

Antimicrobial resistance and epidemiology of ESBLs-producing *Escherichia coli* and *Enterobacter cloacae* isolates from the intensive care unit in an affiliated hospital of University, China

Kun Chen

Army Medical University

Guoliang Yang

Army Medical University

Wenping Li

Army Medical University

Mingcheng Li (✉ limingcheng@beihua.edu.cn)

Beihua University

Research

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Abstract

Background Concerns are increasing over the importance of the hospital intensive care units (ICU) for the transmission of extended spectrum- β -lactamase (ESBLs) -producing *Enterobacteriaceae*. We reported the clinical characteristics and epidemiology of ESBLs isolates collected from a tertiary care hospital in China.

Methods *Escherichia coli* (*E. coli*) and *Enterobacter cloacae* (*E. cloacae*) isolates from ICU infection samples were isolated and identified. Antimicrobial susceptibility profiles and production of ESBLs were determined by using the disk diffusion method and the broth microdilution method. Clonality of isolates was determined by ERIC-PCR techniques.

Results From the included the 223 strains isolated from hospitalized patients with nosocomial infections in ICU during 2016 to 2018, the majority of isolates belonged to Gram-negative *Enterobacteriaceae* including *E. coli* (46.6% of all strains), and *E. cloacae* (46.2% of all strains). 63.25% of samples were separated from sputum or tracheal secretions. All of 207 isolates, ESBL-screen positive *E. coli* was 45.2% (47/104), and 44.7% (46/103) for *E. cloacae*. Resistance rates of ESBLs-producing *E. coli* and *E. cloacae* isolates were 95.5%-91.3% for ampicillin, 80.6%-76.1% for ampicillin/azobactam, 88.1%-28.3% for ciprofloxacin, 89.6%-15.2% for levofloxacin, 34.3%-45.7% for netilmicin, 82.1%-41.3% for compound sulfamethoxazole, 20.9%-43.5% for amikacin, 58.2%-37.0% for gentamicin, 20.9%-69.6% for piperacillin/tazobactam. All of ESBLs-producer isolates resistant to cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime in addition to aztreonam were 100%, whereas the susceptibilities of isolates to imipenem and meropenem were 100%.

Results of ERIC-PCR in all of ESBLs-producing *E. coli* isolates exhibited 11 distinct patterns using a similarity coefficient of 0.8. And one distinct ERIC profiles were observed amongst 46 strains of ESBLs-producing *E. cloacae*. ERIC profiles demonstrated an outbreak of nosocomial infection and ESBLs-producing *E. coli* and *E. cloacae* prevalent in the ICU of this hospital.

Conclusions Our data indicate that the ESBLs-producing *E. coli* and *E. cloacae* clones are circulating in the ICU and constitute a major source for further disseminating in this hospital. It is necessary to increase surveillance and development of adequate prevention strategies.

Introduction

The incidence of infections due to the emergence and dissemination of *Enterobacteriaceae* has rapidly increased for the last several decades. Resistance of *Enterobacteriaceae* to extended-spectrum β -lactams worldwide is becoming a major public health problem [1]. Both *Escherichia coli* (*E. coli*) and *Enterobacter cloacae* (*E. cloacae*) presently are the ESBL producing Gram-negative *Enterobacteriaceae*, which have emerged as the most common cause of hospital-and community-acquired infection admitted to neonatal intensive-care settings [2]. The nosocomial infections of the intensive care units (ICU) that occur in the specific environment in which the treatment and rehabilitation of critically ill patients is frequently

affected [3]. Known risk-factors for onset of infection in ICU with drug-resistant bacteria include: widespread abuse or irrational use of antibiotics, the rapid renewal of antibiotics, pathogens, in the specific ICU environment, critically illness, undergoing surgery, use of invasive medical devices, prolonged hospital stay, etc [4, 5]. Onset of sepsis in ICU is the most common cause of infection-related mortality. Since appropriate antimicrobial treatment is essential for infections in ICU, institutional surveillance of infection-derived bacteria isolates and analysis of their susceptibility to different antimicrobial agents provides crucial information for the choice of empirical antimicrobial therapy. Besides that, the comprehensive analysis of ICU infection epidemiology and the evaluation of the prognosis of the disease are of great significance [6, 7].

The purpose of the current study mainly focused on the bacterial distribution and drug resistance characteristics of ICU infections. Furthermore, we aimed to explore the epidemiology of pathogenic bacteria transmission and diffusion resistance, through retrospective investigation on infectious cases in the ICU of a third-level hospital in Jilin from 2016 to 2018.

Materials And Methods

Settings and collection of specimens

This study was conducted at an affiliated hospital of University, one of the largest hospitals in Jilin of the North-East China, with approximately 2, 000 beds. We carried out a retrospective study of *Enerobacteriaceae*-infected patients in the three ICU settings. 283 patients were enrolled in ICU from June 2016 to June 2018. The specimens were mainly obtained from sputum or tracheal secretions (n = 179), pus (n = 33), blood (n = 43), ascites (n = 20), catheters and drainage tubes (n = 7) and cerebrospinal fluid (n = 2). No repetitive isolates from a single patient were included. This study to collect the clinical samples was approved by ethical committees of Beihua University (formal ethical approval number: Protocol Number 2016-01-01), and written informed consent was gained from all participants in the study prior to the initiation of the study.

Identification and detection of resistance to 17 antibiotics agents

The identification of *E. coli* and *E. cloacae* was identified by using the Vitek 2 Compact System with GN card and ASTGN13 card (bioMérieux, Marcy l'Etoile, France). Susceptibility to a panel of 16 antimicrobial agents was determined according to Clinical and Laboratory Standards Institute recommendations (CLSI, 2012) [8]. The tested antibiotics (AB Biodisk, Solna, Sweden) were included: ampicillin, cefazolin, cefuroxime, ceftazidime, ceftriaxone, cefepime, levofloxacin, netilmicin, aztreonam, ciprofloxacin, amikacin, gentamicin, imipenem, meropenem, ampicillin /azobactam, piperacillin / tazobactam and compound sulfamethoxazole.

MIC values for 17 drugs at 12 concentrations *in vitro* were determined using a micro broth dilution method. *E. coli* ATCC 25922, *K. pneumoniae* 700603 (ESBL positive) and *E. cloacae* ATCC 13047 were

used as quality control strains.

Determination of *ESBLs-producing strains*

Suspected ESBLs-producing strains were screened using cefotaxime (30 µg) and ceftazidime (30 µg) in combination with clavulanic acid (10 µg) according to the CLSI recommended disk diffusion method [8].

Clonality analysis of *E. coli* and *E. cloacae* isolates *by* ERIC

Total DNA extraction from *E. coli* and *E. cloacae*

A single colony was selected from the passage medium and incubated overnight at 37 ° 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 5 minutes. The supernatant was discarded and added to 400 µl dd H₂O and boiled for 10 minutes after mixing. The mixture was cooled and centrifuged at 12, 000 r/min for 5 minutes, and the supernatant was absorbed and stored at - 20° C.

ERIC-PCR amplification

The primer sequences were P1-ATGTAAGCTCCTGGGGATTAC and P2- AAGTAAGTGACTGGGGTGAGCG. The system contained 2 µl of P1 and P2 primers, and the DNA template solution at 3 µg/l. ddH₂O was added to the total reaction system of 50 µl. PCRs were conducted in a GeneAmp PCR system 9600 (Perkin-Elmer, USA) under the following reaction conditions; denaturation at 94 °C for 5 min, denaturation at 94 °C for 30 s, annealing at 56-58 °C for 45 s, extension at 72 °C for 30 s, and 32 cycles at 72 °C after 5 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 for 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). 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 according to the reference [9].

Statistical analysis

Differences in the drug resistance rates of non-ESBLs-producing and ESBLs-producing strains were tested by Chi-square test. All drug resistant data were analyzed using SPSS13.0 statistical software. A value of $P < 0.05$ was considered as statistically significant.

Results

Epidemiological characteristics of specimens and isolates

From the included the 223 strains isolated from hospitalized patients with nosocomial infections in ICU during 2016 to 2018, the majority of isolates belonged to Gram-negative *Enerobacteriaceae* including *E. coli* (46.6% of all strains), which accounted for 24 strains collected in 2016, 55 strains in 2017 and 25

strains in 2018; *E. cloacae* (46.2% of all strains), which accounted for 20 strains collected in 2016, 61 strains in 2017 and 22 strains in 2018; others were not included in the study (data not shown).

The clinical distribution of the specimens was mainly composed of sputum or tracheal secretions accounting for 63.3% of the samples, followed by skin and purulent infections(11.5%), blood 15.2%, ascites 7.1%, catheters and drainage tubes 2.4% and cerebrospinal fluid 0.5%.

Determination of ESBLs phenotypes

In the primary screening test, 72 strains of *E. coli* in the inhibitory zone to ceftazidime with a diameter of ≤ 22 mm, and the inhibitory zone to ceftriaxone, which was ≤ 25 mm in diameter, were highly suspected ESBLs-producing bacteria. It was confirmed that 67 strains of *E. coli* alone were detected in the inhibitory zone to cephalosporin which had a diameter of ≥ 5 mm. At the same time, the diameter of the inhibitory zone to clavulanic acid was ≥ 5 mm.

All of 103 strains of *E. cloacae*, 61 strains of *E. cloacae* to ceftazidime and ceftriaxone were highly suspected to produce ESBLs in the screening test. The inhibitory zone to ceftazidime with a diameter of ≤ 22 mm and the inhibitory zone to ceftriaxone which was ≤ 25 mm in diameter were highly suspected to produce ESBLs. It was confirmed that there were 46 strains of *E. cloacae* isolates whose inhibitory zone diameter to cephalosporin was ≥ 5 mm. Meanwhile, the diameter of the inhibitory zone to clavulanic acid was ≥ 5 mm.

Antimicrobial susceptibility of *E. coli* and *E. cloacae*

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

The susceptibilities of non-ESBLs-screen positive *E. coli* and *E. cloacae* isolates were 89.2%-91.3% to ampicillin, 97.3%-96.5% to ampicillin/azobactam, 94.6% -94.7% to cefazolin, 89.2%-96.5% to cefuroxime, 97.3%-98.3% to ceftazidime, 97.3%-98.3% to ceftriaxone, 91.9%-92.3% to cefepime, 89.2%-100.0% to aztreonam, 32.4%-68.4% to ciprofloxacin, 37.8%-91.2% to levofloxacin, 70.3%-56.1% to netilmicin, 43.2%-61.4% to compound sulfamethoxazole, 62.2%-71.9% to amikacin, 43.2%-68.4% to gentamicin, 97.3%-93.0% to piperacillin/tazobactam respectively (Table 1, Table 2). Only the susceptibilities to imipenem and meropenem were 100%.

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

Antimicrobiol agents	ESBL positive (n = 67)		ESBL negative (n = 37)		χ^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

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

Antimicrobiol agents	ESBL positive (n = 46)		ESBL negative (n = 57)		χ^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

Next, we investigated a local difference of antibiotic resistance between ESBLs and non-ESBLs strains. There was significant difference between non-ESBLs-producing strains and ESBLs-producing strains ($P < 0.05$) except the susceptibility to ciprofloxacin ($P \geq 0.05$).

Determination of MIC values

The MIC values of 67 strains of ESBLs-producing *E. coli* against ampicillin, ceftriaxone, cefazolin, aztreonam, cefixime and ceftazidime were more than 256 $\mu\text{g/ml}$. The MIC for both levofloxacin and cotrimoxazole was greater than 128 $\mu\text{g/ml}$. Ampicillin/sulbactam and piperacillin/tazobactam were highly resistant to ampicillin / sulbactam (MIC = 32 $\mu\text{g/ml}$).

The MIC of 46 strains of ESBLs-producing *E. cloacae* against ampicillin, ceftriaxone, cefazolin, aztreonam, cefixime and ceftazidime was all greater than 256 $\mu\text{g/ml}$. The MIC of amikacin, netilmicin, ampicillin/sulbactam and piperacillin/tazobactam was 32 $\mu\text{g/ml}$.

Clonality of isolates analysis by ERIC-PCR

Clonality of isolates analysis of all the ESBLs-producing strains was investigated by ERIC-PCR typing. Based on the ERIC-PCR typing, one distinct ERIC profiles were observed amongst 46 strains of ESBLs-producing *E. cloacae*, showing that these isolates were of the equal clones (Fig. 1). We selected a representative of the same band in 67 ESBLs-producing *E. coli* isolates from different samples for dendrogram cluster analysis, revealed 11 distinct patterns using a similarity coefficient of 0.8. An obvious clonal association was found within these strains, of which 54 (80.6%) were of the identical clones (Figs. 2 and 3), indicating an outbreak situation. The history of the same drug-resistant strains in these ICU patients was similar. Most of the drug-resistant strains with the same clones had more infections in the lower respiratory tract, and 76% of patients had a history of mechanical ventilation.

Discussion

Antibacterial drugs have been administered extensively in clinic, especially in the treatment of ICU patients. However, the widespread use of antibacterial drugs produces further resistant bacteria, which is becoming a serious public health concern [10]. The abuse of this antibacterial drug causes the body to produce multi-drug resistance and the risk of super bacterial infection increases. The rational application of antibacterial drugs in the clinic effectively prevents the spread of drug-resistant strains and more comprehensive understanding of drug resistance mechanisms, and epidemiology of drug-resistant strains has much potential to improve the cure rates of ICU patients [11].

The majority of ICU isolates collected in the participating multicenters consisted of *E. coli* and *Klebsiella pneumoniae* in addition to *E. cloacae* in China [12, 13]. In this study, ESBLs-producing and non-ESBLs-producing strains of *E. coli* showed significant differences in resistance to cephalosporins with ESBLs-producing strains having significantly higher resistance rates. In contrast, there was no obvious

difference between the ESBLs-producing and non-ESBLs-producing strains concerning ciprofloxacin. In the comparison to the sensitivity of cephalosporin antibiotics with sulbactam and tazobactam, the drug resistance was extremely lower than that of cephalosporins alone. This group of studies fully demonstrated that if the production of ESBLs can be reduced as much as possible, the resistance of *E. coli* can also be greatly reduced.

E. coli and *E. cloacae* belong to conditional pathogens that are part of the normal intestinal flora but can cause infections of the respiratory and urinary tracts [14, 15]. An epidemiological study has reported that the detection rate of *E. coli* and *E. cloacae* producing ESBLs was increasing, 44% in Singapore, 37% in China [16]. Both *E. coli* and *E. cloacae* producing ESBLs are closely related to the drug resistance of antibiotics, and the same type of bacteria are likely to carry a variety of ESBLs, leading to multi-drug resistance [17, 18]. Studies have also shown that there are multiple drug resistance mechanisms in *E. coli* and *E. cloacae*, which include mutations in Amp C enzymes and porin loss, which has been described in previous literatures [19, 20]. However, we did not explore molecular mechanisms of resistances.

In this study, we collected samples from different parts of the patients and found that *E. coli* and *E. cloacae* were most common in infections of the respiratory tract and skin. The resistance of *E. cloacae* to 17 antimicrobial agents was similar to that of *E. coli*. All of them exhibited high resistance to cephalosporins, whilst they were highly sensitive to carbapenems. Because the northeast part of China is the most colded region with the highest incidence of respiratory system diseases, antimicrobial overuse might be an explanation for the antimicrobial agents susceptibility difference in this area.

Clinically, the application of a large number of high-grade cephalosporin antibiotics in the treatment of ICU patients with nosocomial infections and unreasonable use of the third-generation cephalosporins have resulted in a large number of drug-resistant strains [21, 22]. Patients who are ineffectively treated with advanced cephalosporins or β -lactamase inhibitors can only further use carbapenem antibacterial drugs in treating Gram-negative bacteria [23].

Carbapenems have the strongest antimicrobial activity, broadest antimicrobial spectrum and belong to atypical beta-lactam antimicrobial agents. The low toxicity and high stability of beta-lactamase make them one of the best options for the treatment of severe infections when other antimicrobial agents are ineffective [24, 25].

In this study, both *E. coli* and *E. cloacae* were highly sensitive to carbapenems, and no significant difference in resistance between ESBLs-producing and non-ESBLs-producing strains was observed, indicating that carbapenems could be used as an option in the treatment of ESBLs-resistant strains. In contrast to data collected from centers in east China, the reduced susceptibilities of *E. coli* and *E. cloacae* indicated a local carbapenem resistance [26]. Taken together, the carbapenem overuse might be an explanation for the difference because of the developed areas with the higher incomes [27].

In the epidemiological analysis of nosocomial infections in the ICU, ERIC-PCR was used to identify isolates EIC-PCR fingerprints. Results of ERIC-PCR in all of ESBLs-producing *E. coli* isolates exhibited 11

distinct profiles, and one distinct ERIC profiles were observed amongst 46 strains of ESBLs-producing *E. cloacae*. Since the establishment of ERIC-PCR technique by versalovic *et al.* in 1991, it has played an important role in the identification and epidemiological investigation of Gram-negative bacteria. In 2001, Matsumoto *et al.* applied two different methods, ERIC-PCR and PFGE, to detect 23 clinical isolates of *Berkholderia* onions. The results indicated that two methods have similar detection abilities and reproducibility [28, 29]. ERIC profiles demonstrated an outbreak of nosocomial infection and ESBLs-producing *E. coli* and *E. cloacae* prevalent in the ICU of this hospital.

The study found that ESBLs-producing bacteria were resistant to aminoglycoside antibiotics such as levofloxacin, and were more resistant to the above two types of antibacterial drugs than those with high resistance to cephalosporin antibiotics. The resistance to synthetic antibiotics such as sulfonamides was also lower than that of simple ampicillin. In the clinical application of antibiotics, the initial use of advanced cephalosporin antibiotics, especially the unreasonable use of the third generation cephalosporins, is the beginning of habitual therapy. These practices cause us to ignore the role of aminoglycosides and other antibacterial drugs that lead to common clinical drug resistance. In order to control the production and spread of ESBLs from the source, more reasonable use of antibacterial drugs should be followed by limiting the routine empirical application of high-level antibiotics by medical personnel. Publicity and education on the use of antibacterial drugs amongst health care workers should be strengthened along with improvements in the management of pharmacies and hospital ICUs [30, 31]. These measures could effectively cut off the source of infection and a mode of transmission of ESBLs bacteria. In addition, this could guide medical staff to rationally use antimicrobial agents [32]. This study has some limitations. Firstly, the genotypic or molecular data of all strains were not documented. Secondly, the molecular epidemiology was not included. Further studies are needed to confirm the genetic types and the mechanism of transmission.

Conclusions

our findings indicate that the ESBLs-producing *E. coli* and *E. cloacae* clones are circulating in the ICU and constitute a major source for further disseminating in this hospital. Carbapenems are the reasonable choice in the treatment of ESBLs-producing bacteria. It is necessary to increase surveillance and development of adequate prevention strategies.

Abbreviations

ICU: intensive care units; ESBLs: extended spectrum- β -lactamase; *E. cloacae*: *Enterobacter cloacae*; *E. coli*: *Escherichia coli*; ERIC: Enterobacterial repetitive intergenic consensus.

Declarations

Acknowledgement

Not applicable.

Authors' contributions

Ken Chen conceived, designed the experiments and wrote a draft manuscript. Mingcheng Li analyzed, interpreted the results of the experiments and revised the manuscript. Guoliang Yang and Wenping Li performed the experiments. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are available in this published article.

Ethics approval and consent to participate

This study to collect the clinical samples was approved by ethical committees of Beihua University (formal ethical approval number: Protocol Number 2016-01-01), and written informed consent was gained from all participants in the study prior to the initiation of the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

Author details

¹Department of Clinical Nursing, School of Nursing, Army Medical University, Chongqing, 400042, China

²Department of Ultrasound, XinQiao Hospital, Army Medical University, Chongqing, 400042, China

³Department of Obstetrics and Gynecology, DaPing Hospital, Army Medical University, Chongqing, 400042, China

⁴School of Laboratory Medicine, Beihua University, Jilin, 132013, China

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Figures

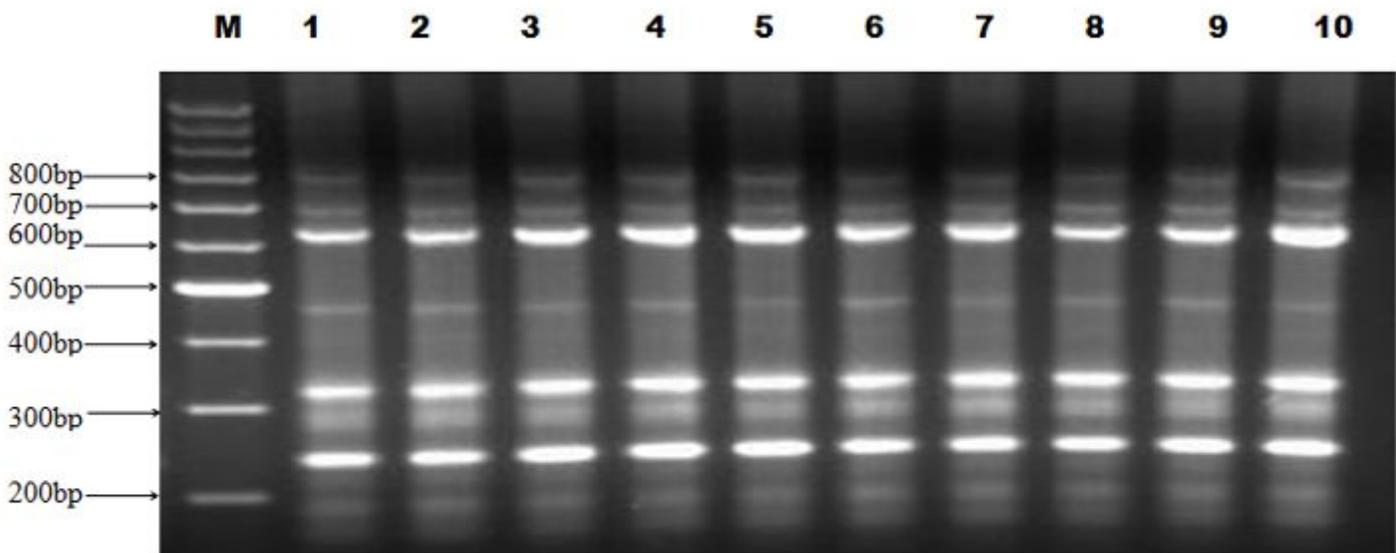


Figure 1

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

Footnotes for Figure 1 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 catheters and drainage tube; 11-13: strains isolated from sputum or tracheal secretions; ERIC: Enterobacterial repetitive intergenic consensus

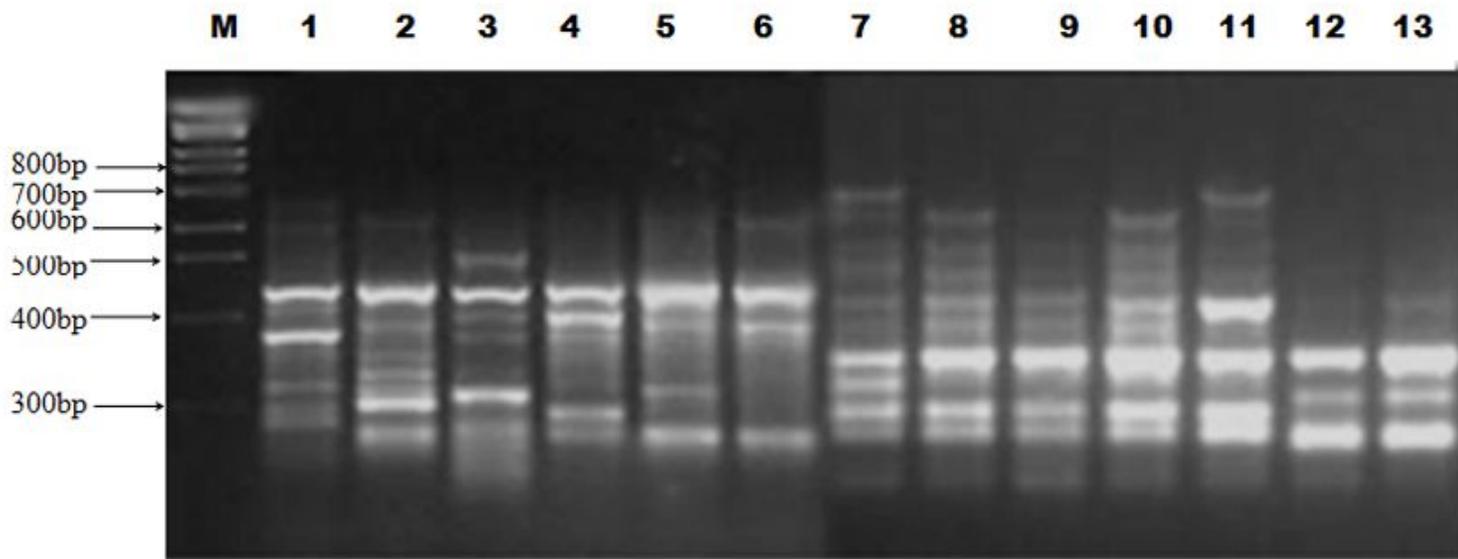


Figure 2

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

Footnotes for Figure 2 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 catheters and drainage tube; 11-13: strains isolated from sputum or tracheal secretions; ERIC: Enterobacterial repetitive intergenic consensus

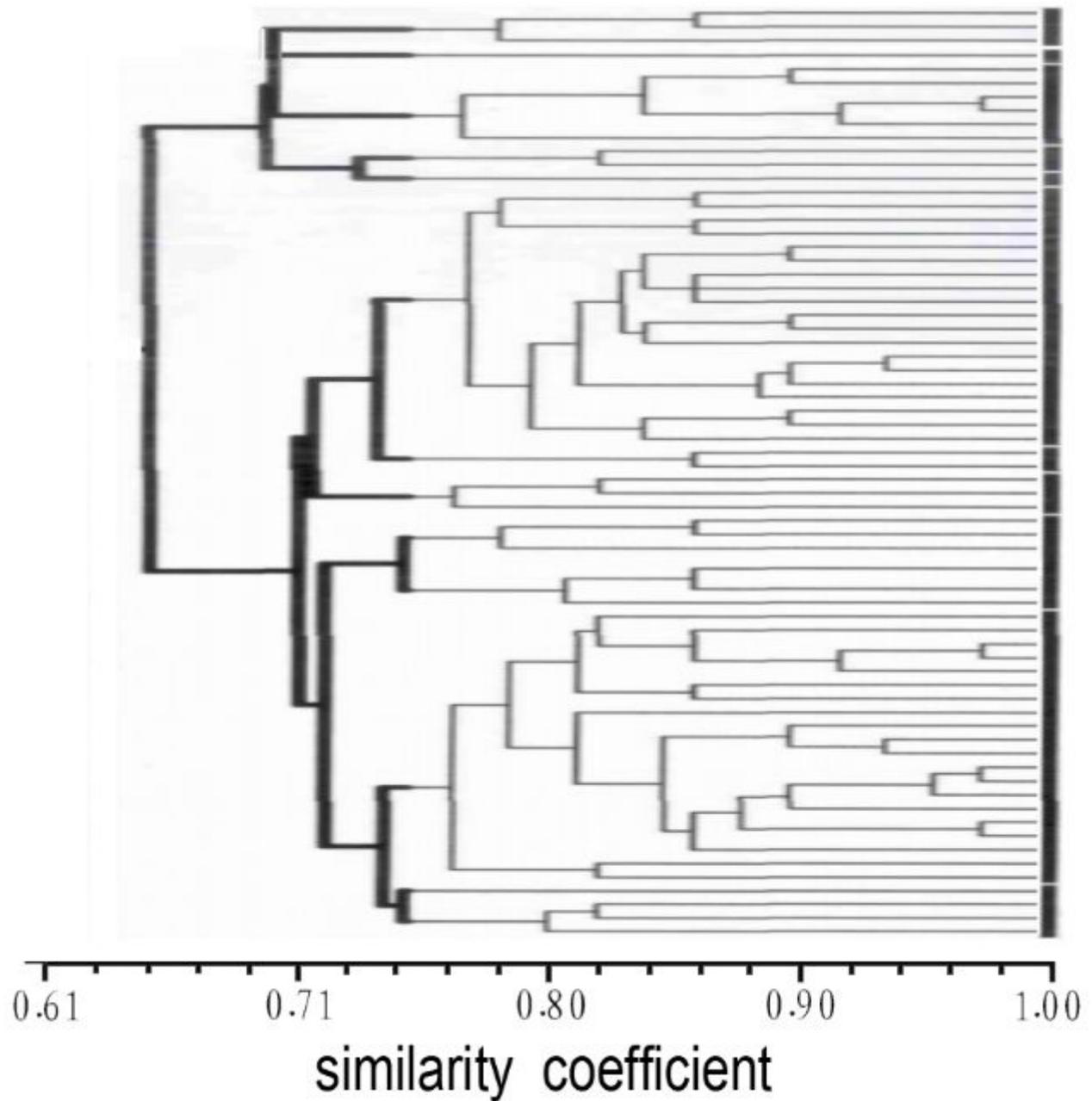


Figure 3

Dendrogram from ERIC-PCR analysis in ESBLs-producing E. coli isolates Footnotes for Figure 3 Note: The scale bar showed the similarity values. ERIC: Enterobacterial repetitive intergenic consensus

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