Original Article

Comparison of extended spectrum β-lactamases-producing *Escherichia coli* with non-ESBLs-producing *E.coli*: drug-resistance and virulence

Sha Li[1], Yan Qu[2], Dan Hu[2], Yong-xin Shi[3]

[1] *Department of Nursing, Qingdao University Medical College, Qingdao 266021, China*
[2] *ICU, Qingdao Municipal Hospital, Qingdao 266071, China*
[3] *Department of Clinical Laboratory, Qingdao Municipal Hospital, Qingdao 266071, China*

Corresponding Author: *Yan Qu, Email: qdquyan@yahoo.com.cn*

BACKGROUND: The virulent factors of *Escherichia coli* (*E.coli*) play an important role in the process of pathopoiesis. The study aimed to compare drug-resistant genes and virulence genes between extended spectrum β-lactamases (ESBLs)-producing *E.coli* and non-ESBLs-producing *E.coli* to provide a reference for physicians in management of hospital infection.

METHODS: From October 2010 to August 2011, 96 drug-resistant strains of *E.coli* isolated were collected from the specimens in Qingdao Municipal Hospital, Qingdao, China. These bacteria strains were divided into a ESBLs-producing group and a non-ESBLs-producing group. Drug sensitivity tests were performed using the Kirby-Bauer (K-B) method. Disinfectant gene, qacEΔ1-sull and 8 virulence genes (CNF2, hlyA, eaeA, VT1, est, bfpA, elt, and CNF1) were tested by polymerase chain reaction (PCR).

RESULTS: Among the 96 *E.coli* isolates, the ESBLs-producing *E.coli* comprised 46 (47.9%) strains and the non-ESBLs-producing *E.coli* consisted of 50 (52.1%) strains. The detection rates of multiple drug-resistant strain, qacEΔ1-sull, CNF2, hlyA, eaeA,VT1, est, bfpA, elt, and CNF1 in 46 ESBLs-producing *E.coli* isolates were 89.1%, 76.1%, 6.5%, 69.6%, 69.6%, 89.1%, 10.9%, 26.1%, 8.7%, and 19.6%, respectively. In the non-ESBLs-producing *E.coli* strains, the positive rates of multiple drug-resistant strain, qacEΔ1-sull, CNF2, hlyA, eaeA, VT1, est, bfpA, elt, and CNF1 were 62.0%, 80.0%, 16.0%, 28.0%, 64.0%, 38.0%, 6.0%, 34.0%, 10.0%, and 24.0%, respectively. The difference in the detection rates of multiple drug-resistant strain, hlyA and VT1 between the ESBLs-producing *E.coli* strains and the non-ESBLs-producing *E.coli* strains was statistically significant ($P<0.05$).

CONCLUSION: The positive rate of multiple drug-resistant strains is higher in the ESBLs-producing strains than in the non-ESBLs-producing strains. The expression of some virulence genes hlyA and VT1 varies between the ESBLs-producing strains and the non-ESBLs-producing strains. Increased awareness of clinicians and enhanced testing by laboratories are required to reduce treatment failures and prevent the spread of multiple drug-resistant strains.

KEY WORDS: ESBLs-producing *Escherichia coli*; Non-ESBLs-producing *E.coli*; Drug-resistant genes; Virulence genes; Multiple drug-resistant

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INTRODUCTION

Escherichia coli (*E.coli*), one of the most common gram-negative blood culture isolates in hospitalized patients, has become a very important pathogen associated with hospital infection.[1] The deadly *E.coli* outbreak killed persons and spread across Europe in May 2011. Multidrug-resistant *E. coli* (MDR-ECO) strains have increased with the application of broad-spectrum antibiotics. A large

number of drug-resistant genes have been reported.[2] Since ESBLs-producing *Enterobacteriaceae* were first reported in 1983 from Germany, a steady increase of these strains has been reported worldwide.[3] ESBLs producers are clinically resistant to many β-lactams,[4] while non-ESBLs producers still keep a good sensitivity to most β-lactams. *E.coli* possess many kinds of virulence factors such as intimin (encoded by the eaeA gene),[5] cytotoxic necrotizing factor 1 (CNF1), cytotoxic necrotizing factor 2 (CNF2), hemolysin (encoded by the hlyA gene),[6] bundle-forming pilli (encoded by the bfpA gene),[7] heat-labile toxin (encoded by the elt gene), heat-stable toxin (encoded by the est gene),[8] and verotoxin1 (encoded by the VT1 gene).[9]

The virulent factors of *E.coli* play an important role in the process of pathopoiesis. This study was undertaken to compare the difference in drug-resistant genes and virulence genes between the ESBLs-producing strains and the non ESBLs-producing strains from clinical samples by using polymerase chain reaction (PCR).

METHODS

Bacterial strains

Drug-resistant *E.coli* isolates were collected from clinical specimens at Qingdao Municipal Hospital within 11 months between October 2010 and August 2011. They were identified by different physiological and biochemical standard methods.[8] Drug sensitivity tests were performed by the Kirby-Bauer (K-B) method which was recommended by the WHO, and the judgement was made by the explaination standard of Cinical and Laboratory Standards Institue (CLSI). Multiple drug-resistant strain was resistant to three (e.g. aminoglycosides, sulphonamides, macrolides) or more than three antibiotics. Selected bacteria stains were stored at –70 ºC. *E.coli* O157:H7 were used as a reference strain in the study.

DNA extraction

Boiling with double distilled water was adopted for DNA extraction. Two to 3 colonies of isolated bacterial strains were placed into a tube containing 100 μL double distilled water. DNA was extracted at 100 ºC for 10 minutes, then the cells were pelleted by centrifugation. The supernatant containing DNA was taken out and stored at –20 ºC.

Polymerase chain reaction (PCR)

DNA supernatant was used for PCR analysis. PCR was performed in a final reaction volume of 25 μL, which comprised 15.3 μL sterile milliQ water, 2.5 μL 10×reaction buffer, 2 μL deoxynucleoside triphosphates (dNTPs), 1 μL (each) reverse and forward primers, 0.2 μL of Taq DNA polymerase, and 3 μL of bacterial lysate (supernatant with template DNA). Amplifications were performed with the GeneAmp PCR System 9600 (Perkin-Elmer Applied Biosystems, Inc., Foster City, Calif.). PCR amplification was started with initial denaturation at 94 ºC for 5 minutes, then 35 cycles of denaturation at 94 ºC for 30 seconds, annealing at 54 ºC for 30 seconds, extension at 72 ºC for 1 minute. A final elongation at 72 ºC for 10 minutes was also conducted. Finally, PCR amplification products were stored at 4 ºC. Primers used and annealing temperature in PCR are listed in Table 1.

The PCR amplification product was separated by submarine gel electrophoresis on 1.2% agarose, stained

Table 1. Oligonucleotide primer pairs used in this study

Primer	Oligonucleotide sequences (5'–3')	Product (bp)	Annealing temperature (ºC)	Reference number
qacEΔ1-sull	F:TAGCGAGGGCTTTACTACTAAGC R: ATTCAGAATGCCGAACACCG	300	55	10
CNF1	F: GGCGACAAATGCAGTATTGCTTGG R: GACGTTGGTTGCGGTAATTTTGGG	552	64	11
CNF2	F:GTGAGGCTCAACGAGATTATGCACTG R: CCACGCTTCTTCTTCAGTTGTTCCTC	839	55	11
elt	F:GGCGACAGATTATACCGTGC R:CGGTCTCTATATTCCCTGTT	450	52	8
est	F: ATTTTTCTTTCTGTATTGTCTT R:CACCCGGTACAGGCAGGATT	190	50	8
bfpA	F: AATGGTGCTTGCGCTTGCTGC R: GCCGCTTTATCCAACCTGGTA	324	50	8
hlyA	F: AGCTGCAAGTGCGGGTCTG R: TACGGGTTATGCCTGCAAGTTCAC	569	54	12
eaeA	F: TGAGCGGCTGGCATGAGTCATAC R: TCGATCCCCATCGTCACCAGAGG	241	54	11
VT1	F: ACGTTACAGCGTGTTGCRGGGATC R: TTGCCACAGACTGCGTCAGTRAGG	121	54	11

with ethidium bromide,and gel image was captured digitally with UV transillumination.

Statistical analysis

The data of the study were analyzed by using the SPSS 17.0. The Chi-square test was used to compare the difference of virulence between the ESBLs-producing *E.coli* and the Non-ESBLs-producing *E.coli*. $P<0.05$ was considered statistically significant.

RESULTS

Multiple resistance analysis

A total of 96 *E.coli* isolates were obtained from clinical samples, including urine (n=49), sputamentum (n=30), blood (n=6), gastric fluid (n=1), bile (n=3), drain (n=1), liquor puris (n=1), synovia (n=1), pucture fluid (n=1), pharynx swab (n=1), and ascites (n=2). Among the 96 patients, 40 were male and 56 were female, with a mean age of 65.2 years.

These isolated bacterial strains were divided into a ESBLs-producing *E.coli* group and a non-ESBLs-producing *E.coli* group according to the extended spectrum β-lactamases. The ESBLs-producing *E.coli* group consisted of 46 strains, and the non-ESBLs-producing *E.coli* group 50 strains. Multiple drug resistance was detected by antibiotic sensitivity test (Table 2). Of the 96 isolates,73 (76.0%) were multiple drug-resistant, and the rest were non multiple drug-resistant. In *E.coli* with ESBLs, 91.3% strains were multiple drug-resistant, while in *E.coli* without ESBLs 62.0% were multiple drug-resistant ($P<0.05$). Consequently, the rate of multiple drug resistance in the ESBLs-producing *E.coli* group was higher than that in the non-ESBLs-producing *E.coli* group.

Table 2. Distributions of multiple drug-resistant strain in *E.coli* with ESBLs and without ESBLs

Parameters	ESBLs+ (n=46)	ESBLs– (n=50)	Total *E.coli* (n=96)
*MDR-ECO	42	31	73
#Non MDR-ECO	4	19	23

*MDR-ECO: *E.coli* resistant to three or more antibiotics; #Non MDR-ECO: *E.coli* resistant to one or two antibiotics.

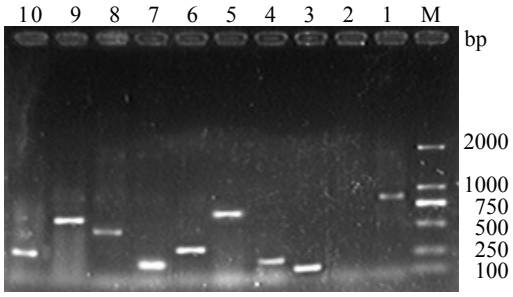

Figure 1. Polymerase chain reaction (PCR)-amplified products. M: DL2000 DNA marker; 1: CNF2 (839bp); 2: negative control; 3: VT1 (121bp); 4: eaeA (241bp); 5: hlyA (569bp); 6: bfpA (324bp); 7: est (190bp); 8: elt (450bp); 9: CNF1 (552bp); 10: qacEΔ1-sull (300bp).

Comparison of gene detection rate between the ESBLs-producing *E.coli* group and the non-ESBLs-producing *E.coli* group

The results PCR in this study are shown in Figure 1. Among the examined 96 drug resistant strains, qacEΔ1-sull was the most frequently detected gene, accounting for 78.1%. Thirty-five (76.1%) of the 46 ESBLs-producing *E.coli* strains were qacEΔ1-sull positive; the 40 (80.0%) of the 50 non ESBLs-producing *E.coli* strains were qacEΔ1-sull positive. The detection rate of the qacEΔ1-sull gene was not statistically different (χ^2=0.215, $P>0.500$). The positive number of CNF2, hlyA, eaeA, VT1, est, bfpA, elt, and CNF1 in the ESBLs-producing *E.coli* group was 3, 32, 32, 41, 5, 12, 4, and 9, respectively. The positive number of CNF2, hlyA, eaeA, VT1, est, bfpA, elt, and CNF1 in the non-ESBLs-producing *E.coli* group was 8, 14, 32, 19, 3, 17, 5, and 12, respectively (Table 3). The positive rates of hlyA and VT1 in the ESBLs-producing *E.coli* group were significantly higher than those in the non-ESBLs-producing *E.coli* group ($P<0.05$). The detection of other six virulence genes between the two groups of *E.coli* was not statistically significant.

DISCUSSION

Disinfection is an important measure to prevent and control hospital infection.[13] In the condition of the disinfectant genes expressed by *E.coli*, disinfection would

Table 3. The detection of virulence genes in *E.coli* with ESBLs and without ESBLs

E.coli	Virulence genes															
	CNF2		hlyA		eaeA		VT1		est		bfpA		elt		CNF1	
	+	%	+	%	+	%	+	%	+	%	+	%	+	%	+	%
ESBLs+	3	6.5	32	69.6	32	69.6	41	89.1	5	10.0	12	26.1	4	8.7	9	19.6
ESBLs–	8	16.0	14	28.0	32	64.0	19	38.0	3	6.0	17	34.0	5	10.0	12	24.0

not live up to the expected effect.[14,15] *E.coli* are a common pathogen of nosocomial infection.[16] With the wide use of antibiotics, drug resistance becomes more and more serious. But it is more difficult to prevent nosocomial infection if it is caused by mutiplle drug resistance of *E.coli*. Since multiple drug-resistant ESBLs-producing *E.coli* infection is increased gradually, it is very difficult to cure.[17] More effort thus should be taken to prevent nosocomial infection outbreak. Studies indicated that the drug resistance rate of ESBLs-producing *E.coli* was higher than that of non-ESBLs-producing *E.coli*.[18,19] It was also reported that the rate of multiple drug-resistant strain was higher in ESBLs-producing *E.coli* than in non ESBLs-producing *E.coli*, and that multiple drug-resistant strains occurred frequently in drug-resistant strains.[20] Hence more attention should be paid clinically to ESBLs-producing *E.coli*, and antibiotics should be used rationally according to the results of antimicrobial susceptibility tests. Multiple drug-resistance may be related to the extensive use of spectrum β-lactamases. However, its mechanism is still uncertain. Nosocomial infection is due to *E.coli* virulence genes.[21] The strains that are subjected to continuous antimicrobial challenges become resistant to some drugs but are likely to become more virulent than their predecessors. Thus it is very important to underststand the virulence genes of *E.coli*. In the present study we compared several virulence genes between the ESBLs-producing *E.coli* group and the non-ESBLs-producing *E.coli* group. After verification, the positive rates of hlyA and VT1 in the ESBLs-producing *E.coli* group were higher than those in the non ESBLs-producing *E.coli* group. This study revealed drug-resistance and virulence of the ESBLs-producing *E.coli* group were more serious than those of the non ESBLs-producing *E.coli* group. Chacón et al[22] found that virulence factors may play an important role in pathogensis, and that the detection of virulence genes is a crucial step in determining the potential pathogenicity. Since clinical laboratories only do drug sensitive test without analysis of bacteria virulence, clinicians could not estimate the toxicity of *E.coli*. This study revealed the virulence of ESBLs-producing *E.coli* provides evidence for clincians.

In conclusion, the results of this study suggest that drug resistance and expression of virulence genes in ESBLs-producing *E.coli* are more serious than in non -ESBLs-producing *E.coli*. Therefore, attention should be paid to the ESBLs-producing *E.coli* strains in order to prevent the outbreak of nosocomial *E.coli* infection in related departments.

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