Frequency and characteristics of ESBL-producing *Escherichia coli* isolated from Mexican fresh cheese

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Abstract

Fresh cheese is one of the most consumed products of the dairy industry in México. This study aimed to determine the frequency, antimicrobial resistance, and genetic characteristics of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in fresh cheese. From fresh cheese samples, *E. coli* was isolated and the production of ESBL, the antibiotic resistance patterns, the frequency of resistance genes, and the genetic relationship were analyzed. Sixty ESBL-producing *E. coli* belonging to phylogenetic groups A, C, and B1 were isolated and were highly resistant to the beta-lactam antibiotics, tetracycline, streptomycin, and trimethoprim-sulfamethoxazole. The bla_{CTX-M} gene was detected in all isolates, either alone or in combination with bla_{TEM} and bla_{SHV}. Similarly, a high frequency of resistance genes *tetA*, *strA*, and *strB* and class 1 integrons were found. According to their ERIC-PCR fingerprints, the *E. coli* were clustered into eight groups. In conclusion, a high frequency of ESBL-producing, genetically diverse, and multidrug-resistant *E. coli* was found in fresh cheese. The presence of ESBL-producing *E. coli* in fresh cheese constitutes a public health issue because these bacteria may be pathogenic or contribute to the dissemination of resistance genes to other pathogenic and non-pathogenic bacteria.

Keywords: bacteria; cheese; antibiotic resistance; extended-spectrum beta-lactamases; integrons.

Practical Application: Understanding the antimicrobial resistance characteristics of bacteria present in fresh cheese could contribute to developing strategies to evaluate risks and track sources of contamination.

1 Introduction

In Mexico, the most popular artisanal cheese is fresh cheese, whose consumption represents 80% of the total cheese intake. It is produced from unpasteurized cow milk because the native microbiota of the milk contributes to the organoleptic characteristics of the final product, although it also represents health risks to consumers (de la Rosa-Hernández et al., 2018). In addition, due to its high nutrient and moisture content, cheese is an excellent culture medium for various microorganisms, both beneficial and undesirable, from milk or acquired during the manufacturing process. Therefore, it has often been associated with outbreaks of infections and food poisoning (Reséndiz et al., 2012; Chávez-Martínez et al., 2019).

Escherichia coli is a Gram-negative bacterium commonly found in the intestine of humans and animals, where it colonizes without causing harm, although several human pathogenic variants have been described (Denamur et al., 2021). In cattle, *E. coli* colonize the intestine without symptoms and can cause infection in the udder. Also, it is a regular inhabitant of the cow's settings. Accordingly, *E. coli* can be introduced into the milk from the environment, udder, milking equipment, and during or after milking (Metz et al., 2020). Therefore, unpasteurized milk and its derivatives have a high potential to transmit microorganisms such as *E. coli*, which are frequently resistant to antibiotics. Additionally, antibiotic-resistant extended-spectrum

beta-lactamase (ESBL)-producing *E. coli* detection has increased in cattle, as well as in the food production chain derived from these animals, thus constituting a serious public health issue worldwide (Verraes et al., 2013; Palmeira & Ferreira, 2020).

Antibiotics are essential for combating bacterial infections, although their overuse or misuse has led to the evolution of resistant bacteria. The most widely used antibiotics for treating Gram-negative bacterial infections belong to the beta-lactam category, which includes penicillins, cephalosporins, monobactams, and carbapenemics (Tepeli and Zorba, 2018). Therefore, bacteria have developed or acquired various strategies, such as ESBL production, to resist the harmful effects of these compounds. The main ESBL families are CTX-M, SHV and TEM, of which CTX-M is the most frequent in clinical, community, environmental, food, and livestock origin bacteria (Castanheira et al., 2021). CTX-M-encoding genes are commonly associated with mobile genetic elements, such as transposons, integrons, and conjugative plasmids. In addition, they are frequently associated with genes that confer resistance to other antibiotics, such as aminoglycosides and fluoroquinolones (Cantón et al., 2012).

In Mexico, the dairy industry is a very important economic activity of the livestock sector, fresh cheese being one of the most consumed products in different regions. However, due to the production and distribution chain characteristics, cheese can be contaminated at

any stage during the process (González-Córdova et al., 2016; de la Rosa-Hernández et al., 2018). In addition, no studies have focused on the frequency and characteristics of ESBL-producing bacteria in fresh cheese. Therefore, this study aimed to determine the frequency of ESBL-producing *E. coli* in fresh cheese from western Mexico, as well as analyze their antimicrobial resistance profiles and the resistance gene and integron frequency.

2 Materials and methods

2.1 E. coli enumeration in fresh cheese

Between June 2019 and August 2020, 100 fresh cheese samples were collected from 10 outlets located in two municipal markets in the western area of Michoacán, Mexico. Decimal dilutions were prepared from each sample according to the Mexican regulation (Norma Oficial Mexicana, 1994a). Briefly, 10 g sample was mixed with 90 mL peptone water (1×10^{-1}), homogenized for 10 min using a magnetic stirrer, and serially diluted to 1×10^{-5} suspension. *E. coli* presence was determined according to the national standard (Norma Oficial Mexicana, 1994b), for which 100 μL of the 1×10^{-4} and 1×10^{-5} suspensions were inoculated in red violet bile lactose agar (VRBA) supplemented with 4-methylumbelliferyl-β-D-glucuronide (MUG; Difco, USA) and incubated at 37 °C for 24 h. The plates were observed under ultraviolet (UV) light and fluorescent colonies indicated the presence of *E. coli*.

2.2 ESBL-producing E. coli selection

ESBL-producing bacteria were selected by incubating 100 μL of the 1×10^{-1} dilution, on MacConkey agar (BD Bioxon, Mexico) with 2 μg/mL cefotaxime (MCA-Ctx; Cayman, Canada) at 37 °C for 20 h. After bacterial growth, a single colony was selected for further analysis. ESBL production was confirmed using the double-disk technique (Clinical and Laboratory Standards Institute, 2020). Briefly, sensidiscs with 30 μg cefotaxime and 30/10 μg cefotaxime/clavulanic acid, or 30 μg ceftazidime and 30/10 μg ceftazidime/clavulanic acid were used. A ≥ 5 mm increase in the inhibition halo diameter of the discs with the two antibiotics from that of the disc with individual antibiotic was interpreted as a positive result (Clinical and Laboratory Standards Institute, 2020).

2.3 Molecular identification and phylogenetic grouping of ESBL-producing E. coli

ESBL-producing bacteria were inoculated on Chromocult® coliform agar (CHROMagar, France) and bile lactose red violet agar supplemented with MUG. Violet and fluorescent colonies, respectively, which represent *E. coli* morphology, were selected. The bacterial identity was confirmed by PCR amplifying the *lacZ*, *uidA*, *cyd*, and *lacY* gene fragments with the oligonucleotides and amplification conditions described previously (Horakova et al., 2008). The phylogenetic group of the identified ESBL-producing *E. coli* was assigned as previously described (Clermont et al., 2013).

2.4 Determination of antimicrobial resistance profiles of ESBL-producing bacteria

Antibiotic susceptibility was tested through dilution assays on Mueller–Hinton agar according to previously reported protocols (Clinical and Laboratory Standards Institute, 2020). Briefly, the bacteria were inoculated in 2 mL Mueller–Hinton broth (MHB) and incubated at 37 °C for 20 h. Subsequently, the bacterial cultures were diluted in MHB to match the turbidity of the tube 0.5 McFarland standard (1×10^8 CFU/mL), deposited in a 96-well plate, and inoculated on Mueller–Hinton agar and ampicillin (Ap), azithromycin (Azm), carbenicillin (Cb), cefixime (Cfx), cefotaxime (Ctx), ceftazidime (Caz), ciprofloxacin (Cip), chloramphenicol (Cm), streptomycin (Stp), gentamicin (Gm), kanamycin (Km), meropenem (Mem), tetracycline (Tc), or trimethoprim/sulfamethoxazole (Tmp/Smx) at the desired concentrations using a 96-tip replicator. The antibiotics used were purchased from Sigma-Aldrich (USA) and Cayman (Canada). Colistin (Cl) and polymyxin B (PB) assays were performed on MHB ELISA plates. In addition, resistance analysis was complemented with disk diffusion assays for aztreonam (Atm), imipenem (Ipm), levofloxacin (Lvx), and ticarcillin (Tic). Then, the bacteria were classified as resistant or susceptible according to the established criteria (Clinical and Laboratory Standards Institute, 2020).

2.5 Molecular analysis of the ESBL type produced by E. coli

The type of beta-lactamase produced by the ESBL-producing *E. coli* was also determined by PCR amplification of the bla_{CTX-M}, bla_{SHV}, and bla_{TEM} genes as described previously (Jiménez-Mejía et al., 2017). *E. coli* ATCC 25922 (negative control), *Klebsiella pneumoniae* ATCC 700603 (bla_{SHV+}), *E. coli* MC70 (bla_{CTX-M+}), and *E. coli* MC61 (bla_{TEM+}) were included as controls (Jiménez-Mejía et al., 2017). The distribution of the main bla_{CTX-M} gene clusters, such as bla_{CTX-M1}, bla_{CTX-M2}, bla_{CTX-M8}, bla_{CTX-M9}, and $bla_{CTX-M25}$, was also determined as described previously (Woodford et al., 2006). Even more, the CTX-M alleles were confirmed in 20 randomly selected CTX-M gene-positive *E. coli*. Accordingly, a PCR-amplified 550 bp fragment was sequenced by capillary sequencing at the LABSERGEN genomic services laboratory (Irapuato, Gto, Mexico) and the sequences were compared by BLAST (National Center for Biotechnology Information, 2022).

2.6 Presence of resistance genes and genetic linkage

Streptomycin (*strA* and *strB*) and tetracycline (*tetA* and *tetB*) resistance gene frequency was also analyzed by PCR as described previously (Jiménez-Mejía et al., 2017). In addition, the class 1 and 2 integron frequency was determined by PCR amplification of *intI1* and *intI2* genes (Mazel et al., 2000). The genetic diversity analysis was performed by ERIC-PCR with ERIC1 and ERIC2 oligonucleotides and the conditions used for amplification were described previously (Mohapatra et al., 2007).

3 Results

3.1 E. coli isolation, identification, and phylogenetic grouping

E. coli was detected in 70% of cheese samples (Log_{10} 4-6.6 CFU/g). Meanwhile, lactose-fermenting bacteria isolated from 71% of samples grew in MCA-Ctx, suggesting that they produce ESBL. A representative isolate colony was taken from each sample and ESBL production was confirmed in 68 isolates using double-disk

assays. Among these, 60 showed typical *E. coli* morphology (violet colonies on Chromocult® coliform agar and fluorescence on VRBA supplemented with MUG). The 60 *E. coli* isolates were identified at molecular level by PCR amplification of the *uidA*, *lacZ, lacY* and *cyd* gene fragments. The phylogenetic group assignment showed that 91.7%, 6.7%, and 1.6% of *E. coli* belonged to groups A, C, and B1, respectively.

3.2 Antibiotic resistance pattern of ESBL-producing E. coli

A susceptibility analysis to 20 antibiotics indicated that all isolates were resistant to ampicillin, carbenicillin, cefixime, cefotaxime, tetracycline, ticarcillin, and trimethoprim/sulfamethoxazole. Moreover, 96.7%, 93.3%, 68.3%, and 51.7% of the isolates were resistant to streptomycin, aztreonam, ceftazidime, and gentamicin, respectively. Interestingly, all isolates were susceptible to colistin, polymyxin B, and meropenem. Furthermore, high susceptibility rates were observed for imipenem (98.3%), azithromycin (91.7%), levofloxacin (80%), kanamycin and ciprofloxacin (66.7%), and chloramphenicol (63.3%). Whereas, the analysis of resistance profiles for *E. coli* isolates showed that all isolates were multidrug-resistant as they resisted 8 to 15 antibiotics from different classes (Table 1).

3.3 Analysis of resistance genes and integrons in E. coli

ESBL genes were amplified in all 60 ESBL-producing *E. coli*, and bla_{CTX-M}, bla_{CTX-M}/bla_{TEM}, and $bla_{CTX-M}/bla_{SHV}/bla_{TEM}$ were detected in 44 (73.4%), 14 (23.3%), and 2 (3.3%) isolates, respectively. In addition, all bla_{CTX-M} genes were found to belong to the $bla_{CTX-M-1}$ group, which was also confirmed by sequencing bla_{CTX-M} fragments of 20 randomly selected *E. coli* isolates (Table 2).

All bacterial isolates possessed at least one *tetA* and *tetB* tetracycline resistance gene. For example, *tetA*, *tetB*, and *tetA–tetB* combination were found in 52 (86.7%), 3 (5%), and 5 (8.3%) isolates, respectively. In addition, both *strA* and *strB* genes were detected in 53 isolates (88.3%) and the remaining 7 isolates (11.7%) were negative for the presence of both genes. Class 1 integrons were detected in 91.7% of the isolates, while both class 1 and 2 integrons were detected in 5% of the isolates and the remaining 3.3% of the isolates did not possess class 1 or 2 integrons (Table 2).

The ERIC-PCR analysis of the ESBL-producing *E. coli* revealed diverse band patterns with similarity higher than 69%, and at 80% of band patterns similarity, the isolates were distributed into eight groups (Supplementary material, Figure S1).

4 Discussion

This study reports that 70% of fresh cheese samples were contaminated with *E. coli*. The origin of these bacteria probably can be traced to milk or contamination during the manufacturing process, transport, and product distribution, since these are generally sold in markets with inadequate sanitary conditions (Husan & Çadirci, 2019). Other studies conducted in Mexico has determined the frequency of *E. coli* in fresh cheese to be 40-63%, some of which can be pathogenic and resistant to antibiotics (González-Montiel & Franco-Fernández, 2015; de la Rosa-Hernández et al., 2018).

E. coli colonizes humans and animals, which reflects its genetic plasticity to adapt to different niches; therefore, a classification system of seven phylogenetic groups A, B1, B2, C, D, E, and F has been established (Clermont et al., 2013). In this regard, 91.7%, 6.6%, and 1.6% of *E. coli* isolated from fresh cheese and characterized in this study belonged to phylogenetic group A, C, and B1, respectively. Thus, the source of contamination could be the cattle, the cattle environment or cheese production and distribution chain environment, since *E. coli* isolates from phylogenetic group A are environmental and frequently cause bovine mastitis in Mexico (Jiménez-Mejía et al., 2017). Previous reports have indicated that *E. coli* isolated from cheese produced using unpasteurized milk in Brazil mostly belong to phylogenetic group A (54.4%) (Ribeiro et al., 2016).

The wide use of antibiotics has caused the rapid evolution of bacterial resistant to antibiotics, and ESBL-producing bacteria represent a very important public health issue worldwide (Castanheira et al., 2021). This study reported that 60% of cheese samples were contaminated with ESBL-producing *E. coli*. Previous studies have reported that the frequency of ESBL-producing bacteria isolated from cheese in other countries is variable. For example, 19% (42/222) milk and cheese samples were contaminated with ESBL-producing *E. coli* in Egypt (Ombarak et al., 2018), while 67% of samples were contaminated with ESBL-producing Gram-negative bacteria in Brazil (Silva et al., 2020). In Turkey, 26.6% of cheese samples were contaminated with bacteria belonging to the ESBL-producing *Enterobacteriaceae* family (Husan & Çadirci, 2019). To the best of our knowledge, this is the first study on the frequency of ESBL-producing *E. coli* in fresh cheese in Mexico. Although our sampling area is small, the results described here

Table 1. Antimicrobial resistance of ESBL-producing *E. coli* isolated from fresh cheese.

Antibiotic	Resistance (%)	Susceptibility (%)
Ampicillin	100	0
Aztreonam	93.3	6.7
Azithromycin	8.3	91.7
Ceftazidime	68.3	31.7
Carbenicillin	100	0
Cefixime	100	0
Ciprofloxacin	33.3	66.7
Colistin	0	100
Chloramphenicol	36.7	63.3
Cefotaxime	100	0
Gentamicin	51.7	48.3
Imipenem	1.7	98.3
Kanamycin	33.3	66.7
Levofloxacin	20	80
Meropenem	0	100
Polymyxin B	0	100
Streptomycin	96.7	3.3
Tetracycline	100	0
Ticarcillin	100	0
Trimethoprim-Sulfamethoxazole	100	0

Table 2. Phylogenetic group, resistance pattern and antibiotic resistance genes of *E. coli*.

E. coli isolate	Phylogenetic group	Resistance pattern	bla_{CTX-M}	bla_{TEM}	bla_{SHV}	*intI1*	*intI2*	*strA*	*strB*	*tetA*	*tetB*
1.1	A	ApAtmCazCbCfxCmCtxKmStpTcTicTmp-Smx	+			+		+	+	+	
2.1	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
6.1	A	ApAtmCazCbCfxCtxGmStpTcTicTmp-Smx	+	+		+		+	+	+	
10.1	A	ApAtmCazCbCfxCipCmCtxStpTcTicTmp-Smx	+					+	+	+	
13.2	A	ApAtmCazCbCfxCipCtxStpTcTicTmp-Smx	+	+		+		+	+	+	
21.2	A	ApAtmCbCfxCipCmCtxGmKmStpTcTicTmp-Smx	+	+		+				+	
22.1	A	ApAtmCazCbCfxCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
27	A	ApAtmCbCfxCtxGmStpTcTicTmp-Smx	+	+		+		+	+	+	
29	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+				+	+
30	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+	+		+		+	+	+	+
31	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+	+		+		+	+		+
34	A	ApAtmCazCbCfxCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
35	A	ApAtmCazCbCfxCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
37.1	A	ApAtmCazCbCfxCipCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
39.1	A	ApAtmCazCbCfxCipCmCtxGmKmStpTcTicTmp-Smx	+			+		+	+	+	
40.1	A	ApAtmCazCbCfxCtxGmStpTcTicTmp-Smx	+			+	+			+	
41.1	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
42	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+	+		+		+	+	+	
43	A	ApAtmAzmCazCbCfxCipCmCtxLvxStpTcTicTmp-Smx	+	+	+	+		+	+	+	
45	A	ApCbCfxCipCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
46	A	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+	+	
47	A	ApAtmCazCbCfxCipCmCtxGmKmStpTcTicTmp-Smx	+	+		+				+	
48	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
51	A	ApAtmCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
52	A	ApAtmAzmCbCfxCmCtxTcTicTmp-Smx	+					+	+	+	
53	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
54.1	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
57	C	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+	+	
58	A	ApAtmCbCfxCipCmCtxGmKmStpTcTicTmp-Smx	+			+		+	+	+	
59	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
60.2	C	ApAtmCazCbCfxCtxIpmStpTcTicTmp-Smx	+	+		+				+	
61	A	ApAtmCbCfxCmCtxStpTcTicTmp-Smx	+			+	+	+	+	+	+
63	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	+
65	A	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+	+	
67.1	C	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+	+	
68.1	A	ApAtmCbCfxCipCmCtxGmStpTcTicTmp-Smx	+	+		+		+	+	+	+
69.2	A	ApAtmCbCfxCmCtxGmKmStpTcTicTmp-Smx	+			+		+	+	+	
70.1	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
71.1	A	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+	+	
73.1	A	ApAtmCazCbCfxCmCtxGmKmStpTcTicTmp-Smx	+			+		+	+	+	
75.1	C	ApAzmCbCfxCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
76.1	A	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+		+
77.1	A	ApAtmCazCbCfxCmCtxKmStpTcTicTmp-Smx	+			+		+	+	+	
79.1	A	ApAtmCazCbCfxCtxKmLvxStpTcTicTmp-Smx	+			+					+
81.2	A	ApAtmCazCbCfxCipCtxGmKmLvxStpTcTicTmp-Smx	+			+	+			+	
83.1	A	ApAtmCbCfxCmCtxGmKmStpTcTicTmp-Smx	+	+		+		+	+	+	
84.1	A	ApAtmCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
85.1	A	ApAtmCbCfxCtxStpTcTicTmp-Smx	+	+		+		+	+	+	
86.1	A	ApAtmCbCfxCipCtxGmLvxTcTicTmp-Smx	+			+		+	+	+	
87.1	A	ApAtmCbCfxCtxGmKmStpTcTicTmp-Smx	+			+		+	+	+	
88.2	A	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+	+	+
90.1	A	ApAtmCazCbCfxCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
91.2	A	ApCbCfxCtxStpTcTicTmp-Smx	+	+		+		+	+	+	
92.2	A	ApAtmCbCfxCtxStpTcTicTmp-Smx	+	+		+		+	+	+	
93.1	A	ApAtmCazCbCfxCtxStpTcTicTmp-Smx	+	+	+	+		+	+	+	
94.2	A	ApAtmCazCbCfxCipCmCtxGmKmLvxStpTcTicTmp-Smx	+			+		+	+	+	
95.1	A	ApAtmCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
98.2	B1	ApAzmCbCfxCtxStpTcTicTmp-Smx	+			+		+	+	+	
99.1	A	ApAtmCazCbCfxCtxGmStpTcTicTmp-Smx	+			+		+	+	+	
100.2	A	ApAtmAzmCazCbCfxCtxGmStpTcTicTmp-Smx	+			+		+	+	+	

Abbreviations: Ap, Ampicillin; Atm, Aztreonam; Azm, Azithromycin; Caz, Ceftazidime; Cb, Carbenicillin; Cfx, Cefixime; Cip, Ciprofloxacin; Cm, Chloramphenicol; Ctx, Cefotaxime; Gm, Gentamicin; Ipm, Imipenem; Km, Kanamycin; Lvx, Levofloxacin; PB, Polymyxin B; Stp, Streptomycin; Tc, Tetracycline; Tic, Ticarcillin; Tmp-Smx, Trimethoprim-Sulfamethoxazole.

provide an insight of the issues in this productive sector, and reflect the need to analyze cheeses from other regions to obtain a complete picture of the national situation.

CTX-M, TEM, and SHV frequencies often vary greatly between studies. In this work, the most frequent ESBL type was CTX-M (76.7%), and to a lesser extent their combinations with TEM and SHV. In agreement with this, the most frequent ESBL genes in *Enterobacteriaceae* isolated from dairy products from Egypt was bla_{CTX-M} (48%), followed by bla_{TEM} (44%) and bla_{SHV} (14.8%) (Gaffer et al., 2019); while the ESBL genes in *Enterobacteriaceae* isolated from cheeses in Turkey included bla_{CTX-M} (43.2%), bla_{TEM} (26.3%), and bla_{SHV} (10.8%) (Husan & Çadirci, 2019). In contrast, the main type of ESBL genes in *E. coli* isolated from cheese in Slovakia was bla_{TEM} (33.3%) and bla_{SHV} (8.8%), while bla_{CTX-M} was not detected (Vrabec et al., 2015).

ESBL-producing bacteria frequently have other mechanisms that confer resistance to different antibiotics such as tetracyclines and aminoglycosides (Cantón et al., 2012). ESBL-producing *E. coli* isolated from fresh cheese were multidrug-resistant and resisted antibiotics from three or more different classes. For example, they were highly resistant to beta-lactam antibiotics as well as tetracycline, streptomycin, and trimethoprim-sulfamethoxazole. Recent studies have found an association between multi-antibiotic-resistance and ESBL production in *E. coli* from bovine mastitis samples (Jiménez-Mejía et al., 2017), as well as from in the cattle environment samples (Palmeira & Ferreira, 2020). According to the above, in *E. coli* from cheese, the tetracycline and streptomycin resistance genes were found in 100% and 88.3% of isolates. Furthermore, class 1 integrons were present in 96.7% of *E. coli* and class 2 integrons were present in 5% of isolates. A previous study has described that the frequency of class 1 and 2 integrons in bacteria from cheese samples was 77 and 97%, respectively (Paula et al., 2018), while in another study, class 1 integrons were found in 42.4% *E. coli* isolated from cheese (Ombarak et al., 2018). Therefore, integrons, mainly class 1 integrons, are associated with resistance genes against beta-lactam, aminoglycosides, chloramphenicol, quinolones, sulfonamides, and macrolides (Mazel, 2006).

According to the fingerprints patterns, the 60 isolates were classified into eight groups based on 80% similarity. This shows that there is no predominance of a particular *E. coli* genotype in the study area. In addition, the bacterial distribution in the groups and the origin of samples or resistance profile were not correlated, indicating heterogeneity among bacteria. In this regard, studies of genetic diversity of *E. coli* isolated from milk and cheese show that these bacteria are genetically diverse (Campos et al., 2009).

In conclusion, the data presented in this study show a high prevalence of multidrug-resistant ESBL-producing *E. coli* in the fresh cheese produced and consumed in the study area, which constitute a serious public health issue because these bacteria can cause infections or transfer antibiotic resistance genes to pathogenic variants.

Conflict of interest

All authors have no potential conflicts of interest.

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Supplementary material

Supplementary material accompanies this paper.

Figure S1. The ERIC-PCR study of the ESBL-producing bacteria yielded several 250-4,000 bp bands and their comparison revealed that the similarity was higher than 66% in the 60 E. coli isolates. Meanwhile, the bacteria were distributed into eight groups (I–VIII) with 80% similarity, of which groups V and VI include 22 and 18 isolates, respectively, while the remaining groups contain 10 or less isolates each.

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