents and Chemotherapy. 2013; 57: 3012-3020.

[21] Han SH, Kim YA, Wang M, Lee Y, Chung H, Yum JH, *et al.* Comparison of the genetic structures surrounding qnrA1 in Korean *Enterobacter cloacae* and Chinese *Escherichia coli* strains isolated in the early 2000s: evidence for qnrA mobilization via Inc HI2 type plasmid. The Journal of Microbiology. 2012; 50: 166-169.

[22] Qu H, Wang X, Ni Y, Liu J, Tan R, Huang J, *et al.* NDM-1-producing *Enterobacteriaceae* in a teaching hospital in Shanghai, China: IncX3-type plasmids may contribute to the dissemination of blaNDM-1. International Journal of Infectious Diseases. 2015; 34: 8-13.

[23] Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. Antimicrobial Agents and Chemotherapy. 2009; 53: 2227-2238.

[24] Yang Q, Zhang H, Wang Y, Xu Y, Chen M, Badal RE, *et al.* A 10 year surveillance for antimicrobial susceptibility of *Escherichia coli* and *Klebsiella pneumoniae* in community- and hospital-associated intra-abdominal infections in China. Journal of Medical Microbiology. 2013; 62: 1343-1349.

[25] Du N, Liu S, Niu M, Duan Y, Zhang S, Yao J, *et al.* Transmission and characterization of bla NDM-1 in *Enterobacter cloacae* at a teaching hospital in Yunnan, China. Annals of Clinical Microbiology and Antimicrobials. 2017; 16: 58.

[26] Charfi K, Grami R, Ben Jeddou A, Messaoudi A, Mani Y, Bouallegue O, *et al.* Extended-spectrum β-lactamases and plasmid-mediated quinolone resistance in enterobacterial clinical isolates from neonates in Tunisia. Microbial Pathogenesis. 2017; 110: 184-188.

[27] Zhang R, Ichijo T, Hu Y, Zhou H, Yamaguchi N, Nasu M, *et al.* A ten years (2000-2009) surveillance of resistant *Enterobacteriaceae* in Zhejiang Province, China. Microbial Ecology in Health and Disease. 2012; 23.

[28] Chanawong A, M'Zali FH, Heritage J, Xiong J, Hawkey PM. Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14, among *Enterobacteriaceae* in the People's Republic of China. Antimicrobial Agents and Chemotherapy. 2002; 46: 630-637.

[29] Matsumoto M, Suzuki Y, Miyazaki Y, Tanaka D, Yasuoka T, Mashiko K, *et al.* Enterobacterial Repetitive Intergenic Consensus Sequence-Based PCR (ERIC-PCR); its ability to differentiate streptococcus pyogenes strains and applicability to the study of outbreaks of streptococcal infection. The Tohoku Journal of Experimental Medicine. 2001; 194: 205-212.

[30] Woodford N, Turton JF, Livermore DM. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiology Reviews. 2011; 35: 736-755.

[31] Alibi S, Ferjani A, Boukadida J. Molecular characterization of extended spectrum beta-lactamases produced by Klebsiella pneumoniae clinical strains from a Tunisian hospital. Medecine et Maladies Infectieuses. 2015; 45: 139-143.

[32] Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, *et al.* Prevalence and spread of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in Europe. Clinical Microbiology and Infection. 2008; 14: 144-153.

[33] Luo Y, Yang J, Ye L, Guo L, Zhao Q, Chen R, *et al.* Characterization of KPC-2-Producing *Escherichia coli*, *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Klebsiella* oxytoca isolates from a Chinese hospital. Microbial Drug Resistance. 2014; 20: 264-269.