JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Correlation between class 1 integron of *Escherichia coli* and multidrug resistance in lower respiratory tract infection

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Abstract

Introduction: Class 1 integrons are mobile genetic elements considered to be responsible for the transfer of multidrug resistance. This study aimed to investigate the distribution of type 1 integrons in multidrug-resistant*Escherichiacoli*from hospital-acquired lower respiratory tract infections.

Methodology: Clinical strains of *E. coli* were isolated from patients with hospital-acquired lower respiratory tract infections in the emergency intensive care unit from January to December 2014. Drug sensitivity testing was performed using the Kirby-Bauer method. The combination disk method was used to detect extended-spectrum β-lactamase (ESBL), and polymerase chain reaction (PCR) amplification was used to detect the *intI1* gene.

Results: Among 58 *E. coli* strains, resistance to β-lactam antibiotics ranked as follows: imipenem (0.0%), cefoperazone/sulbactam sodium (25.9%), ceftazidime (37.9%), and cefepime (39.7%); other β-lactam antibiotic resistance rates were all > 50%. The resistance rates to amikacin, ciprofloxacin, gentamicin, and cotrimoxazole were32.8%, 63.8%, 70.7%, and 81.0%, respectively. In total, 31 (53.4%) isolates were positive for class 1 integron and carried 4 different sizes of amplification fragments: 800, 1,600, 1,900, and 2,600 bp. Among 43 ESBL-positive isolates, 27 (62.8%) also carried class 1 integron; among 15 ESBL-negative isolates, 4 carried class 1 integron (26.7%). The positive rate for class 1 integron in ESBL-producing strains was significantly higher than that in non-ESBL-producing strains. The rates of resistance of integron-positive isolates to ceftriaxone, cefotaxime, amikacin, ciprofloxacin, and cotrimoxazole were significantly higher than those in integron-negative isolates.

Conclusions: Class 1 integrons are widely distributed in *E. coli* and are associated with multidrug resistance.

Key words: hospital-acquired infection; lower respiratory tract infection; *Escherichia coli*; integron; extended-spectrum β-lactamase.

J Infect Dev Ctries 2017; 11(8):604-610. doi:10.3855/jidc.8247

(Received 13 February 2016 – Accepted 16 December 2016)

Introduction

Escherichia coli is one of the main causes of nosocomial infections. With the extensive use of antibiotics, resistant strains of *E. coli* are becoming more common, which makes anti-infective treatment difficult. Previous studies have shown that 65% of clinical isolates of *E. coli* produce extended-spectrum β-lactamases (ESBLs) in China[1]. ESBL-producing strains are resistant to β-lactams, fluoroquinolones, and aminoglycosides [2] and cause hospital- and community-acquired infections.

In recent years, integrons have been shown to play a key role in horizontal transmission of antibiotic resistance genes in bacteria. As movable genetic elements, integrons capture foreign drug-resistance genes via integrase, which are expressed under the control of upstream promoters [3]. Based on the integrase coding sequence, there are currently nine classes of integrons, of which class 1 integrons are the most commonly encountered type clinically, playing an important role in transferring drug resistance [4]. Class 1 integrons carry the integrase gene (*intI1*), which codes for the site-specific recombinase responsible for cassette insertion. The integrase gene also includes the *attI1* site, where the cassettes are integrated and a promoter, Pc, is responsible for the transcription of the cassette-encoded gene. The gene cassette contains a single antibiotic resistance gene and a 59-base element (or *attC* site) downstream of the gene, which is responsible for recombination events. Among clinical isolates of *E. coli*, those with resistance to up to three antibiotics are integron negative; those with resistance to 12–14 antibiotics are integron positive; and the total number of resistant antibiotics in integron-positive isolates has significantly shifted to the right [5]. Understanding the molecular mechanism of resistance genes may help in the introduction of new antimicrobial strategies and some preventive procedures to prevent further spreadof resistance determinants among the pathogens. We detected a class 1 integron in 58 isolates

of *E. coli* from hospital-acquired lower respiratory tract infections to explore its role in multidrug resistance and its prevalence in the region.

Methodology

Strain source

A total of 58 strains of *E. coli* were isolated from sputum samples from patients with hospital-acquired lower respiratory tract infections in the emergency intensive care unit (EICU) of the hospital from January to December 2014. The hospital is a large, comprehensive, university-affiliated hospital, treating more than 5,000 patients each year, 2,500 in the EICU. All isolates were identified with the API-20E identification system (BioMerieux-Vitek, Hazlewood, USA.). *E. coli* ATCC 25922 standard strain was purchased from the NationalClinicalLaboratoryCenter and used as a control.

Drug sensitivity test

The Kirby-Bauer disk diffusion method was used to detect piperacillin, amikacin, ciprofloxacin, aztreonam, ceftriaxone, ceftazidime, cefotaxime, cefepime, cefoperazone/sulbactam sodium, imipenem, gentamicin, and cotrimoxazole. Drug sensitivity test paperswerepurchased from Oxoid (Basingstoke, UK).The results were interpreted according to the standard criteria recommended by Clinical and Laboratory Standards Institute (CLSI) in 2010 [6], and the strains that were moderately sensitive to drugs were considered drug resistant in statistical analysis. *E. coli* ATCC 25922 was used as a quality control strain.

Combined disk method to detect ESBLs

Disks containing 30 μg of cefotaxime or ceftazidime, and disks containing a combination of the two drugs plus 10 μg of clavulanic acid (HiMedia, Mumbai, India) were placed independently, 30 mm apart, on a lawn culture of 0.5 McFarland opacity of the test isolate on a Mueller-Hinton agar (Oxid, Basingstoke, UK) plate and incubated for 18–24 hours at 35°C. Isolates were considered ESBL positive if the inhibition zone measured around one of the combination disks after overnight incubation was at least 5 mm larger than that of the corresponding cephalosporin disk. *K. pneumoniae*ATCC 700603 was used as the standard strain.

Identification of class 1 integron by PCR

Primers: PCR primers from Bado *et al*. were employed [7]. The amplified fragment of class 1 integrase (IntI) was 587 bp, using upstream primer IntI-F: 5'-TGATGGCGACGCACGAC-3' and downstream primer IntI-B: 5'-TTGGGCAGCAGCGAAGT-3'. The conserved sequences at the 5'- and 3'-ends of the integron were selected, and the PCR amplification primers of the variable region of the resistance gene cassette were designed. Upstream primer was 5'CS-F: 5'-GGCATCCAAGCAGCAAGC-3' and downstream primer was 3'CS-B: 5'-AAGCAGACTTGACCTGAT-3'. PCR primers were synthesized from Shanghai BioEngineering Technology Services Co., Ltd. (Shanghai, China).

Template preparation: Fifty microliters of overnight bacterial culture was centrifuged at 7,104 g for 1 minute, and the supernatant was discarded. Sterile water of equal volume was added, shaken, and mixed. The bacterial suspension was added to boiling water for 10 minutes, then centrifuged at 15,984 g for 1 minute. Finally, the supernatant was preserved as a template for PCR amplification.

PCR amplification conditions were as follows: the total volume was 50 μL, containing 1× PCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 0.1 mmol/L for each primer, 1.25 U of Taq DNA polymerase, and 100 ng DNA template. The PCR for amplification of the integrase gene was carried out as follows: 94°Cfor 5 minutes, 94°Cfor 40 seconds, 58°Cfor 40 seconds, and 72°C for 45 seconds for 35 cycles, followed by final extension at 72°Cfor 5 minutes. The PCR for amplification of the variable region of the resistance gene cassette of integron was as follows: 94°C for 5 minutes, 94°C for 1 minutes, 55°C for 1 minute, and 72°C for 3 minutes for 35 cycles, with final extension at 72°C for 5 minutes. The amplification products underwent 0.8% agarose gel electrophoresis; the results were observed under UV light.

PCR product sequencing

PCR product purification and sequencing were done by Shanghai BioEngineering Technology Services Co., Ltd. (Shanghai, China). Sequencing results were performed gene homology analysis in GenBank sequence database.

Statistical analysis

SPSS version 11.5 (IBM, Armonk USA) statistical software was used for data processing. The integron detection results were divided into integron-positive and integron-negative groups. The drug sensitivity test results of the two groups were analyzed by the χ^2 test in a two-by-two table.$P < 0.05$ was considered significantly different.

Results

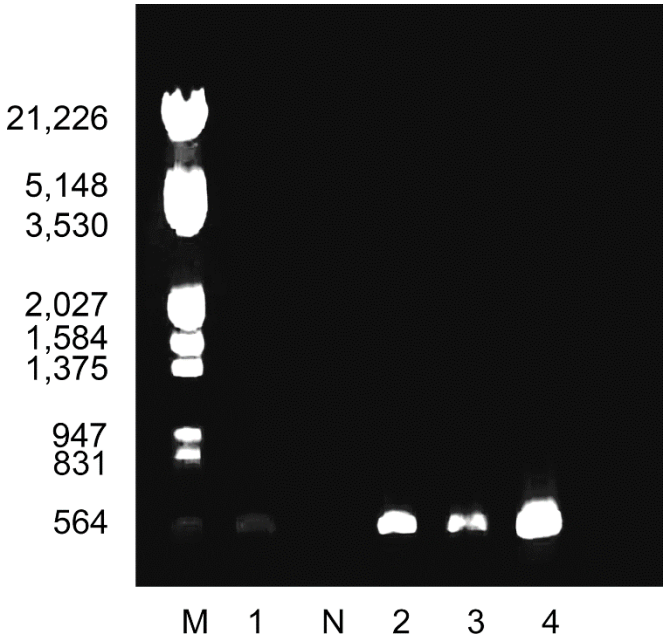
Monitoring of antibiotic resistance

Following culture of 962 sputum specimens, 374 (38.9%) yielded pathogenic bacteria growth. Gram-negative bacteria accounted for 73.8% (276/374), with 58 (21.0%) identified as *E. coli*; Gram-positive bacteria accounted for 13.9% (52/374), and fungi accounted for 12.3% (46/374). The rates of resistance to β-lactam antibiotics in the 58 clinical isolates of *E. coli* were as follows: imipenem 0.0%, cefoperazone/sulbactam sodium 25.9%, ceftazidime 37.9%, and cefepime 39.7%; rates of resistance to other β-lactam antibiotics were all > 50%; and rates of resistance to amikacin, ciprofloxacin, gentamicin, and cotrimoxazole were 32.8%, 63.8%, 70.7%, and 81.0%, respectively. There was a high incidence of multidrug resistance among the isolates (Tables 1 and 2).

Bacteria carrying integrons

PCR amplification of class 1integrase sequences in 58 strains of *E. coli* showed that 31 were positive and the positive rate was 53.4%. Further amplification of the insertion region of the integron cassette showed that there were 4 different amplified fragments of integrons (650–2,600 bp, Figures 1 and 2). Among them, 1 isolate contained a 650-bp integron, 8 contained a 1,600-bp integron, 17 contained a 1,900-bp integron, and 5 contained a 2,600-bp integron. Inserted gene cassette of integron sequence analysis showed 2,600-bp integron harbored aminoglycoside acetyltransferase gene (*aacA4*) chloramphenicol efflux protein coding gene

Figure 1.PCR amplification results of integrase sequence.



M: markers; N: negative control; 1, 2, 3 and 4: from clinical isolates.

Table 1. Antibiotic resistance in 58 strains of *E. coli*.

Antibiotics	Sensitive	Resistant	Resistance rate (%)
Piperacillin	12	46	79.3
Ceftriaxone	29	29	50.0
Ceftazidime	36	22	37.9
Cefotaxime	23	35	60.3
Cefepime	35	23	39.7
Amikacin	39	19	32.8
Ciprofloxacin	21	37	63.8
Aztreonam	23	35	60.3
Imipenem	58	0	0
Cefoperazone Sulbactam sodium	43	15	25.9
Gentamicin	17	41	70.7
Cotrimoxazole	11	47	81.0

Table 2. Distribution of drug resistance patterns in *E. coli* (%).

Drug resistance pattern	Strains	Constituent ratio
Multiple resistance	28	48.30
Double resistance	20	34.50
Single resistance	7	12.10
Sensitive	3	5.17
Total	**58**	**100.00**

Single resistance: resistance to only a kind of antibiotic (amino glycopeptides, beta lactams,quinolones, cotrimoxazole); double resistance: resistance to two kinds of antibiotics; multiple resistance: resistance to three kinds of antibiotics.

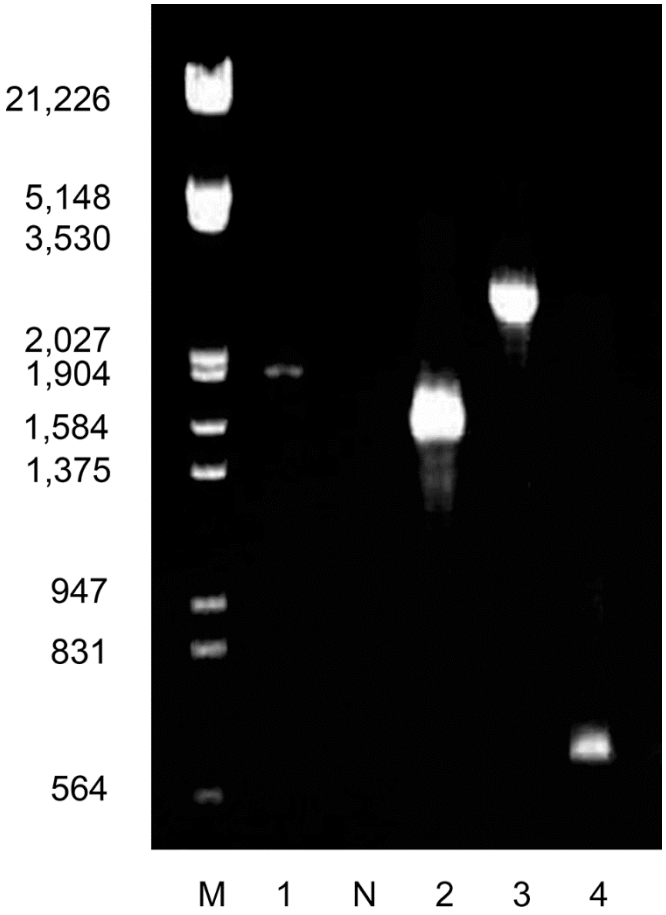
(*cmlA4*); 1,900-bp integron harbored dihydrofolate reductase gene (*dfrA17*) coding function being not clear (*ORF5*)- aminoglycoside adenylyl transferase gene (*aadA5*).

Among 43 ESBL-positive isolates, 27 (62.8%) also carried a class 1 integron, and among 15 ESBL-negative isolates, 4 carried a class 1 integron (26.7%). In the highly resistant clinical isolates, the positive rate of class 1 integron in ESBL-positive strains was significantly higher than that in ESBL-negative strains ($\chi^2 = 5.832$; $p < 0.05$). The patients infected with strains that carried 1 integrons had used third-generation cephalosporins or fluoroquinolone drugs before the bacteria were isolated.

Relationship between E. coli integron and drug-resistant phenotypes

Among 58 clinical isolates of *E. coli*, integron-positive isolates showed a high resistance rate (> 60%) to antibiotics other than ceftazidime, cefepime, imipenem, and cefoperazone/sulbactam sodium. In particular, for ceftriaxone, cefotaxime, amikacin, ciprofloxacin, and cotrimoxazole, the resistance rate of integron-positive isolates was significantly higher than that in integron-negative isolates(χ^2=8.385, 8.113, 4.650, 5.353, 6.785, respectively, $p < 0.05$, Table 3).

Figure 2.PCR amplification results of insertion region of gene cassette in integron.



M: markers; N: negative control; 1, 2, 3, 4: from clinical isolates.

Table 3. Correlation between class I integron and antibiotic resistance in *E. coli*.

Antibiotics	Integron-positive isolates resistance rate (%) (n = 31)	Integron-negative isolates resistance rate (%) (n = 27)	χ^2	P value
Piperacillin	80.6	77.8	0.072	> 0.05
Ceftriaxone	67.7	29.6	8.385	< 0.05
Ceftazidime	41.9	33.3	0.454	>0.05
Cefotaxime	77.4	40.7	8.113	< 0.05
Cefepime	41.9	37.0	0.145	> 0.05
Amikacin	45.2	18.5	4.650	< 0.05
Ciprofloxacin	77.4	48.1	5.353	< 0.05
Aztreonam	61.3	59.3	0.025	> 0.05
Imipenem	0	0	-	-
Cefoperazone/sulbactam sodium	29.0	22.2	0.349	> 0.05
Gentamicin	74.2	66.7	0.395	> 0.05
Cotrimoxazole	93.5	66.7	6.785	< 0.05

Discussion

Many studies have indicated that class 1 integrons play a crucial role in the dissemination of antibiotic resistance in Gram-negative bacteria and occur frequently in *E. coli* by carrying and capturing genes via site-specific recombination catalyzed by specific integrase genes [8]. Class 1 integrons aid in gene transference in the capture and distribution of gene cassettes among clinical Gram-negative bacilli [9]. In the present study, among 58 strains of *E. coli*, 53.4% were positive for class 1 integron. However, there are some differences in the detection rate of class 1 integrons in *E. coli* isolated from different regions [10]. The detection rate of class 1 integron in clinical Gram-negative strains was about 43% in Western and Central Europe, > 50% in the Netherlands, ~59% in France, and up to 75% in Gram-negative strains resistant to aminoglycosides in the United States. In Greece, the positive rates for gene *intI* were 49.2%, 26.2%, and 11.1% in *E. coli* isolates from local birds, inpatients, and outpatients, respectively [11]. This may be because clinical isolates of *E. coli* showed some selection bias, leading to a significant regional difference in the distribution of integrons, or because in different laboratories, different primers were used for PCR amplification of integrons. Nowadays, most research uses PCRs targeted at the 5'CS integrase gene, while some uses PCRs targeted at 3'CS; 3'CS shows indefinite lengths, and some class 1 integrons even have no 3'CS, leading to a low detection rate. Many studies have shown that class 1 integrons exist widely in Gram-negative bacteria, including Enterobacteriaceae, *Pseudomonas*, *Vibrio*, and non-fermenting Gram-negative bacteria. Class 1 integrons are present at a higher frequency in Gram-negative bacteria that are resistant to aminoglycoside antibiotics [12].

According to the literature, the size of class 1 integrons is 500–3,000 bp. It increases in size over time, probably because it captures more gene cassettes. Our study found 4 different sizes of integron amplified fragments: 650, 1,600, 1,900 and 2,600 bp, in *E. coli*. The sequence analysis of the inserted gene cassette of the integrons showed that: the 2,600-bp integron harbored aminoglycoside acetyltransferase gene (aacA4)andchloramphenicol efflux protein coding gene (cmlA4); the 1,900-bp integron harbored dihydrofolate reductase gene (dfrA17) andaminoglycoside adenylyltransferase gene (aadA5). The larger gene fragment of integrons contained more inserted resistance genes, suggesting that this clinical isolate was resistant to multiple antibiotics.

In recent years, rates of ESBL-producing *E. coli* have increased annually, because ESBLs can promote the spread of resistance genes in bacteria via conjugation, transformation, and transduction, causing serious nosocomial infection [13]. The prevalent type of ESBLs is not identical in different regions. Our study showed that the rate of class 1 integron positivity was 62.8% in ESBL-producing *E. coli*, which was higher than 26.7% in the non-ESBL strains, indicating that class 1integrons are present widely in this region but are more concentrated in the ESBL-producing strains, which is consistent with the results in the literature [14]. Some ESBL-encoding genes may be located inside class 1integron; therefore, the ESBL-producing strains carrying class 1integron show a high capacity for dissemination and the integrons participate in drug resistance of ESBL strains, inducing severe multidrug resistance, to which more attention should be paid clinically.

With respect to drug-sensitive phenotypes, our study indicated that class 1integron was closely associated with multidrug resistance of *E. coli*. The strains carrying class 1 integron had a higher rate of resistance to ciprofloxacin, amikacin, and cotrimoxazole compared with the strains carrying no integrons. Meanwhile, the rate of resistance to third-generation cephalosporins (ceftriaxone and cefotaxime) was significantly higher in strains with class 1 integron than in the strains without the integron. This may be because class 1 integron is often located on a transferable resistance plasmid, which also carriesβ-lactamase-encoding resistance genes. Integrons are also related to multidrug resistance. The strains carrying integrons are more prone to multidrug resistance. This study shows that the positive rate of multidrug resistance is highest among class 1 integron-positive strains; all sensitive strains are mainly integron-negative strains, single-resistance strains, and double-resistance strains. Multidrug-resistant strains are mainly integron positive strains, especially double resistant and multi-drug resistant strains were all integron positive strains. Each strain was resistant to 5.8 kinds of drugs, on average, in the integron-negative group and 10.2 kinds of drugs in the integron-positive group, indicating that the integron plays a role in the dissemination of drug-resistant *E. coli* in hospital-acquired lower respiratory tract infections.

In the natural environment without antibiotics, the occurrence of integron is only 3.6%, and more than half of the integrons carry no antibiotic resistance genes [15]; therefore, it can be concluded that the ability of integrons to capture gene cassettes is related to

antibiotic selection pressure. In our study, the patients who carried I integron 1-positive strains infection had used three generations of cephalosporin or fluoroquinolone drugs before the separation of bacteria.Clinically, multidrug resistance of bacteria is related to the accumulation of resistance genes by integron-gene cassette systems [16,17]. Currently, novel antibiotic resistance gene cassettes are observed, such as blaIMP (encoding resistance to imipenem and broad-spectrum β-lactam antibiotics) and aacA7 (encoding resistance to new aminoglycoside drugs such as amikacin and netilmicin). These gene cassettes do not have enough selection pressure or time to be widespread, but the integrons have a high capacity to capture and accumulate gene cassettes, so perhaps in the near future, these new antibiotic resistance gene cassettes will become popular [18-20]. Integrons will continue to affect antibiotic efficacy.

Conclusions

The prevalence of class 1 integrons in ESBL-producing *E. coli* strains is high. According to the results of the present study, the presence of class 1 integrons and ESBLs together mediates the resistance of *E. coli*isolates to most antibacterial agents; it is therefore very important to identify and control the resistant strains. Further research is needed to investigate the characteristics, types, and carried gene cassettes of integrons in clinical isolates to control the dissemination of bacterial drug resistance, reduce the external environmental factors inducing the production and molecular evolution of integrons, prevent the emergence of resistant strains, and provide a reliable experimental basis for the clinically rational use of antibiotics.

Acknowledgements

This work was funded in part by Liaoning Science and Technology Project of China(grant 2011225020).

Authors' contributions

WY designed the search strategy, participated in the data collection and data analysis, and drafted the manuscript.KBB participated in the design of the study andperformed culture and identification of all isolates.YWP and ZX performed identification of class 1 integron by PCR and helped to draft the manuscript. All authors read and approved the final manuscript.

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Conflict of interests: No conflict of interests is declared.