Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Analysis of drug resistance genes of integrons in clinical isolates of *Escherichia coli* from elderly bloodstream infections

Wei Liu, Fangjian Zhao, Jian Chen*

Second Department of laboratory, Hunan Provincial People's Hospital (The First Affiliated Hospital of Hunan Normal University), Changsha, Hunan410006, China

ARTICLE INFO

Original paper

Article history:
Received: December 12, 2022
Accepted: March 18, 2022
Published: June 30, 2022

Keywords:

Elderly patients, bloodstream infection, Escherichia coli, integrated gene, drug resistance.

ABSTRACT

This experiment was carried out to provide a basis for the treatment of clinical bloodstream infections by analyzing the drug resistance characteristics and integrated gene distribution of Escherichia coli in bloodstream infections in elderly patients. For this aim, E. coli were collected for bacterial identification and drug sensitivity testing from bloodstream infections in elderly patients in the hospital from January 2016 to December 2019. ESBLs positive strains were assayed for genotypes and their integron carriage rates by PCR amplification. The characteristics and differences of various genotype rates were compared and analyzed. Results showed that a total of 230 E. coli strains were isolated. The detection rate of ESBLs-producing bacteria was 37.39 %. ESBLs-producing E. coli showed a high rate of resistance to cefepime, levofloxacin, cotrimoxazole, and ticarcillin/clavulanic acid (>40%). The resistance rate of 230 strains of E. coli to meropenem, minocycline, amikacin, gentamicin and cefoxitin was less than 20%. Among the ESBLs-producing E. coli in bloodstream infections in elderly patients, CTX-M-9 accounted for 27.91%, CTX-M-2 for 17.44%, and SHV for 13.95%. The detection rate of type I integrated genes was 41.30%, and type II and III integrated genes were not detected. ESBLs-producing genotyping-positive bacteria were detected with more than 50% of type I integrated genes. It was concluded that type I integrated genes in ESBLs-producing E. coli isolated from elderly patients carried resistance genes such as CTX-M-9 and CTX-M-2 aggravating multi-drug resistance in bacteria.

Doi: http://dx.doi.org/10.14715/cmb/2022.68.6.11

Introduction

Elderly patients have decreased immunity due to aging and a decreasing function of tissues and organs. At the same time, the chances of bloodstream infections increase because of frequent hospitalization for various invasive clinical medical operations (1,2). Moreover, bloodstream infections in the elderly are characterized by higher severity, less obvious symptoms and high mortality. Therefore, clinicians are paying more and more attention to the drug-resistant characteristics of pathogenic bacteria in bloodstream infections in elderly patients.

The β-lactam ring is a structure with antibacterial activity for a variety of antibacterial drugs such as penicillins, cephalosporins and monocyclic lactams, which can cause damage to the bacterial cell wall. However, β-lactamases can bind to the β-lactam ring and change the binding target of antibacterial drugs to bacteria, thus reducing or even eliminating their antibacterial activity (3). Clinical studies have shown that extended-spectrum β-lactamases (ESBLs) are an important mechanism for the development of bacterial resistance (4).

As a common causative agent of bloodstream infections, *E. coli* is located first in the rate of isolation of Enterobacteriaceae in the Chinese Bacterial Resistance Surveillance Study (5). ESBLs produced by *E. coli* can spread drug resistance genes among bacteria by integrating genes into a mobile gene element and plasmid-mediated. Moreover, they have the function of capturing drug-resistant gene cassettes. This has led to a rising trend of hospital infections and drug resistance, which has become a problematic issue for physicians (6,7).

In this study, we retrospectively analyzed the clinical data and drug resistance characteristics of 230 strains of elderly patients with *E. coli* bloodstream infections and amplified the genotypes of ESBLs-producing genotypes to identify the changes in drug resistance and the distribution composition of ESBLs genotypes so as to provide a reference basis for clinical treatment and prevention of ESBLs-producing bacterial infections.

Materials and Methods

General information

9712 blood culture specimens were collected from January 2016 to December 2019 among elderly patients (aged >60 years) sent for fever in various clinical departments of our hospital. Clinically confirmed 1160 pathogenic bacteria were isolated. Drug-resistant data were collected according to the criteria for 230 strains of *E. coli* infection, including 121 males and 109 females aged 60 to 97 years, with a mean age of (64.99 ± 12.94) years. The study was approved by the hospital ethics committee with the informed consent of the patients.

Inclusion criteria: Patients who met both clinical diagnostic criteria and pathogenic, diagnostic criteria were

* Corresponding author. Email: chenjian9240@126.com

included according to the 2001 Diagnostic Criteria for Hospital Infections (Trial) of the Ministry of Health of the People's Republic of China (8). Clinical criteria: Patients with fever > 38.0 °C or < 36.0 °C with chills, combined with one of the following: invasive portal or migratory lesions; systemic toxicity without obvious foci of infection; hepatosplenomegaly with rash, hemorrhagic spots, and neutrophilia with leftward nuclear shift on blood analysis; systolic blood pressure below 90 mm Hg or more than 40 mm Hg drop from the original systolic blood pressure. *Pathogenic diagnostic criteria:* pathogenic microorganisms were isolated by blood culture. *Exclusion criteria:* Age <60 years; The same strain was isolated from the same patient for 2 consecutive times. The second result was not included in the statistics.

Instruments and reagents

Instruments included BD9120 automated blood culture instrument (BD), VITEK-2 bacterial identification system (Mérieux, France), API real-time fluorescence quantitative Step one gene amplification instrument (USA), and a gel imaging system (Bio-Rad, USA), and Helena electrophoresis instrument (USA). DNA Marker, *Taq* DNA polymerase, agarose and amplification reagents were purchased from Shanghai Bao Biological Company. Ceftazidime (Lot No. CT0142B), ceftazidime/clavulanic acid (Lot No. 231- 754), cefotaxime (Lot No. CT0166B), cefotaxime/clavulanic acid (Lot No. 231752) paper sheets were from Oxoid (UK).

Specimen collection

All blood culture specimens were collected in a standardized manner. Blood was collected from 5 ml to 10 ml, injected into special blood culture enrichment bottles, and put into BD91200 automatic blood culture instrument for culture.

Judgment of results

When the instrument alarm was positive, transient isolation culture was performed in the blood plate, while the smear was done for Gram staining. The growth of bacteria on the blood plate was positive, and no bacterial growth on the blood plate was a false positive. After five days, no alarm was detected by the instrument, and no bacterial growth on the culture of the transfected blood plate was considered negative, whereas any bacterial growth was considered false negative.

Bacterial identification and drug sensitivity test

Bacteria were isolated by conventional methods. They were identified as *E. coli* and *Klebsiella* pneumoniae by the fully automated microbiological analyzer. The drug sensitivity test results were judged according to the 2017 American Clinical Laboratory Standardization Institute (CLSI) guidelines (6). The quality control strain was *E. coli* ATCC25922.

ESBL phenotype confirmation assay

Measurements were performed using the VITEK2 Compact automated microbiology analyzer and its accompanying drug sensitivity card. The results were interpreted according to the CLSI standards. The minimum inhibitory concentration (MIC) of ceftriaxone, ceftriaxone/clavulanic acid, ceftazidime and ceftazidime/clavulanic acid were determined, respectively. The MIC ratio of clavulanic acid-free to clavulanic acid-containing strains was ≥8, which could be confirmed as ESBL-producing strains.

Integrator DNA template extract preparation

The bacteria to be tested were incubated on blood plates for 18~24h. 6~8 colonies were taken in 200μL of sterilized double-distilled water and ground to make a fresh bacterial suspension. The prepared bacterial DNA template was boiled at 95°C for 10min, placed at -20°C for 5min, centrifuged at 13000r/min for 10min, and the supernatant was taken to make the integron DNA template extract (stored at -20°C refrigerator).

Polymerase chain reaction (PCR) amplification

ESBLs-producing *E. coli* genes and integrated genes were designed with reference to the literature. The primer sequences for amplification were synthesized by Beijing Sanbo Yuanzhi Biological Company. The specific sequences are shown in Table 1. A gene PCR reaction vo-

Table 1. PCR amplification primer sequences.

Gene	Primer sequence (5'→3')	Product size (bp)	Annealing temperature
TEM	F ATAAAATTCTTGAAGAC R TTACCAATGCTTAATCA	1075bp	58°C
SHV	F GGGTTATTCTTATTTGTCGC R TTAGCGTTGCCAGTGCTC	931bp	56°C
CTX-M-9	F GTGACAAAGAGAGTGCAACGG R ATGATTCTCGCCGCTGAAGCC	856bp	62°C
CTX-M-1	F TTAGGAARTGTGCCGCTGYA R CGATATCGTTGGTGGTRCCAT	688bp	60°C
CTX-M-2	F CGTTAACGGCACGATGAC R CGATATCGTTGGTGGTRCCAT	404bp	60°C
Intl1	F GGTCAAGGATCTGGATTTCG R ACATGCGTGTAAATCATCGTC	456bp	62°C
Intl2	F CACGGATATGCGACAAAAAGGT R GTAGCAAACGAGTGACGAAATG	789bp	62°C
Intl3	F AGTGGGTGGCGAATGAGTG R TGTTCTTGTATCGGCAGGTG	433bp	60°C

lume of 25μl was composed of 1μl of each primer, 2μl of DNA template, 2.5μl of 10×PCR buffer (Mg2+), and 2.0μl of dNTP (2.5 mmol/L). The reaction was amplified under the action of *Taq* DNA polymerase 0.5 U, with the conditions of pre-denaturation at 95°C for 5 min, 94°C for 30 s, 50°C for 40 s, 72°C for 40 s, for a total of 35 cycles, and final extension at 72°C for 10 min, using an American API real-time fluorescence quantitative Step one gene amplification instrument. Their PCR products were analyzed by agarose gel electrophoresis under a gel imager to observe and record the results.

Sequencing and analysis of amplification products

The amplified products were sent to Huada Gene Biotechnology Co., Ltd. for bi-directional sequencing. The sequencing results were corrected and spliced in DNA star software. The gene sequences of the variable region of the integrator were analyzed by BLAST comparison in Gen Bank database.

Statistical processing

The data of drug resistance of *E. coli* were statistically analyzed by Whonet 5.6 and SPSS 22.0 software. The cardinality test was used to count the rates, and the differences were indicated as statistically significant according to $P < 0.05$. Trend analysis was performed by applying the Armitage linear trend $\chi 2$ test with $\alpha = 0.05$ and $P < 0.05$ as a statistically significant difference.

Results and discussion

Detection and distribution of *E. coli* in elderly bloodstream infections

Of 9712 blood culture specimens sent for fever in elderly patients, 1160 strains of pathogenic bacteria were isolated, with an isolation rate of 11.94%. Among them, 230 strains of *E. coli* were isolated, accounting for 19.83%. The isolation rate of pathogenic bacteria increased gradually with time ($\chi 2 = 7.493$. $p < 0.05$), while the proportion of *E. coli* in the pathogenic bacteria did not increase year by year ($\chi 2 = 1.096$ $p = 0.295$) (Table 2). The patients were mainly from the intensive care unit, neurosurgery and general surgery (Figure 1).

Analysis of resistance to ESBLs-producing and non-ESBLs-producing *E. coli* in bloodstream infections in elderly patients

The number of ESBLs-producing strains was 86 out of 230 strains of *E. coli* from bloodstream infections in elderly patients, with a positivity rate of 37.39%. The resistance rate of 230 strains of *E. coli* to meropenem, minocycline, amikacin, gentamicin and cefoxitin was less than 20% by comparing the resistance rate of non-ESBLs-producing and ESBLs-producing *E. coli*. There was a significant difference in the rate of resistance to ticarcillin/clavulanic acid, ceftriaxone, ampicillin, cefuroxime ceftazidime, cefepime, lomefloxacin, aminotrans, levofloxacin, meropenem, cefoxitin, and amikacin between ESBLs-producing and non-ESBLs-producing *E. coli* ($P<0.05$). There was no significant difference between ESBLs-producing *E. coli* to piperacillin/tazobactam, minocycline, gentamicin and cotrimoxazole ($P>0.05$) as shown in Table 3.

Table 2. Comparison of positive bloodstream infection rate and percentage results of *E. coli* in elderly patients.

Year	Number of specimens	Number of positives	Positive rate	Number of *E. coli*	Positive rate
2016	2048	217	10.6	40	18.4
2017	2303	262	11.4	49	18.7
2018	2547	316	12.5	62	19.6
2019	2814	365	12.9	79	21.6

Genetic testing of ESBLs-producing *E. coli* in bloodstream infection from elderly patients

86 strains of *E. coli* infected in the blood stream of elderly patients were tested by PCR amplification with designed primers for ESBL-producing resistance genes. The ESBL-producing *E. coli* drug resistance gene amplification electrophoresis results were as follows: 8 strains were not amplified to positive results, and 78 positive strains

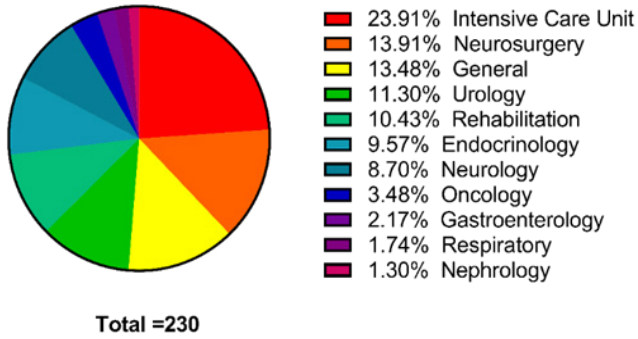

Figure 1. Clinical departmental distribution of elderly patients with *E. coli* bloodstream infection.

Table 3. Comparison of drug resistance rates of ESBLs-producing and non-ESBLs *E. coli* in bloodstream infections in elderly patients (%).

Antibiotics	ESBLs-producing *E. coli* (n=86)		Non-ESBLs-producing *E. coli* (n=144)		χ^2	*p*
	Number of resistant bacteria	Drug resistance rate	Number of resistant bacteria	Drug resistance rate		
Ampicillin	86	100	126	87.5	11.663	0.001
Cotrimoxazole	51	59.3	72	50	1.873	0.171
Cefuroxime	81	94.18	85	59.03	33.14	0.005
Ceftriaxone	83	96.5	74	51.39	50.601	0.005
Lomefloxacin	42	48.83	46	31.95	6.505	0.011
Levofloxacin	38	44.18	38	26.38	7.708	0.005
Aminotransom	80	93.02	54	37.5	68.259	0
Cefepime	39	45.34	39	27.08	8.015	0.011
Ticarcillin / Clavulanic acid	36	41.86	31	21.52	10.782	0.001
Ceftazidime	83	96.5	38	26.38	106.193	0
Gentamicin	8	9.3	26	18.05	3.275	0.07
Minocycline	2	2.32	0	0	1.219	0.27
Cefoxitin	15	17.44	12	8.33	4.311	0.038
Piperacillin / tazobactam	31	36.04	41	28.47	1.436	0.231
Meropenem	10	11.62	0	0	14.821	0.005
Amikacin	12	13.95	8	5.55	4.783	0.029

were detected. CTX-M-9 was detected in 27.91%, followed by CTX-M-2 in 17.44%, and SHV in 13.95%. Two strains were detected with CTX-M-2, CTX-M-9 and SHV genes, four strains were detected with both CTX-M-1 and CTX-M-2 genes, and 10 strains were detected with both CTX-M-9 and SHV genes, whose genotype distribution ratios were shown in Figure 2.

Detection of integrated genes for bloodstream infection with *E. coli* in elderly patients

Among the 230 strains of *E. coli* infected with blood streams from elderly patients, 95 strains showed 491bp of amplification products, with a detection rate of 41.30%, and no type II or III integrations were detected. The results of PCR amplification electrophoresis of *E. coli* type I integrated genes are shown in Figure 3.

Comparison of drug resistance rate of *E. coli* integrated gene-positive strains and negative strains

The results of 16 antimicrobial drug resistance tests were analyzed for 95 *E. coli* integrate-positive and 135 *E. coli* integrate-negative strains. The resistance rate of 16 antimicrobial drugs was significantly higher in the integrate-positive than in the integrate-negative *E. coli* (except gentamicin). Among them, the resistance rates of *E. coli* to antibacterial drugs were below 20% for meropenem, amikacin, cefoxitin, minocycline and gentamicin. See Table 4.

Relationship between ESBLs genotyping and type I integrated gene positivity in bloodstream infection with *E. coli* in elderly patients

The ESBLs genotyping-positive strains were analyzed with the detection of type I integrated gene-positive bacteria (Figure 4). The results showed that 83.3% of the positive CTX-M-9 genotypes detected type I integrated genes, and more than 50% of the positive ESBLs genotypes detected type I integrated genes.

E. coli is a common clinical pathogen as well as a com-

Table 4. Resistance rates of antibacterial drugs for *E. coli* in integrated gene-positive and -negative strains.

Antibacterial drugs	Integrating gene-positive *E. coli* (n=95)		Integrating gene-positive *E. coli* (n=95)		χ^2	*p*
	Number of resistant bacteria	Drug resistance rate	Number of resistant bacteria	Drug resistance rate		
Ampicillin	95	100	110	81.48	19.738	0.01
Cotrimoxazole	55	57.89	72	53.33	0.469	0.439
Cefuroxime	91	95.78	85	62.96	33.445	0.02
Ceftriaxone	88	92.63	74	54.81	38.294	0.005
Lomefloxacin	45	47.37	46	34.07	4.122	0.042
Levofloxacin	41	43.15	38	28.14	5.571	0.018
Aminotrans	88	92.63	54	40	65.389	0.01
Cefepime	45	47.37	39	28.89	8.214	0.004
Ticarcillin / clavulanic acid	36	37.89	31	22.96	6.022	0.014
Ceftazidime	86	90.52	38	28.15	87.322	0.01
Gentamicin	7	7.36	26	19.26	6.415	0.011
Minocycline	2	2.11	0	0	2.867	0.9
Cefoxitin	16	16.84	10	7.4	4.95	0.026
Piperacillin / tazobactam	34	35.79	39	28.88	1.226	0.268
Meropenem	11	11.58	0	0	13.973	0.005
Amikacin	15	15.78	6	4.44	8.65	0.005

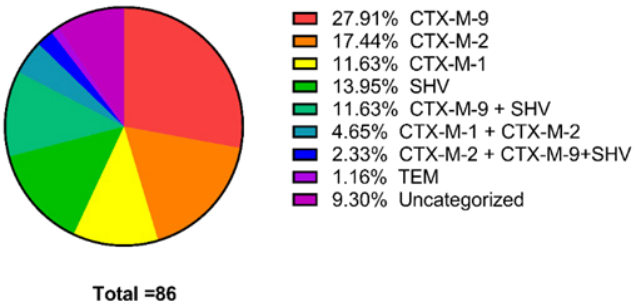

Figure 2. The genotype composition ratio of ESBLs-producing *E. coli* (%).

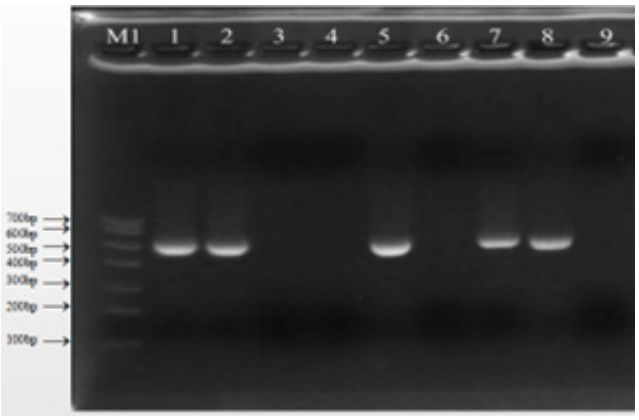

Figure 3. Electrophoresis diagram of type I integrated gene amplification. M1 for DNA marker bands, 1.2.5.7.8 for positive bacteria, 3.4.6.9 for negative bacteria.

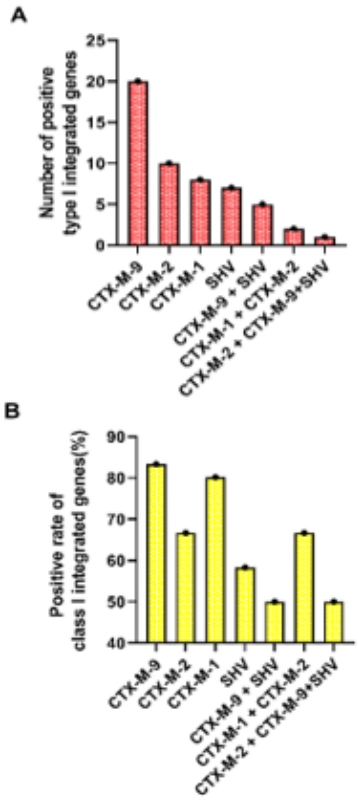

Figure 4. Genotyping and distribution of the number of positive (A) and positive rates (B) of class I integrated genes in ESBLs..

mon causative bacterium of hospital-acquired infections (9). Statistics from our hospital for 2018-2021 showed that the rate of bloodstream infections in the elderly was increasing year by year. This may be due to the decrease in immune function and the increase in underlying diseases in the elderly with age. Also, elderly patients are frequently hospitalized and subjected to invasive procedures, increasing the risk of infection. With the widespread use of extended-spectrum antibacterial drugs, the detection rate of drug resistance and ESBLs-producing *E. coli* has been increasing (10,11,12). The statistics of our hospital in the past 4 years showed that its detection rate was increasing. This result is consistent with the report of Chinese bacterial drug resistance surveillance about Gram-negative bacteria surveillance in 2017-2018 (13). Therefore, it is important to standardize invasive procedures in elderly patients and strengthen the management of geriatric intensive care units to minimize the incidence of blood catheter-related bloodstream infections.

ESBL is a derivative of serine protease. It enables *E. coli* to hydrolyze penicillin, cephalosporins and monocyclic β-lactamase antibacterial drugs. Moreover, it can recombine drug resistance genes in multiple ways, aggravating the spread of resistance genes among bacteria which is the root cause of bacterial multi-drug resistance (14, 15). The present study showed that the detection rate of ESBLs-producing *E. coli* was 37.39% in bloodstream infections in elderly patients, which was lower than the detection rate of ESBLs-producing bacteria reported by Xia Fei et al (16, 17). This may be related to the different elderly populations studied. The analysis of the resistance of ESBLs-producing and non-ESBLs-producing *E. coli* to antimicrobial drugs showed that ESBLs-producing *E. coli* had a lower resistance rate to carbapenems and β-lactamase inhibitors and aminoglycosides. Therefore, carbapenem-based antimicrobial drugs can be used as the first choice for the treatment of ESBLs-producing *E. coli* strains in elderly patients with bloodstream infections with *E. coli.* Although the resistance rate of amikacin and gentamicin was also less than 20%, these drugs can only be used as an alternative to carbapenem antibacterial drugs because of their greater renal damage. In addition, they were often ineffective when applied alone and were generally used in combination with carbapenems or enzyme inhibitors as an anti-infective treatment option.

In this study, genotyping of ESBLs showed that the genotype of ESBLs-producing *E. coli* in our elderly patients with bloodstream infection was mainly CTX-M, and CTX-M-9 was the most predominant genotype which was different from what has been reported (18). The genotyping results were dominated by single genotypes, and only eight strains with more than two ESBLs genotypes were present at the same time, which was similar to the related report (19). There were some differences in the genetic background and functional characteristics among the various ESBLs genotypes. In particular, CTX-M ESBLs-producing *E. coli* showed better susceptibility only to carbapenems and β-lactamase inhibitors.

The mechanism of drug resistance in *E. coli* is not only ESBL-producing but also the result of multiple effects of ESBLs, integrons and plasmids to produce bacterial drug resistance. ESBLs-producing *E. coli* genes are often transferred between plasmids or between plasmids and chromosomes by transformation, transduction, splicing and translocation in different species of bacteria under the action of integrons, which makes ESBLs bacteria more generated and spread. After the reassembly of integrons, many resistance genes can be aggregated on the same resistance plasmid, which is an important factor in exacerbating the emergence of multi-drug resistant bacteria. It has been reported that the integrons of *E. coli* were mainly type I integrons (20), and the detection rate of classes II and III was low. In the present study, the results of amplification of integron genes of ESBLs-producing strains were similar to those reported, with more than 50% of strains carrying type I integrons and significantly higher integrons in ESBLs genotype-positive strains than in negative strains. The above results indicate that not only type I integrons exist in ESBL-producing *E. coli* infected with blood in our elderly patients but also it has been fully involved in the mechanism of β-lactamase resistance, resulting in more serious bacterial drug resistance. It suggests that we need to choose sensitive drugs carefully under the guidance of bacteriology and drug sensitivity tests, so as to reduce the effect of antibiotics on integrons, decrease the production of drug-resistant bacteria specifically multidrug-resistant bacteria, and effectively control sepsis and chronic infections such as diabetic foot ulcers (21-23).

This study investigated the drug resistance characteristics and integration gene distribution of *E. coli* in bloodstream infections in elderly patients. It was found that type I integrated genes in ESBLs-producing *E. coli* isolated from blood in elderly patients carry resistance genes such as CTX-M-9 and CTX-M-2 aggravating the multi-drug resistance of bacteria, which provides a basis for clinical treatment of bloodstream infections and application of antibiotics by physicians.

Acknowledgments

Topic Research: Study on the genotype of Integron and drug resistance of ESBLs-producing Escherichia coli in the elderly (Jian Chen, Grant No: 17C0967)

Interest conflict

The authors declare that they have no conflict of interest.

References

1. Ciesielczuk H, Betts J, Phee L, Doumith M, Hope R, Woodford N, Wareham DW. Comparative virulence of urinary and bloodstream isolates of extra-intestinal pathogenic Escherichia coli in a Galleria mellonella model. Virulence. 2015;6(2):145-51. doi: 10.4161/21505594.2014.988095. PMID: 25853733; PMCID: PMC4601332.
2. Conceição RA, Ludovico MS, Andrade CG, Yano T. Human sepsis-associated Escherichia coli (SEPEC) is able to adhere to and invade kidney epithelial cells in culture. Braz J Med Biol Res. 2012 May;45(5):417-24. doi: 10.1590/s0100-879x2012007500057. Epub 2012 Apr 12. PMID: 22488222; PMCID: PMC3854282.
3. Dolejska M, Villa L, Hasman H, Hansen L, Carattoli A. Characterization of IncN plasmids carrying bla CTX-M-1 and qnr genes in Escherichia coli and Salmonella from animals, the environment and humans. J Antimicrob Chemother. 2013 Feb;68(2):333-9. doi: 10.1093/jac/dks387. Epub 2012 Oct 11. PMID: 23060365.
4. Markovska R, Schneider I, Ivanova D, Mitov I, Bauernfeind A. Predominance of IncL/M and IncF plasmid types among CTX-M-ESBL-producing Escherichia coli and Klebsiella pneumoniae in Bulgarian hospitals. APMIS. 2014 Jul;122(7):608-15. doi:

10.1111/apm.12204. Epub 2013 Dec 5. PMID: 24303846.

5. Laupland KB, Church DL. Population-based epidemiology and microbiology of community-onset bloodstream infections. Clin Microbiol Rev. 2014 Oct;27(4):647-64. doi: 10.1128/CMR.00002-14. PMID: 25278570; PMCID: PMC4187633.
6. McDanel J, Schweizer M, Crabb V, Nelson R, Samore M, Khader K, Blevins AE, Diekema D, Chiang HY, Nair R, Perencevich E. Incidence of Extended-Spectrum β-Lactamase (ESBL)-Producing Escherichia coli and Klebsiella Infections in the United States: A Systematic Literature Review. Infect Control Hosp Epidemiol. 2017 Oct;38(10):1209-1215. doi: 10.1017/ice.2017.156. Epub 2017 Jul 31. PMID: 28758612.
7. Cho SY, Kang CI, Cha MK, Wi YM, Ha YE, Chung DR, Lee NY, Peck KR, Song JH; Korean Network for Study on Infectious Diseases. Clinical Features and Treatment Outcomes of Bloodstream Infections Caused by Extended-Spectrum β-Lactamase-Producing Escherichia coli Sequence Type 131. Microb Drug Resist. 2015 Aug;21(4):463-9. doi: 10.1089/mdr.2014.0261. Epub 2015 Mar 10. PMID: 25756854.
8. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. J Clin Microbiol. 2021 Nov 18;59(12):e0021321. doi: 10.1128/JCM.00213-21. Epub 2021 Sep 22. PMID: 34550809; PMCID: PMC8601225.
9. Liang X, Xiao H. Clinical and pathogenic analysis of 193 cases of community-acquired bloodstream infections. Chin J Infect Chemother 2019;19(01):6-11.
10. Bartoletti M, Antonelli M, Bruno Blasi FA, Casagranda I, Chieregato A, Fumagalli R, Girardis M, Pieralli F, Plebani M, Rossolini GM, Sartelli M, Viaggi B, Viale P, Viscoli C, Pea F. Procalcitonin-guided antibiotic therapy: an expert consensus. Clin Chem Lab Med. 2018 Jul 26;56(8):1223-1229. doi: 10.1515/cclm-2018-0259. PMID: 29874192.
11. Wang HJ, Chen SP, Huang XW, Zheng SF. Analysis of bacterial distribution and drug resistance in community-acquired bloodstream infections in the elderly. Clinic Edu General Med 2019;17(06):513-515.
12. Li C, Lv Y, Zheng B. et al. Chinese bacterial drug resistance surveillance study 2017 -2018 Gram-negative bacteria surveillance report. Chin J Clinic Pharmacol 2019; 35(10):2508-2528.
13. Xia F, Qin T, Zhang MW, et al. Clinical characteristics and risk factors analysis of bloodstream infections caused by ESBL-producing Escherichia coli. Chin J Hospital Pharma 2020;40(03):305-309.
14. Clasen J, Birkegård AC, Græsbøll K, Folkesson A. Evolution of TEM-type extended-spectrum β-lactamases in Escherichia coli by cephalosporins. J Glob Antimicrob Resist. 2019 Dec;19:32-39. doi: 10.1016/j.jgar.2019.03.010. Epub 2019 Apr 30. PMID: 31048029.
15. McDanel J, Schweizer M, Crabb V, Nelson R, Samore M, Khader K, Blevins AE, Diekema D, Chiang HY, Nair R, Perencevich E. Incidence of Extended-Spectrum β-Lactamase (ESBL)-Producing Escherichia coli and Klebsiella Infections in the United States: A Systematic Literature Review. Infect Control Hosp Epidemiol. 2017 Oct;38(10):1209-1215. doi: 10.1017/ice.2017.156. Epub 2017 Jul 31. PMID: 28758612.
16. Raja NS. Emerging clinical role of pivmecillinam in the treatment of urinary tract infections caused by Extended Spectrum βeta-lactamase (ESBL) producing Enterobacteriaceae. Int J Clin Pract. 2019 Sep;73(9):1-5. doi: 10.1111/ijcp.13387. Epub 2019 Jul 30. PMID: 31243859.
17. Chen YF, Dong YQ, Chen F, et al. Genetic analysis of CTX-M-1 type variant strains of Escherichia coli producing ultra-broad-spectrum β-lactamases. Guizhou Medicine 2018;42(04):404-405+513.
18. Tu FL, Huang YM, Lin Y, et al. Exploring the relationship between drug resistance of ESBLs-producing Escherichia coli and TEM-type drug resistance genes. Southwest Military Med 2018;20(02):104-109.
19. Martins N, Picão RC, Adams-Sapper S, Riley LW, Moreira BM. Association of class 1 and 2 integrons with multidrug-resistant Acinetobacter baumannii international clones and Acinetobacter nosocomialis isolates. Antimicrob Agents Chemother. 2015 Jan;59(1):698-701. doi: 10.1128/AAC.02415-14. Epub 2014 Oct 27. PMID: 25348522; PMCID: PMC4291415.
20. Mercer RG, Zheng J, Garcia-Hernandez R, Ruan L, Gänzle MG, McMullen LM. Genetic determinants of heat resistance in Escherichia coli. Front Microbiol. 2015 Sep 9;6:932. doi: 10.3389/fmicb.2015.00932. PMID: 26441869; PMCID: PMC4563881.
21. Alavi M, Thomas S, Sreedharan M. Modification of silica nanoparticles for antibacterial activities: mechanism of action. Micro Nano Bio Asp. 2022; 1(1): 49-58.
22. Abbas-Al-Khafaji ZK, Aubais-aljelehawy Qh. Evaluation of antibiotic resistance and prevalence of multi-antibiotic resistant genes among Acinetobacter baumannii strains isolated from patients admitted to al-yarmouk hospital. Cell Mol Biomed Rep. 2021; 1(2):60-68.
23. Aubais aljelehawy Qh, Hadi Alshaibah LH, Abbas Al- Khafaji ZK. Evaluation of virulence factors among Staphylococcus aureus strains isolated from patients with urinary tract infection in Al-Najaf Al-Ashraf teaching hospital. Cell Mol Biomed Rep. 2021; 1(2):78-87.