Short Communication

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Detection of qacEΔ1, qacG, qacE, qacF resistance genes in Escherichia coli producing broad-spectrum beta-lactamases to benzalkonium chloride

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ABSTRACT

BACKGROUND AND OBJECTIVE: The resistance genes of quaternary ammonium compounds(qac) play an important role in the resistance of gram-negative bacteria producing broad-spectrum beta-lactamases to disinfectants. The aim of this study was detection of qacEΔ1, qacG, qacE, qacF resistance genes in *Escherichia coli* producing broad-spectrum beta-lactamases to benzalkonium chloride.

METHODS: This study cross sectional-descriptive was conducted on 150 clinical samples of selected hospitals in Arak. ESBL strains were identified by using phenotypic methods of disc diffusion and combinatory disc method and evaluating the SHV, TEM, CTXM1 genes by genotyping method. The PCR was performed to determine the resistance genes qacEΔ1, qacG, qacE and qacF.The electrophoresis of PCR products and the MIC of benzalkonium chloride were relative to *E. coli* producing ESBL. Antibiotic pattern of Escherichia coli (ESBL), quadruple ammonium resistance genes and benzalkonium chloride MIC were also investigated.

FINDINGS: This study showed that 60% of *Escherichia coli* were ESBL producer. The qacEΔ1 genes were observed in all of them and qacE, qacF, qacG genes were not found in any of the strains**.** The strains had MIC range from 32 to 64 mg/l for benzalkonium chloride. Resistance to carbapenems (33.33%) was observed.

CONCLUSION: This study showed that qacEΔ1 resistance gene and resistance to disinfectant benzalkonium chloride increased. Also increased resistance to the antibiotics studied were observed in *E. coli* ESBL strains.

KEY WORDS: *Escherichia Coli, Beta-Lactamase, Benzalkonium Chloride, Disinfectant.*

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Introduction

Beta-lactamases are enzymes that by hydrolysis of the beta-lactam ring inactivate these antibiotics and thereby cause resistance to the beta-lactam antibiotics. A new class of these enzymes, called broad-spectrum beta-lactamases, encodes genes that are transposable on large conjugated plasmids and can simultaneously transfer resistance genes to other antimicrobial agents, including bacterial resistance to antibiotics (1-4). Numerous reports from different regions of the country show the prevalence of broad-spectrum beta-lactamase-producing organisms from 40 to 80% among gram-negative bacteria (5-7), which has been of concern to physicians.

Infection with these bacteria has been associated with higher mortality rates, increased hospital stay, and increased treatment costs (8). Escherichia coli is one of the common pathogens of nosocomial infections that has become resistant beta-lactam antibiotics including cephalosporin due to the acquisition of plasmids encoding broad-spectrum beta-lactamases. Three genes of beta-lactamases including SH,V CTXM1 and TEM are mostly found in Escherichia coli (9).

In recent years, significant efforts have been made to promote health and infection control in hospitals and in the community, leading to increased use of disinfectants. For example, disinfectants based on quaternary ammonium compounds such as benzalkonium chloride are widely used in hospitals to disinfect surfaces and prevent infections because these compounds have broad antimicrobial activity (10,11). In gram-negative bacteria, qacE, qacEΔ1, sugE (P), qacF and qacG genes encode resistance to quaternary ammonium compounds (QACs genes) that are located on removable genetic elements (12-14).

As a result of the deletion mutation on qacE, the qacEΔ1 gene is formed that encodes resistance to disinfectants such as quaternary ammonium compounds. The qacF gene is genetically similar (68%) to the qacE gene (15). In gram-negative bacteria, qacE and qacEΔ1 genes are much more prevalent than other QACs genes (16,17). Among ESBL isolates isolated from clinical specimens, there was a significant relationship between the presence of qacE and qacEΔ1 resistance genes with antibiotic resistance (18). Escherichia coli isolated from clinical sources also showed a high MIC compared to quaternary ammonium compounds, which is associated with antibiotic resistance (19). Few studies have been performed on the abundance of quaternary ammonium resistance genes in ESBL Escherichia coli. Since the control of bacterial diseases depends on the use of antibiotics, the spread of antibiotic resistance phenomenon is raising public concern and is one of the public health problems.The present study was performed to investigate the antibiotic pattern, the prevalence of ESBL Escherichia coli, the distribution of qacE, qacG, qacF and qacEΔ1 resistance genes in this bacterium and to determine the minimum inhibitory concentration of benzalkonium chloride compared to ESBL Escherichia coli isolated from clinical specimens.

Methods

Samples: This cross-sectional study was approved by the Ethics Committee of Arak University of Medical Sciences under code ARAK.MU.REC.IR.294.1396 and was performed on 150 clinical samples (urine, blood, sputum and ulcer) in 1397 patients referred to selected training hospitals in Arak. Samples collected from hospitals were transferred to the University Microbiology Laboratory by the Stuart Transition Environment. Samples were cultured on blood agar and Meccanaki medium (Merck, Germany) for bacterial identification. Escherichia coli were identified using biochemical methods such as gram staining, growth on VP, MR, SIM, TSI, OF, Simon citrate, urea, lysine decarboxylase (20).

Determination of susceptibility pattern of Escherichia coli isolates by Disk diffusion agar: Escherichia coli strains were investigated on the Müller Hinton Agar medium (Merck, Germany) in terms of antibiotic susceptibility pattern by disk diffusion method (kirby-bauer method) and based on Clinical Laboratory Standards Institute guidelines (2018CLSI). Antibiotics (Mast, UK) included: Nitrofurantoin (300 µg), Tri-Metoprim Sulfamethoxazole (5 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), cefepime (30 µg), nalidixic acid (30 µg), amikacin (30 µg), cefotaxime (30 µg), cefazolin (30 µg), cefaxime (5 µg), imipenem (10 µg), meropenem (10 µg) Tetracycline (30 µg), cefoxitin (5 µg), ceftazidime (30 µg), ceftriaxone (30 µg). E-coli 25922 was also used as control strain. After disking, plates were incubated for 24 h at 37 °C, then the diameter of the growth zone around each disk was interpreted based on the 2018 CLSI tables (21).

Phenotypic confirmation of ESBL producing isolates: Combination disk method was used to confirm the phenotypic characterization of ESBL isolates. In the above method, discs (Mast, UK) of ceftazidime (30 µg),

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ceftazidime-clavonic acid (10-30 µg), cefotaxime (30 µg) and cefotaxime-clavonic acid (10-30 µg) were used. The suspension was made from a bacterium equivalent to Half-McFarland and cultured on Müller Hinton agar medium (Merck, Germany). The discs were then placed on the perimeter. After 24 h incubation at 37 ° C, the diameter of the growth zone around each disk was measured. As the diameter of the bacterial growth zone around the compound disc was 5 mm higher than the single antibiotic growth zone around the single disk, the bacterium had broad-spectrum beta-lactamase enzymes (ESBLs), and the result was a positive bacterial phenotypic test. The standard strain of Escherichia coli ATCC 35218 was used to control the quality of ESBL isolates (21). Then ESBL Escherichia coli isolates were stored in Luria Brittany Broth (LBB) medium containing 20% glycerol at -20 °C.

Genotypic identification of ESBL strains: Identification of SHV, TEM and CTXM1 beta-lactamase genes was performed by PCR using specific primers prepared (Table 1) (22).

Identification of qacE, qacEΔ1, qacF and qacG genes by polymerase chain reaction (PCR): After preparation of a fresh culture from stored ESBL-producing Escherichia coli isolates, DNA extraction using kit (Experiment Kit, Iran) was done. The sequences of primers for construction was delivered to Gene Fanavaran Co. (TAG Copenhagen's sole agent in Iran). Then, the identification of qacE, qacEΔ1, qacF and qacG genes in ESBL Escherichia coli isolates was performed with the help of specific primers prepared (Table 2) (23).

PCR products electrophoresis: The PCR product obtained for each gene was identified by electrophoresis on 1% agarose gel. The bands were then observed and photographed using a UV transducer and gel dock (Fig 1).

Determination of Minimum Inhibitory Concentration: Minimum inhibitory concentration of ESBL Escherichia coli isolates relative to benzalkonium chloride 4% was determined by microdilution broth in accordance with CLSI 2018 guidelines. In this method, serial dilutions of the disinfectant were prepared. The suspension was made from a bacterium equivalent to half McFarland. Finally, bacterial concentration was 5 *105 in each well. The microplate was then incubated at 37 °C for 24 hours. Serial dilutions ranged from 1/2 to 1/1024 and the concentration range for MIC determination was 0.125 to 1024 mg/L. E. coli ATCC 25922 was used as quality control (21). Antibiotic pattern, prevalence of ESBL Escherichia coli, distribution of qacE, qacG, qacF and qacEΔ1 resistance genes in this bacterium and minimum inhibitory concentration of benzalkonium chloride were evaluated and STATA 13 software was used to calculate the number and percentage.

Table 1. Characteristics of the beta-lactamase gene primers used in PCR

Primer	Sequence(5 to 3)	Amplicon Size(bp)	Annealing Temperature
TEM	ATGAGTATTCAACATTTCCG CTGACAGTTACCAATGCTTA	867	58
SHV	GATGAACGCTTTCCCATGATG CGCTGTTATCGCTCATGGTAA	214	61
CTXM1	AAGACTGGGTGTGGCATTGA AGGCTGGGTGAAGTAAGTGA	670	52

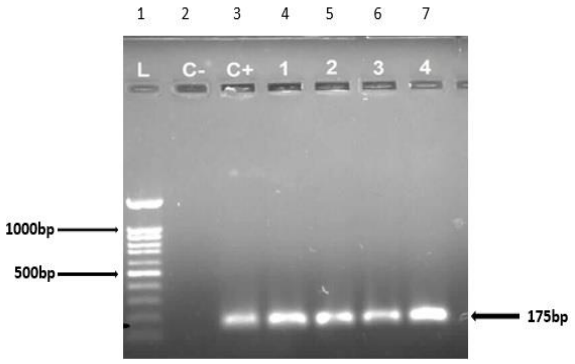

Figure 1. PCR results of the qacEΔ1 gene in ESBL strains of Escherichia coli., Column 1: 100 bp size marker, column 2: negative control strain (without qacEΔ1 gene), column 3: positive control strain (with qacEΔ1 gene), Column 4 to 7: Genotype-positive strains with qacEΔ1 resistance gene

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Table 2. The qac primers used in this study

Gene	Sequence (5'-3')	Annealing temperature (elongation time)	PCR product size (bp)	Accession no.
qacEΔ1	AATCCATCCCTGTCGGTGTT CGCAGCGACTTCCACGATGGGGAT	56°C (30s)	175	JN596280 JN566044
qacE	AAGTAATCGCAACATCCG CTACTACACCACTAACTATGAG	50°C (30s)	258	X68232 HQ875011
qacF/H/I	GTCGTCGCAACTTCCGCACTG TGCCAACGAACGCCCACA	60°C (30s)	229	FJ160769 JN596279
qacG	TGCCAACGAACGCCCACA AACGCCGCTGATAATGAA	56°C (30s)	122	FJ950725 AF288045

Results

Samples included 123 urines (82%), 11 blood (7.3%), 11 ulcers (7.3%) and 5 sputa (3.4%). Of the 150 samples studied, 130 Escherichia coli were isolated. 78 isolates (60%) of the 130 E. coli isolates were ESBL positive. The results of genotypic testing revealed that all ESBL positive Escherichia coli had CTXM1 gene. 45 isolates (57.69%) had SHV gene and 55 (70.51%) isolates had TEM gene. According to the antibiotic susceptibility pattern of ESBL Escherichia coli, there was the least resistant to nitrofurantoin and resistance to most antibiotics was increased, including carbapenem, which was 33.33% (Fig 2).

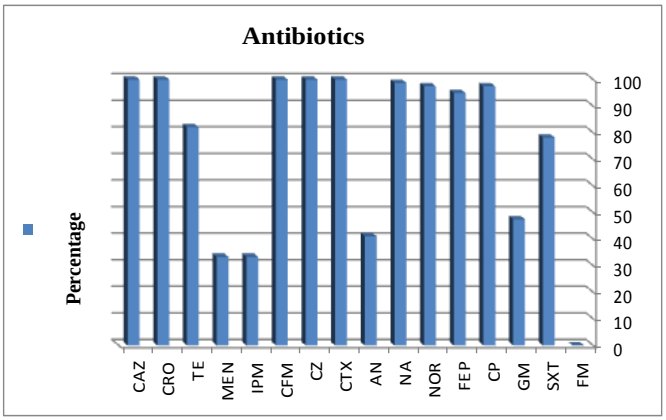

Figure 2. Frequency distribution of resistance pattern of Escherichia coli-ESBL isolates to antibiotics studied

FM (Nitrofurantoin), SXT (trimethoprim sulfomethoxazole), GM (gentamicin), CP (ciprofloxacin), FEP (cefepime), NOR (neuromycin), NA (nalidixic acid), AN (amikacin), CTX (cefotaxime) (Cefazolin), CFM (cefexime), IPM (imipenem), MEN (meropenem), TE (tetracycline), CRO (ceftriaxone), CAZ (ceftazidime).

The sensitivity results of the benzalkonium chloride disinfectant showed that the MIC in ESBL strains was between 32-64 mg l that 43.64% of the samples had 64 mg/l concentration and 56.36% of samples had 32 mg/l concentration. The qacEΔ1 gene was found in all ESBL E. coli isolates, and none of the qacG, qacE, qacF genes were detected in any of the isolates.

Discussion

In this study, the qacEΔ1 gene was detected in all isolates (100%). The qacE, qacEΔ1, qacF, qacG resistance genes are encoded on the plasmid or integron that can induce resistance to QACs by the efflux pump (12,13,15). ESBL Escherichia coli is able to withstand a greater amount of disinfectants by expressing the resistance gene such as the qacEΔ1 gene to quaternary ammonium compounds.

Therefore, excessive use of disinfectant not only does not eliminate or eliminate the bacteria but can also lead to resistance of ESBL bacteria to disinfectants. In the study of Canal et al., the qacEΔ1 gene was detected in all isolates (100%) (24). Shafaati et al. reported the abundance of the qacEΔ1 gene among ESBL-producing Escherichia coli isolates from urine samples about 94% (18). Mahzonieh et al. reported 91.5% of qacEΔ1 resistance genes in gram-negative bacteria (25). In this study, all ESBL strains had minimum inhibitory concentrations ranging from 32 to 64 mg/L for benzalkonium chloride. In the study of Guo et al., the minimum inhibitory concentration of ESBL strains was reported to be between 8 to 128 mg/L (23).

In the study of Liu et al., the minimum inhibitory concentration of benzalkonium chloride for S. baumannii and Klebsiella was 8.32 mg/l and 128-32, respectively (26). Given the increased inhibitory concentration of quaternary ammonium compounds of benzalkonium chloride in this study, there is more need to pay attention to the choice of disinfectant and further research on its use and also is necessary the proper use of antiseptics and disinfectants in hospitals for the

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effectiveness of disinfectants. The present study showed that the most effective antibiotic for ESBL Escherichia coli is nitrofurantoin, which was consistent with the study by Amiri et al. in 2015 and Ranjbaran et al. (5, 27). Resistance to carbapenems has also increased compared to previous studies (7,26,28), indicating an increase in carbapenem use in hospitals. It should be noted that carbapenems are often the preferred option for the treatment of ESBL-producing bacterial infections as well as the treatment of severe infections acquired from the hospital. Therefore, unnecessary use, and irrational administration of antibiotics, can lead to drug resistance.

Therefore, the high prevalence of ESBL-producing Escherichia coli and its increased qac resistance genes, as well as resistance to broad-spectrum antibacterial agents (carbapenems) in Enterobacteriaceae, are of concern in this study. There is a need for infection control measures to rationally manage antibiotic use and rapid identification of beta-lactamase producing strains. In order to prevent the increase of drug resistance in different regions, it is recommended to conduct continuous research in each area. Because rational administration of antibiotics helps the effectiveness of the treatment process. On the other hand, increasing the minimum inhibitory concentration in this study indicates that proper disinfectants should be used in hospitals in order to be effective disinfectants. Use of disinfectants at concentrations lower than the inhibitory concentration may increase the prevalence of bacteria resistant to disinfectants, which may spread the infection to patients and the community. Despite the prevalence of QAC resistance genes in Escherichia coli, the mechanism of resistance to disinfectants and the relationship between resistance genes and antibiotic susceptibility in Escherichia coli isolates have not been well understood. Therefore, increasing the recognition of the resistance genes of quaternary ammonium compounds in ESBL Escherichia coli, proper use of disinfectants, as well as investigating the microbial resistance patterns in this bacterium, may help the effectiveness of infection control process in hospitals.

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