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Investigation of TEM and SHV Beta-Lactamase Genes in *Escherichia coli* Isolated from Strawberry Samples in Sanandaj, Iran

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HIGHLIGHTS

- The most susceptibility and resistance of *Escherichia coli* to antibiotics were related to chloramphenicol and trimethoprim/sulfamethoxazole, respectively.
- Six out of 21 *E. coli* isolates were Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli*.
- Four out of six ESBL-producing *E. coli* isolates included bla_{TEM} gene, while bla_{SHV} gene was not found.

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Acronyms and abbreviations
ESBL=Extended-Spectrum Beta-Lactamase
PCR=Polymerase Chain Reaction

ABSTRACT

Background: When animal manures are used, food products may include pathogenic bacteria, especially *Escherichia coli*. The major aim of the current study was to investigate TEM (bla_{TEM}) and SHV beta-lactamase (bla_{SHV}) genes in *E. coli* isolated from strawberry samples in Sanandaj, Iran.

Methods: In this study, 150 strawberry samples were collected from farms (traditional), greenhouses, and packages in Sanandaj, Iran. *E. coli* contamination was done using routine culture methods. Then, isolates were investigated for Extended-Spectrum Beta-Lactamase (ESBL) production and bla_{TEM} or bla_{SHV} genes using phenotypic and genotypic methods, respectively.

Results: The most susceptibility and resistance of *E. coli* to antibiotics were related to chloramphenicol and trimethoprim/sulfamethoxazole, respectively. Out of 21 isolates of *E. coli*, eight were resistant to ceftazidime and cefotaxime; from which, six isolates were ESBL-producer. Furthermore, Polymerase Chain Reaction (PCR) analysis of six ESBL-producing *E. coli* isolates showed that four isolates included bla_{TEM} gene, while no isolates included bla_{SHV} gene.

Conclusion: In this study, multiple antibiotic resistance patterns were seen in *E. coli* isolates, especially ESBL patterns in *E. coli* isolated from strawberries produced in Iran.

Introduction

Various etiological factors can cause food-borne diseases, including bacteria, viruses, parasites, biological toxins, and chemicals (Wu et al., 2018). In most cases, bacteria grow in foods and hence foods lose their appealing presence. For example, bacteria that produce pigments can change food color. In fact, bacteria are the

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most important microbial factors of the food transmitted diseases. Investigation of bacteria that cause chemical changes in foods is highly important. Identification of improving/inhibiting factors of the bacterial growth and activity is essential in understanding principles of food preservation and decay. Common chemical changes in foods caused by the bacteria include hydrolyses of carbohydrates, proteins, and lipids. To produce energy, bacteria usually use foods in the oxidation-reduction reactions (Addis and Sisay, 2015).

When animal manures are used, food products may include pathogenic bacteria, especially *Escherichia coli* (Castro-Rosas et al., 2012; Laidler et al., 2013; Oliveira et al., 2019; Slayton et al., 2013). Studies by Bayat Makoo et al. (2010) and Soltan Dallal et al. (2013) have shown that beta-lactamase enzymes are the most important factor of resistance to β-lactam antibiotics in Gram-negative bacteria such as *E. coli*. The Extended-Spectrum Beta-Lactamases (ESBLs) includes several enzymes that result in resistance to ceftazidime, cefotaxime, other cephalosporins, and penicillins (Pishtiwan and Khadija, 2019). The TEM, SHV, and CTX-M enzymes are three major types of ESBL. However, ESBL containing bacteria may show resistance to other antibiotics such as sulfonamides, aminoglycosides, as well as quinolones. Naturally, the highlighted genes are transferred by mobile genetic elements such as integrons, plasmids, and transposons or chromosomally encoded within the bacteria (Akpaka et al., 2010). This has resulted in increased infections by these bacteria worldwide (Soltan Dallal et al., 2013).

Therefore, the current study was carried out because of the importance of the highlighted bacteria in food poisoning and also the lack of studies on strawberry contamination. The major purpose of the present research was to investigate TEM (bla_{TEM}) and SHV beta-lactamase (bla_{SHV}) genes in *E. coli* isolated from strawberry samples in Sanandaj, Iran, using Polymerase Chain Reaction (PCR).

Materials and methods

Sample collection

Totally, 150 strawberry samples from farms (traditional), greenhouses, and fruit packages were collected in Sanandaj, Iran, from May to July 2017. These samples were collected under appropriate conditions and immediately transferred to the laboratory.

Isolation and identification of E. coli

Samples were investigated for *E. coli* according to the national standard instructions no. 2946. The *E. coli* ATCC 25922 standard strain was used as positive control. Briefly, 5 g of each sample was weighed in a sterile container next to the flame, mixed with 45 ml of ringer solution, and incubated at room temperature for 20 min. Then, 10 ml of this mixture were added to Durham tubes containing Lauryl sulfate broth (Merck, Germany) and incubated at 37 °C for 48 h. One ml from each sample with turbidity and gas was added to a tube containing *Escherichia coli* broth (EC; Merck, Germany) and incubated at 44 °C for 48 h. In case of gas was produced in tube, media were cultured linearly on MacConkey agar (MAC; Merck, Germany) using sterile loop. After 24 h of incubation at 37 °C, amethystine colonies were identified using differential tests for *E. coli*.

Antibiotic susceptibility assessment

Isolated *E. coli* were assessed for their antibiotic susceptibility schemes using disk diffusion (Kirby-Bauer) method. Antibiotic susceptibility disks of gentamicin, imipenem, ciprofloxacin, chloramphenicol, cefotaxime, ceftazidime, and trimethoprim/sulfamethoxazole (Mast, UK) were used for the assessment. The *E. coli* ATCC 25922 standard strain was used as control.

Phenotypic assessment of ESBL-producing E. coli

To identify ESBL-producing isolates, *E. coli* isolates with resistance to cefotaxime and ceftazidime were tested for ESBL phenotype using combined disk assay as a confirmation test (CLSI, 2018). Briefly, a bacterial suspension equivalent to 0.5 McFarland was prepared from each of the cefotaxime and ceftazidime resistant isolates and cultured on Mueller-Hinton agar media (Merck, Germany). Then, four disks of ceftazidime, ceftazidime/clavulanic acid, cefotaxime, and cefotaxime/clavulanic acid were transferred onto the media plate with appropriate distances. After incubation at 37 °C for 24 h, inhibition zones (mm) around the disks were measured using ruler and results were recorded. A ≥5 mm enhancement in zone diameter for ceftazidime/clavulanate (30/10 µg) and cefotaxime/clavulanate (30/10 µg) compared to ceftazidime (30 µg) and cefotaxime (30 µg) disks were defined as a positive result. *Klebsiella pneumoniae* ATCC 700603 was used as positive and *Escherichia coli* ATCC 25922 as negative controls.

Detection of bla_{TEM} and bla_{SHV} genes by PCR

The ESBL-producing *E. coli* isolates which were previously identified were investigated for bla_{TEM} as well as bla_{SHV} genes using the gene specific primers. The primer sequences included bla_{TEM}-F: ATGAGTATTCAACATTTCCG and also bla_{TEM}-R: GTCACAGTTACCAATGCTTA, generating

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847 bp amplicons in size; as well as *bla*$_{SHV}$-F: GATGAACGCTTTCCCATGATG and also *bla*$_{SHV}$-R: CGCTGTTATCGCTCATGGTAA generating a fragment of 214 bp. Bacterial DNA was extracted using commercial kits based on the manufacturer's instructions (CinnaGen, Iran). The PCR reactions were prepared in a final volume of 25 µl, including 12.5 µl of 1× master mix, 1 µl of each forward and reverse primers in 10 pm concentration, 8.5 µl of sterile water, and 2 µl of the DNA template; and amplification was done in thermo cycler (Bioer, China). After that, PCR products were assessed using agarose gel electrophoresis and visualized under UV light. In each run, *K. pneumoniae* ATCC 7881 was used as positive control and ddH_2O as negative control.

Results

Out of 150 samples, 21 (14%) were contaminated with *E. coli*; of which, 19 and 2 cases were found in farm and packaged samples, respectively. No contamination was seen in greenhouse samples. The most bacterial susceptibility to antibiotics was recorded for chloramphenicol (14 isolates) and the most resistance was related to trimethoprim/sulfamethoxazole (14 isolates) (Table 1; Figure 1).

Out of 21 isolates of *E. coli*, eight were resistant to ceftazidime and cefotaxime; from which, six were ESBL-producer. These ESBL-producing *E. coli* were detected in five traditional cultured and one packaged strawberry samples. Furthermore, molecular analysis of the six ESBL-producing *E. coli* isolates showed that four included *bla*$_{TEM}$ gene, while no isolates included *bla*$_{SHV}$ gene (Figures 2 and 3).

Discussion

In general, reports on the roles of food microbial contaminations show the importance of studying food contaminations, especially those linked to various sources of microorganisms, e.g. water, soil, animals, and humans (Jung et al., 2014). Food-borne diseases are mostly caused by the consumption of contaminated foods and water. In this study, we found ESBL-producing *E. coli* in traditional cultured and packaged strawberry samples. The most antibiotic resistance rate was related to trimethoprim/sulfamethoxazole. Also, our molecular analysis showed *bla*$_{TEM}$ gene in most ESBL-producing *E. coli* isolates. In traditional cultivation of strawberry in Iran, *E. coli* contamination is predictable due to use of animal manures and contaminated surface waters in agricultural fields.

Garcia et al. (2015) reported that 36 (39.6%) out of 91 soil samples, 167 (87.9%) samples out of 190 irrigation water, and 29 (31.5%) samples out of 92 vegetable samples in Philippines were contaminated with *E. coli*. Bacterial contamination of the vegetables in that study was two times greater than that in the present study. In another study by Zurfluh et al. (2015) in the Switzerland, 26 (15.3%) samples out of 169 vegetable samples were contaminated with *E. coli*; closely similar to our findings. In a study by Shenge et al. (2015) on tomatoes in Nigeria, contamination with *E. coli* was reported as 17%, which was slightly greater than that in the present study. Ilic et al. (2008) investigated spinaches in USA using culture methods and reported that 8.9% of the samples were contaminated with *E. coli*; which was lower than that reported by the present study (14%).

Rasheed et al. (2014) reported 23 (76.7%) *E. coli* isolates from 30 vegetable samples in India; from which, two (8.7%) were ESBL-producing bacteria. This value was higher than the value from the present study (28.6%). However, prevalence of ESBL production in the study of Rasheed et al. (2014) was lower than that in the current study (28.6%). Yoon et al. (2010) microbiologically assessed strawberry production using samples from greenhouses and packaging centers to recommend good agricultural practice systems. In general, no *E. coli* and *E. coli* O157:H7 were detected in the samples, which revealed involvement of soil and irrigation water in contaminating vegetables and fruits, including strawberry. Similar results were reported in the study of Day et al. (2019) who evaluated ESBL-producing *E. coli* in food related samples collected from UK. However, 21 *E. coli* isolates (21%) in the current study might be linked to the irrigation with wastewater sources and/or animal originated manures. As we know, presence of coliforms, especially *E. coli* as fecal indicator, in strawberry and other fruits and foods can alert that at least one of the three major stages of plating, harvesting, and storage is contaminated with human or animal feces. Therefore, to prevent *E. coli* outbreaks, preventive measures must be strictly considered.

Bacterial antibiotic resistance is majorly concerned by the health authorities worldwide and the rapid extent of this resistance in various societies has immerged development of food hygiene monitoring systems. In a study by Njage and Buys (2015) on 46 *E. coli* isolates from canal water, river water, and lettuce in South Africa; one (5%) isolate was reported as *bla*$_{TEM}$ gene-carrier *E. coli*. No isolates with *bla*$_{SHV}$ gene were reported. Also, no isolate with *bla*$_{TEM}$ gene was found in 14 samples of river water. However, one (7%) isolate was reported to include *bla*$_{SHV}$ gene. Regarding the lettuce samples, three out of 10 samples were *bla*$_{TEM}$ gene carriers with no isolates

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Table 1: Antibiotic susceptibility pattern of the *Escherichia coli* isolated from strawberry samples produced in Sanandaj, Iran

Antibiotics	No. of isolates		
	Resistant	Intermediate	Susceptible
Trimethoprim/sulfamethoxazole	14	2	5
Gentamicin	9	12	0
Ciprofloxacin	10	1	10
Imipenem	9	5	7
Chloramphenicol	6	1	14
Ceftazidime	8	0	13
Cefotaxime	8	0	13

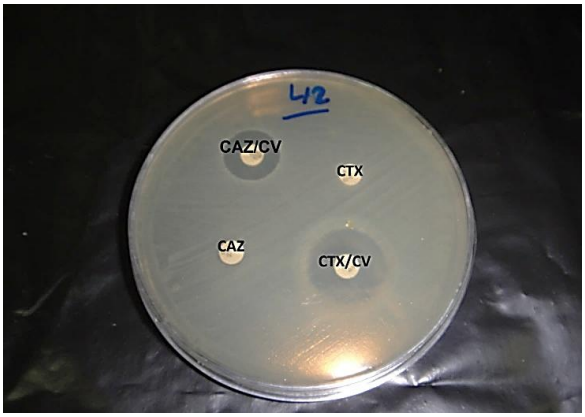

Figure 1: A positive extended-spectrum beta-lactamase-producing *Escherichia coli* using combination disk method (Confirmation test)

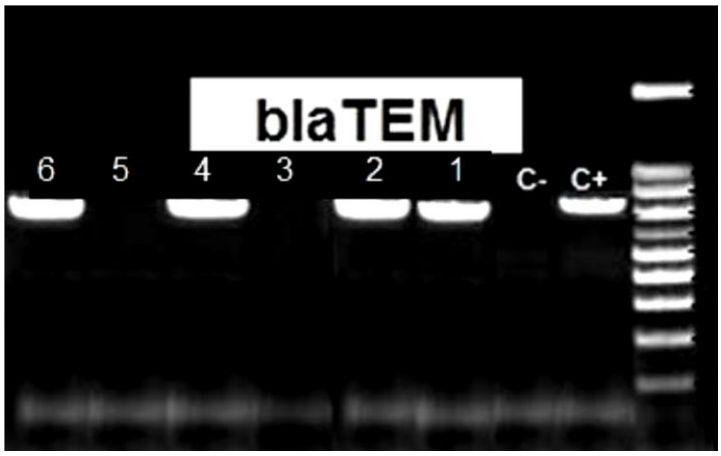

Figure 2: Agarose gel electrophoresis of bla_{TEM} Polymerase Chain Reaction products using 100 bP DNA ladder. Lane C+: positive control; lane C-: negative control; lanes 1, 2, 4, and 6: isolates with bla_{TEM} genes; lanes 3 and 5: isolates without bla_{TEM} gene.

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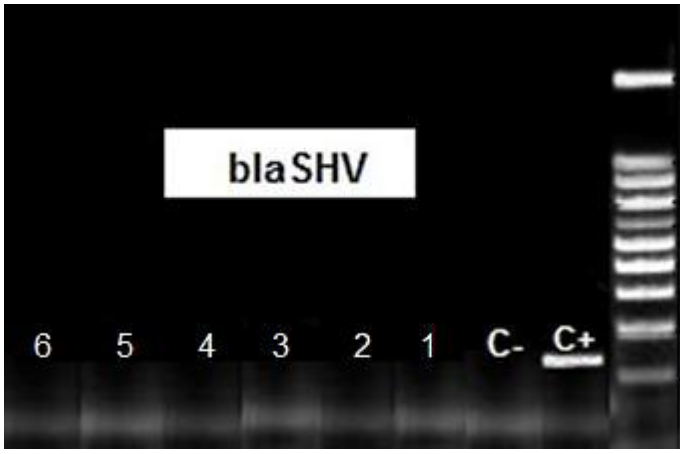

Figure 3: Agarose gel electrophoresis of *bla*$_{SHV}$ Polymerase Chain Reaction products using 100 bp DNA ladder. Lane C+: positive control; lane C-: negative control; lanes 1-6: isolates without *bla*$_{SHV}$ gene.

carrying *bla*$_{SHV}$ gene. Among the isolates, four (8.7%) were *bla*$_{TEM}$ gene carriers and one was *bla*$_{SHV}$ gene carrier; which were lower than those of *bla*$_{TEM}$ gene carriers and more than those of *bla*$_{SHV}$ gene carriers from the present study.

Conclusion

In this study, multiple antibiotic resistance patterns were seen in *E. coli* isolates, especially ESBL patterns in *E. coli* isolated from strawberries produced in Iran. In total, most of the ESBL-producing *E. coli* isolates included *bla*$_{TEM}$. However, the rest of ESBLs-producing isolates might include other encoding genes. Therefore, studies on further resistance encoding genes such as *bla*$_{CTX\text{-}M}$ seem necessary.

Author contributions

M.M.S.D. designed and supervised the study; H.A. did the experimental work and drafted the manuscript; R.R. advised scientifically during the study; R.M.N.F. advised technically during the study and revised the manuscript.

Conflicts of interest

The authors declared that they have no conflicts of interest.

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