

## Characterization of integrons among *Escherichia coli* in a region at high incidence of ESBL-EC

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### ABSTRACT

**Objective:** The aim of study was to investigate the distribution of the integrons in *Escherichia coli* (*E. coli*) isolates, and analyze the possible relationship between the antimicrobial resistance profiles and the integrons.

**Methods:** The antimicrobial profiles of 376 *E. coli* strains were analysed by disk diffusion test. The integron genes and variable regions were detected by PCR. Some amplicons were sequenced to determine the gene cassettes style.

**Results:** Of 376 isolates, 223 isolates (59.3%) were confirmed as ESBL-EC. Comparison to ESBL-negative *E. coli*, the high rates of resistance to the third and fourth generation of cephalosporins, penicillins and amikacin were found in ESBL-EC. Only class 1 was integron detected in the isolates, and the prevalence of it was 66.5%. It was commonly found in ESBL-EC (77.6%, 173/223), which was higher than that of ESBL-negative *E. coli* (50.3%, 77/153) ( $p < 0.001$ ). Six different genes cassettes were detected in this study and were classified into three groups: *dfr17-aadA5*, *dfrA12-aadA2* and *aacA4-CmlA1*. Additionally, more than one gene array harboured in 13.9% isolates of ESBL-EC, while in 9.1% isolates of ESBL-negative *E. coli*.

**Conclusion:** The high incidence of ESBL-EC with resistance to multiple antibiotics were detected in the isolates from Blood stream infection (BSI). More resistant gene cassettes in ESBL-EC may partially underlie the high resistance to amikacin, while no relation exists between the high incidence of ESBL-EC and classes 1- 3 integrons in this region.

**KEY WORDS:** Blood stream infection, ESBL-EC, Integron, Gene cassette.

### Abbreviations:

**BSI:** Bloodstream infection. ***E. coli:*** *Escherichia coli*, **ESBL:** Extended spectrum  $\beta$ -lactamases, **ESBL-EC:** ESBL-producing *E. coli*.

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## INTRODUCTION

Bloodstream infection (BSI) is associated with major morbidity and mortality, and *Escherichia coli* (*E. coli*) is one of the most common microorganisms isolated from this infection.<sup>1</sup> In recent years, the detection rate of extended spectrum  $\beta$ -lactamases (ESBL) in enterobacteriaceae bacteria, specially *Escherichia coli* (*E. coli*) is increasing.<sup>2</sup> It is well known that ESBL-producing organisms are resistant to multiple unrelated antibiotics.<sup>3</sup> As a result, multiple drug-resistant clinical infections of *E. coli* are now common. Integrons are widespread genetic elements responsible for dissemination of antibiotic

resistance among Gram-negative bacteria, being commonly found in plasmids and/or transposons.<sup>4</sup> Recently integrons have become an important drug-resistance mechanism of *E. coli*.<sup>5</sup> ESBL-producing *E. coli* (ESBL-EC) carrying integrons will have more drug resistance genes and resistance to many other drugs.

Knowledge of the characterization of integrons among *E. coli* isolated will contribute to assess the phenotypic and genetic characterization of antimicrobial profiles of the organism in BSI. In this study, we investigated classes 1, 2, and 3 integrons associated integrase genes in *E. coli* isolates from blood to evaluate integron characterization and typing of this organism.

## METHODS

**Bacterial strains:** All strains of *E. coli*, isolated from BSI in the bacteriology laboratory of Weihai Municipal Hospital affiliated to Dalian Medical University, were identified by biochemical tests (API 20E strip; bioMérieux, Craponne, France) from January 2008 to January 2010. Written and informed consent was obtained from all patients, and the study was conducted after approval by the Ethical Committee of Weihai Municipal Hospital affiliated to Dalian Medical University.

**Antimicrobial Susceptibility Testing and extended-spectrum- $\beta$ -lactamase detection:** Antimicrobial susceptibility testing was performed using Kirby-Bauer's disc diffusion method and the results were interpreted according to CLSI.<sup>6</sup>

Screening and confirmatory testing of isolates for ESBL production were carried out according to CLSI.<sup>6</sup> Strains showing zone of inhibition of  $\leq 22$ mm for ceftazidime,  $\leq 27$ mm for cefotaxime, and  $\leq 25$ mm for ceftriaxone were selected for conformational tests of ESBL. The standard strains *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls, respectively.

***E. coli* genomic DNA and plasmid DNA extraction:** After bacteria were harvested, genomic DNA and

plasmid DNA were isolated in all isolates using bacterial genomic DNA extraction kit (DV810A, Takara) and Plasmid Purification Kit (D821A, Takara), respectively. The assays were strictly performed according to manufacture's protocol. Extractions were electrophoresed in 1.0% agarose gel and visualized by staining with ethidium bromide under short UV light.

**PCR for analyzing classes 1, 2 and 3 integrons status in *E. coli*:** The presence of classes 1, 2, and 3 integrons was analyzed in all isolates obtained in this study. Three pair primers were used to obtain the converse regions sequence of the three classes integrase genes respectively, while the variable region of the integrons were determined with another pair primers.<sup>7</sup> All primers are listed in Table-I. Some amplicons of the variable region were sequenced by Life Technologies Corporation. **Statistical analysis:** The statistical calculation was performed using SPSS version 13.0 (SPSS, Chicago, IL, USA). Comparison of categorical variables and percentages between groups was carried out by Chi-square test. *p* value less than 0.05 was considered significant.

## RESULTS

A total of 376 *E. coli* isolates were taken during the study period. Of these, 223 isolates (59.3%) were confirmed as ESBL-EC. None of isolates were all susceptible to antimicrobial agents, and the phenotypic characterization of antimicrobial profiles are shown in Table-II. Among the isolates, the high rate of resistance to levofloxacin, ciprofloxacin and piperacillin were 59.8%, 64.9% and 84.8% respectively, with the low rate of resistance to imipenem and meropenem (0.5% and 0%). Comparison to ESBL-negative *E. coli*, the high rates of resistance to the third and fourth generation of cephalosporins, penicillins and amikacin were observed in ESBL-EC.

**The Prevalence of classes 1, 2 and 3 integrons status in *E. coli*:** The prevalence of classe 1 integron in *E.*

Table-I: Oligonucleotide primers used in the PCR assay.

Primer	Nucleotide sequence (5'-3')	PCR target	Expected size (bp)
Int11F	ACGAGCGCAAGGTTTCGGT	Class 1 integrase gene	565
Int11R	GAAAGGTCCTGGTCATACATG		
Int12F	GTGCAACGCATTTTGCAGG	Class 2 integrase gene	403
Int12R	CAACGGAGTCATGCAGATG		
Int13F	CATTTGTGTTGTGGACGGC	Class 3 integrase gene	717
Int13R	GACAGATACGTGTTTGGCAA		
5'-CS	GGCATCCAAGCAGCAAG	Variable region of integrons	Uncertain
3'-CS	AAGCAGACTTGACCTGAT		

Table-II: Antimicrobial resistance for 376 *E. coli* isolated from BSI (%).

Antimicrobials*	AMZ	AK	SCF	CIP	CRO	FOX	IMP	LEV	MEM	PRL	CTX	CAZ	TZP	FEP
ESBL-EC (223)	26.5	52.0	12.6	78.0	100	26.0	0.9	78.0	0	100	100	60.0	26.0	60.5
ESBL-negative <i>E. coli</i>	13.1	3.9	0	45.8	12.4	8.5	0	33.3	0	62.7	16.3	3.9	0	0
Total	21.0	32.4	7.4	64.9	64.4	18.9	0.5	59.8	0	84.8	66.0	37.2	15.4	35.9

\*Abbreviations in the table: Amoxicillin-clavulanic acid (AMZ); Amikacin (AK); Cefoperazone/sulbactam (SCF); Ciprofloxacin (CIP); Ceftriaxone (CRO); Cefoxitin (FOX); Imipenem (IMP); Levofloxacin (LEV); Meropenem (MEM); Piperacillin (PRL); Cefotaxime (CTX); Ceftazidime (CAZ); Piperacillin-Tazobactam (TZP); Cefepime (FEP)

*coli* isolates was 66.5% (250/376). It was commonly found in ESBL-EC (77.6%, 173/223), which was higher than that of ESBL-negative *E. coli* (50.3%, 77/153) ( $p < 0.001$ ). No amplification products were obtained from any of these isolates when the primers specific for *intI2* or *intI3* were used.

Class 1 integron was detected on genomic and plasmid DNA in 155 isolates (77%, 155/201) of ESBL-EC, but it was detected on genomic or plasmid DNA in 9 isolates respectively. While Class 1 integron was harboured on genomic and plasmid DNA in 29 isolates (37.7%, 29/77) of ESBL-negative *E. coli*, most of others (30 isolates) was harboured on plasmid DNA.

**The Characteristic of Class 1 Integron in Isolates:** Each gene-cassette region in class 1 integrons was amplified to identify characteristic of gene cassette in the isolates. 164 ESBL-EC isolates (94.8%, 164/173) of carrying class 1 integron contained resistant gene cassette, and the other 9 isolates were detected no resistant gene cassette. For ESBL-negative *E. coli*, 56 isolates (72.7%, 56/77) were detected resistant gene cassette.

All these gene cassettes detected were divided into 6 different ones: *dfrA17*, *aadA5*, *dfrA12*, *aadA2*, *aacA4* and *CmlA1*. The proteins encoded by gene cassette may contribute to the resistance of bacteria isolates to trimethoprim (*dfr17* and *dfrA12*), aminoglycosides (*aadA5*, *aadA2*, and *aacA4*), and chloramphenicol (*CmlA1*).

The integrons were classified into three groups according to the length of amplicons (Fig.1). The

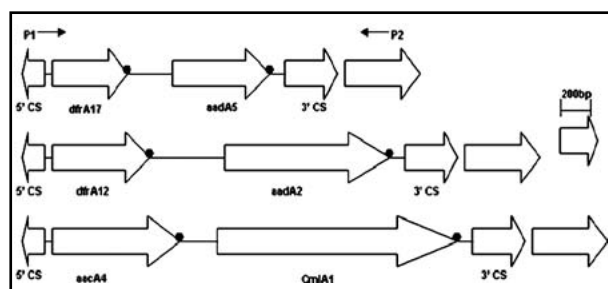


Fig.1: Schematic representation of the genetic elements of Class 1 Integron in *E. coli* isolated from BSI.

*dfr17-aadA5* array was the most prevalent in the isolates which lengths most were 1,600bp and the other two arrays were *dfrA12-aadA2*, and *aacA4-CmlA1* which lengths were 2,000bp and 2,400bp, respectively. More than one gene array harboured in 13.9% (24/173) isolates of ESBL-EC, while in 9.1% (7/77) isolates of ESBL-negative *E. coli*.

## DISCUSSION

Despite the great advances in medical science in the past century, BSI remains a growing public health concern worldwide. *E. coli* is one of the most common pathogens for this kind infection. Over the past few years, a significant increase in the number of ESBL-EC-associated BSI is being reported in several parts of the world.<sup>1,8</sup> In our study, about 60% *E. coli* isolates from BSI were ESBL-EC, which is higher than other reports (7.3% in Hong Kong, 1.5–16.7% in Taiwan).<sup>9,10</sup>

The high rates of resistance to levofloxacin and ciprofloxacin were found in the study, while low rates of resistance to imipenem and meropenem existed in vitro. Carbapenems use has been associated with the low risk of death in cases of serious infections caused by these pathogens.<sup>11,12</sup> Thus, these antimicrobials are first line therapy in patients with serious infections. Additionally, the resistance to amikacin was significantly higher in ESBL-EC than in ESBL-negative *E. coli* except to the third and fourth generation of cephalosporins and penicillins.

Integrons had come under observation in 1989 by Stokes for the first time<sup>13</sup>, which could carry more than forty resistance genes. The resistant gene could spread to other bacteria through the integrons, so it is very important to monitor *E. coli* strains isolates from BSI.

We screened the three classes integrons associated integrases genes by PCR, and found only class 1 integron gene was harboured in *E. coli* isolated from BSI. The prevalence of class 1 integron in *E. coli* isolates was in accordance with other's reports<sup>14</sup>, while the prevalence was higher in ESBL-EC than in ESBL-negative *E. coli*. Additionally, the prevalence

of class 1 integron on genomic and plasmid DNA was higher in ESBL-EC. We found six different gene cassettes in class 1 integron and three arrays in the study. These results also showed that the characterization of integrons in bacteria obviously had regional difference.<sup>15,16</sup> Although the same resistant gene cassettes and arrays were found on ESBL-EC and ESBL-negative *E.coli*, the carrying rate of gene cassettes in ESBL-EC was higher than that in ESBL-negative *E.coli*.

In conclusion, the high incidence of ESBL-EC with resistance to multiple antibiotics were detected in the isolates from BSI. Comparison to ESBL-negative *E.coli*, more resistant gene cassettes in ESBL-EC may partially underlie the high resistance to amikacin, while no relation exists between the high incidence of ESBL-EC and classes 1~3 integrons in this region.

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#### Authors contribution:

**Ming-yi WANG:** Designed the study and did statistical analysis

**Lu-Ming Li, Xiao-Yan Yuan, Hong-Jun Wang:** Contributed in data collection and analysis

**Qin Li, YA-Mei Zhu:** Helped in drafting and revising the manuscript.