

Original Article

# Prevalence of extended-spectrum $\beta$ -lactamase (ESBL)-producing *Escherichia coli* associated with outbreaks of food-borne gastroenteritis in Tehran



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## ABSTRACT

The prevalence of antibiotic resistance has been demonstrated in various food-borne pathogens. Beta-lactam antibiotics are among the first-line antimicrobials that are normally administered in case of gastrointestinal infections. However, *Escherichia coli* (*E. coli*) and some other members of Enterobacteriaceae have indicated broad resistance against such antibiotics thanks to extended-spectrum beta-lactamase (ESBL) enzymes. In this research, 216 stool samples have been screened for ESBL-producing *E. coli*, using phenotypic antibiotic susceptibility tests. ESBL-producing *E. coli* isolates were further screened for the presence of antibiotic-resistance genes CTX-M, SHV, and TEM. Our isolation experiments resulted in 111 *E. coli* isolates among which 41 (36.9%) isolates were found as ESBL. Also, 51.2% of the above ESBL isolates harbored *bla*<sub>TEM</sub>. Furthermore, 18 (43.9%) and 2 (4.9%) of those ESBL isolates had *bla*<sub>CTX-M</sub> and *bla*<sub>SHV</sub> genes, respectively. Our results revealed a detectable prevalence of ESBL *E. coli* in stool samples collected during food outbreaks. Results of such researches can guide how to control the distribution of drug-resistant pathogens in various environments. In this line, the considerable prevalence of ESBL *E. coli* seems to have originated from the wide administration of various beta-lactam antibiotics.

## 1. Introduction

Food-borne diseases tend to be an important public health problem in developing and even developed countries. According to the definitions, a food-borne disease outbreak is when two or more individuals with the same diagnostic symptoms have used the same food or drink source [1].

The World Health Organization (WHO)

estimated that 31 foodborne hazards caused 600 million foodborne illnesses which led to around 420,000 deaths worldwide in 2010 [2]. Also, WHO statistics revealed that the situation is far more concerning in developing countries where considerable numbers of children lose their lives due to food-borne infections [3, 4].

Gram-negative bacteria cause a major part of food-borne infections worldwide. Members

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of the genera *Salmonella*, *Shigella*, *Escherichia*, and *Campylobacter* are among the most prevalent food-borne pathogens [5, 6].

One of the main issues of the global food industry during recent decades seems to be the mass production of various food products to satisfy the growing population [7]. However, mass production of food products can have a diverse array of safety and hygiene issues which may even cause food-borne diseases. Besides those food safety issues, the persistent application of broad-spectrum antibiotics in agriculture which has been in line with the mass production of food has had a major role in the prevalence of multiple drug-resistant (MDR) microbes. Hence, surveying the prevalence of antibiotic resistance in food products can guide health authorities to take preventive measures against food-borne disease outbreaks [7].

*Escherichia coli* (*E. coli*) causes a considerable part of gastrointestinal infections recorded worldwide. *E. coli* is an opportunistic pathogen that can acquire various plasmids carrying different drug-resistant genes. Such plasmids carry not only beta-lactam resistance genes but also genes coding for resistance to various other antibiotics. Thus, this Gram-negative rod shows resistance against a diverse array of antibiotics, including beta-lactam antibiotics (Penicillin, Cephalosporin, Monobactam, Carbapenem) [8].

Production of beta-lactamase enzymes tends to be the main mechanism through which *E. coli* is resistant to beta-lactams [9]. Hence, bacteria often develop resistance to  $\beta$ -lactam antibiotics by synthesizing  $\beta$ -lactamase enzymes that are classified based on their spectrum of activity on various beta-lactam antibiotics. So, broad-spectrum beta-lactamases which have *bla*<sub>shv</sub> and *bla*<sub>TEM</sub> mutations fall in group Ambler class A [9].

Distribution of drug-resistant pathogens can happen through contaminated food, water, and other sources. Food products originating from agricultural facilities which apply antibiotics to increase their productivity can serve as reservoirs of antibiotic resistance genes. Hence, our results shed more light on the importance of such surveying programs to

control food poisoning. Such investigations can have controlling effects on the distribution of beta-lactamase genes among pathogens. In this study, the distribution of broad-spectrum beta-lactamases in *E. coli* isolates originating from food-borne outbreaks was addressed.

## 2. Materials and Methods

### 2.1. Samples, Enrichment, and Isolation

In This study, 216 stool samples from food-borne outbreaks were collected and preserved at 4 °C to be transported to the laboratory. The microbiological assessments were performed to isolate *E. coli* [10, 11]. Briefly, 1 gram of the collected stool samples were aseptically inoculated and homogenized into 9 ml 0.9% saline buffer in glass tubes. Then, aliquots of 50  $\mu$ L were streaked on Mack Candy agar plates.

A loop-full transfer to Xylose Lysine Deoxycholate (XLD) agar plates was performed after 16 hours of incubation at 37 °C. After 24 hours of incubation at 37 °C, suspicious colonies were further streaked on agar media to get pure single colonies. *E. coli* isolates were confirmed based on biochemical tests; and Lactose positive, motile, Lysin positive, Citrate negative, Urea hydrolysis negative, Indole positive, and Methyl red positive colonies were assumed as *E. coli* isolates [10]. Purified isolates which were confirmed as *E. coli* were finally cryopreserved at -20 °C in trypticase soy broth medium supplemented with 10% glycerol [10].

### 2.2. Phenotypic detection of ESBL isolates

#### 2.2.1. Presumptive detection of ESBL-producing *E. coli*

Screening for ESBL isolates, and antibiotic susceptibility of *E. coli* isolates was assessed based on CLSI 2018(M100) standard. Disk diffusion antibiotic susceptibility tests were performed on Mueller Hinton agar plates.

#### 2.2.2. Confirmatory ESBL detection

Ceftazidime-cefotaxime-resistant *E. coli* Isolates were further tested for ESBL using clavulanic acid-combined disks [12] based on the CLSI 2018(M100) standard. In this regard,

Ceftazidime-Cefotaxime-Resistant *E. coli* Isolates were streaked on Trypticase soy agar (TSA) medium. A single pure colony was transferred to 0.9% saline buffer to obtain a cell density similar to 0.5 McFarland turbidity standard. A sterile swab was immersed in the above *E. coli* suspension followed by homogenous inoculation on Mueller-Hinton agar plates.

According to CLSI 2018 standard, the Cefotaxime disk was placed on agar keeping 20 mm distance from the clavulanic acid-Ceftazidime and either clavulanic acid-Cefotaxime combined disks. At least 5 mm larger halos of growth inhibition for combined disks compared to normal disks were inferred as ESBL. Beta-lactamase positive *Klebsiella pneumoniae* ATCC 700603 was used as a control.

### 2.3. DNA extraction

Genomic DNA extraction from the confirmed *E. coli* isolates was performed using the boiling method which is very efficient in the case of Gram-negative bacteria. So, 1 ml of a 24h culture of isolates was centrifuged (10000 r.p.m, 4 °C). The supernatant was discarded and the biomass was suspended in 400 µL distilled deionized water. After a gentle spin, micro-tubes were incubated at 100 °C for 15 min in a boiling water bath followed by immediate transfer to - 20 °C. After 2 minutes, micro-tubes were centrifuged (13000 r.p.m, 4 °C). Supernatants, containing the genomic DNA, were transferred to new micro-tubes [12, 13].

The extracted genomic DNA samples were spectrophotometrically assessed based on absorbance at 260/280 nm wavelengths followed by horizontal electrophoresis of DNA samples on 1% agarose gel, staining and visual observation in the Gel doc system. DNA samples were preserved at -20 C for further experiments [12, 13].

### 2.4. PCR- detection of ESBL-associated genes

Genetic loci including *bla<sub>CTXM</sub>*, *bla<sub>TEM</sub>* and *bla<sub>SHV</sub>* as genetic markers for resistance against beta-lactam antibiotics have been widely tested as genetic markers for ESBL in various enteric bacteria [13].

PCR method using *bla<sub>CTXM</sub>*, *bla<sub>TEM</sub>* and *bla<sub>SHV</sub>* genes specific primers was thus used for screening of ESBL. Primer pairs were first analyzed using the blastN program (National Center for Biotechnology Information (NCBI) available at (<http://ncbi.nlm.nih.gov/BLAST>)). The selected primer pairs were: *bla<sub>TEM</sub>* (750 bp): TAATCAGTGAGGCACCTATCTC, and GAGTATTCAACATTTCCGTGTC [13], *bla<sub>CTXM</sub>* (499 bp): TTTGCGATGTGCAGTACCAGTAA, and CGATATCGTTGGTGCCATA [13], *bla<sub>SHV</sub>* (471 bp): GGTATGCGTTATATTCGCC, and TTAGCGTTGCCAGTGCTC (13). Primers were synthesized by Genfanavar Ltd. Co. (Iran) [13].

The total volume for PCR amplifications was 20 µL, containing 1X Amplicon Master Mix (New England Biolabs Inc, Ipswich, UK), 0.3 mM of each of the above primers, and 0.4 ng of the genomic DNA of *E. coli* isolates. PCR (Eppendorf, Homburg, Germany) temperature cycling was as below: first stage: 95 °C for 5 min (a single cycle); second stage: 95 °C for 40 s, 58 °C for 60 s, 72 °C for 50 s; third stage: 72 °C for 5 min. The PCR products were visualized with Gel electrophoresis in 1% (W/V) agarose gel.

## 3. Results

In this study, 216 stool samples were collected from patients with gastroenteritis symptoms. Based on our isolation experiments, *E. coli* was detected in 111(51.4%) samples. Furthermore, phenotypic antibiotic susceptibility tests based on clavulanic acid-combined disks revealed that among those *E. coli* isolates, 41(36.9%) were presumptive beta-lactamase-producing *E. coli* isolates.

The growth inhibition halo diameter in the above beta-lactamase-producing *E. coli* isolates was more than 5 mm when clavulanic acid disks were used (Table 1). Also, results of confirmatory ESBL detection tests were found in line with the above findings as 41 beta-lactamase-producing *E. coli* isolates were detected as ESBL (Figure 1).

### 3.1. Prevalence of genes encoding for ESBL in isolated *E. coli*

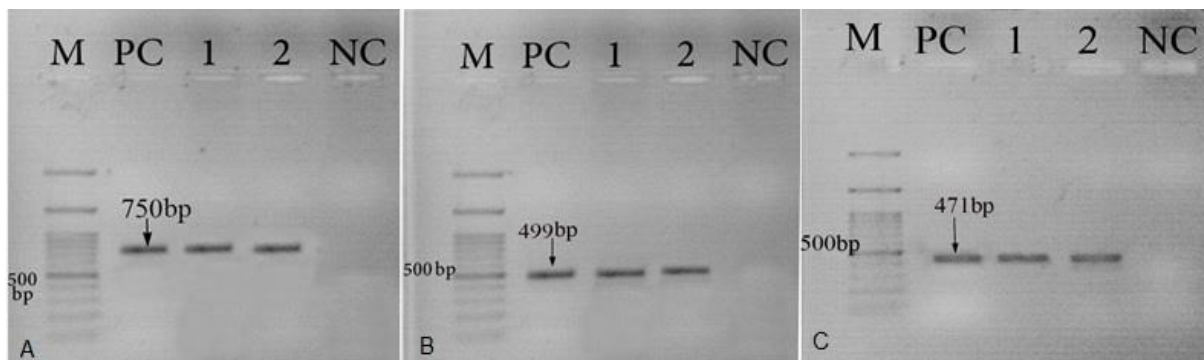
The prevalence of *bla<sub>TEM</sub>* gene was studied which encodes for hydrolysis of broad-

spectrum cephalosporins, including cefotaxime and ceftazidime. The specific band for this region (750 bp) was detected in 21 (51.2%) isolates out of 41 phenotypic beta-lactamase-producing *E. coli* isolates (Figure 2A). Furthermore, according to PCR-amplification tests 18 (43.9%) *E. coli* isolates

harbored *bla*<sub>CTX-M</sub> gene (499 bp) which causes a high resistance against cefotaxime (Figure 2B). Additionally, similar tests revealed that 2 (4.9%) out of 41 beta-lactamase producing *E. coli* isolates harbored *bla*<sub>SHV</sub> gene (471 bp) (Figure 3C).



**Fig. 1.** Confirming test for ESBL using clavulanic acid-combined disks of ceftazidime (30 mg) and cefotaxim (30 mg).



**Fig. 2.** Molecular size marker (M), Positive Control (PC), Negative Control (NC); **A:** 750 bp band for *bla*<sub>TEM</sub> gene; **B:** 499 bp band for *bla*<sub>CTX-M</sub> gene; **C:** 471 bp band for *bla*<sub>SHV</sub> gene.

#### 4. Discussion

The high prevalence of beta-lactam-resistant *E. coli* in farm animals represents a potential reservoir of human infection that transmit to humans through direct or indirect contact [5]. Such an increasing distribution of drug-resistant bacteria can endanger food safety at the societal level.

*E. coli*, is a facultative anaerobe, belonging to Enterobacteriaceae which has been long known as a member of human gastrointestinal

common flora [13]. The World Health Organization (WHO) estimated that 31 foodborne hazards caused 600 million foodborne illnesses which led to around 420,000 deaths worldwide in 2010 [2]. Interestingly, *E. coli* is also known as one of the most prevalent microbes that cause foodborne diseases [2]. In this line, it has been shown that *E. coli* is among the most prevalent causes of food outbreaks in the United States [14, 15]. In the current study, *E. coli* were detected in 51.4% of samples. Hence, our results on the prevalence of *E. coli* in food

outbreaks seem to be in line with the results of similar studies [5]. However, pathogenic species of *Salmonella* and *Campylobacter* have been also introduced as significantly prevalent causes of food outbreaks [16].

Antibiotic-resistant pathogens have been long regarded as an economically threatening problem, globally. Extended-Spectrum  $\beta$ -lactamases (ESBLs) are one of such drug resistance issues which have significant consequences at a societal level. ESBL *E. coli* has been detected in 60% of mortal cases caused by bacteremia. Hence, studying the distribution of ESBL isolates in various environments can help health authorities to take control measures [17]. It has been indicated that multiple drug resistance had been more frequent in *E. coli* when compared to *Salmonella* and *Campylobacter* isolates [18]. It has been shown that the ESBL-producing *E. coli* is nowadays eight times more prevalent when compared to the last two decades with a prevalence of around 2.6% in 2003 that reached 21.1% in 2018 [19].

It has been studied the prevalence of antibiotic resistance-associated genes in ESBL-producing *E. coli* detected in Germany, Britain, and Netherlands [20]. The above research revealed that 66% and 23% of *E. coli* isolates harbored  $bla_{CTX-M}$  and  $bla_{CTX-M-bla_{OXA}}$  genes. Falgenhaur et al. showed that the prevalence of ESBL *E. coli* in poultry is around 29% whereas the prevalence of such pathogens in children can be about 61% [21]. Hence, our results seem to be in line with the findings of similar researches [21-24].

ESBL can be transferred by plasmids. Interestingly, mutations have been reported in TEM and SHV genes which lead to amino acid substitutions in active sites of such  $\beta$ -lactamases [25]. Interestingly, we have detected ESBL-producing *E. coli* isolates that had CTX-M enzymes together with TEM and SHV [26]. CTX-M  $\beta$ -lactamases are reportedly more active against ceftriaxone and cefotaxime than ceftazidime, however, point mutations can improve their activity against ceftazidime [27, 28]. The prevalence of the CTX-M encoding gene has been reported in *E. coli* isolates detected in various samples [28]. The majority of the researches have

reported the prevalence of that gene to be between 40-100% depending on the studied samples [29-32].

Our results revealed that the prevalence of  $bla_{TEM}$  (51.2%) is much higher than that of  $bla_{SHV}$  (4.8%). There are now >90 TEM-type  $\beta$ -lactamases and >25 SHV-type enzyme. Interestingly, most ESBLs are thought to be derived from TEM and SHV enzymes [33, 34].

## 5. Conclusion

Regarding the threatening distribution of antibiotic-resistant pathogens, conducting surveying researches tends to be a priority for associated authorities at the national and international level. Results of such researches can guide how to control the distribution of drug-resistant pathogens in various environments. In this line, the considerable prevalence of ESBL *E. coli* seems to be originated from the wide administration of various  $\beta$ -lactam antibiotics.

## Conflict of Interests

The authors declare that they have no competing interests. All authors have approved the manuscript for publication. This research is original research which has been approved by all authors.

## Ethics approval and consent to participate

No human or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

## Consent for publication

All authors read and approved the final manuscript for publication.

## Informed Consent

The authors declare not used any patients in this research.

## Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Authors' contributions

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