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Screening of Food-borne *Staphylococcus aureus* and *E. coli* Pathogens in Artisanal White Soft Cheese in Delta Region, Egypt

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Abstract

Staphylococcus aureus is considered as one of the leading causes of food-intoxication and on the other hand *E. coli* and particularly, Shiga toxin (ST) producing *Escherichia coli* (STEC) have emerged as important food-borne enteropathogens frequently associating serious to fatal disorders in humans and both species have shown to be resistant to a wide spectrum of antibiotics. The current study was performed to determine the prevalence of two common food-borne pathogens: *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) in white soft cheese being one of the most popular dairy products in Egypt. A total 150 samples of soft cheese were purchased from the market and were microbiologically tested. The *S. aureus* isolates were identified according to recommended biochemical tests, and polymerase chain reaction (PCR) targeting the nuc gene, as a species-specific marker gene for *S. aureus*. Meanwhile, *E. coli* isolates were identified based on biochemical tests, serological tests, haemolytic activity, and multiplex PCR. This has been done to assess capability of producing STs based on targeting *stx1* and *stx2* genes. Result revealed that *S. aureus* and *E. coli* were observed in 66.66% and 36% of samples, respectively. The antimicrobial resistance of isolates was phenotypically investigated against eleven different antibiotics and results showed the presence of a variable multidrug-resistance among isolates against selected antibiotics. These findings highlighted the importance of periodical screening of food-borne pathogens in artisanal products due to lack of strict hygienic measures in markets; the reason that facilitate the contamination of such artisanal products by several food-borne pathogens.

KEYWORDS
Food-borne *Staphylococcus aureus*, *E. coli* , white soft cheese, Shiga toxin

INTRODUCTION

Unripened soft cheese: a type of rennet coagulated cheese frequently produced by farmers and artisanal dairy producers, is being one of the most popular dairy products consumed in Egypt. However, the lack of strict hygienic measures in villages and artisanal regions and due to the intrinsic properties of cheese such as high moisture content, low salt content, and near-neutral pH that make it susceptible to rapid Food-borne spoilage (Alnakip *et al*., 2014; Flynn *et al*., 2021). Worldwide, foodborne diseases are among the foremost economic and public health concerns particularly in susceptible people, such as infants, pregnant women, children and elderly. In this sense, *S. aureus* and *E. coli* are the most incriminated pathogens causing foodborne outbreaks associated with the consumption of milk and dairy products (Marta Sánchez-Rubio *et al*., 2018; Alnakip *et al*., 2019).

Staphylococcus aureus is considered as one of the leading causes of food-intoxication due to production of a wide range of heat-stable enterotoxins, as well as toxic shock syndrome toxin type-1 (TSST-1) (Aydin *et al*., 2011; Crago *et al*., 2012; Bayoumi *et al*., 2018; Alnakip *et al*., 2019). According to the European Food Safety Authority, staphylococcal enterotoxins (SE) in foods products are among the top 10 microbial toxins causing the highest number of hospitalizations in strong-evidence outbreaks (Schwendimann *et al*., 2021). Furthermore, according to the WHO report, the disease caused by *S. aureus* enterotoxins (SAEs) is the second most common cause of foodborne disease.

The presence of coliforms in cheese and their relation to entropathogenic *E. coli* in soft cheeses has received considerable attention in previous studies (Abou-Donia 1986; Schrade and Yager, 2001; El-Baradei *et al*., 2007). *E. coli* is responsible for several outbreaks of diarrhea in children and adults after ingestion of contaminated milk and dairy products (Marta Sánchez-Rubio *et al*., 2018; Hamdy *et al*., 2023). Different studies showed that 1-5% of food-borne infections were related to consumption of milk and dairy products, that 53% of cases of food-borne infections caused by contaminated cheese (Schrade and Yager, 2001).

Shiga toxin (ST) producing *Escherichia coli* (STEC) have emerged as important food-borne enteropathogens frequently associating serious to fatal disorders in humans and shown to be resistant to a wide spectrum of antibiotics.

There are at least 200 serotypes of *E. coli* that are capable of producing Shiga toxins (STs) and are known as STs producing *E. coli* (STEC) (Nataro and Kaper, 1998; Pradel *et al*., 2000) but only a few are related to severe human diseases and most infections are caused by O157:H7 serotype (Boyce *et al*., 1995; Law, 2000). Other major STEC serogroups associated with pathogenicity include O111, O26, O6, OX3, O91 and O103; which have

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been identified in bovine feces and in food samples (Paton and Paton, 1998; Pradel *et al*., 2000). STEC, particularly O157:H7 serotype was emerged as important food-borne pathogens associated with various human diseases, including watery diarrhea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in humans (Paton and Paton, 1998; Fitzpatrick, 1999; Law, 2000).

The rapid and accurate identification of food borne bacteria is a fundamental issue, since several species are responsible for serious problems in human and veterinary field The traditional phenotypic tools involved in bacterial diagnosis have a lot of drawbacks attributed to time-consuming nature, number of required materials and poor diagnostic accuracy compared to molecular approaches. So, polymerase chain reaction (PCR) targeting the nuc gene; as a species-specific marker gene for *S. aureus*. and multiplex PCR which assess the capability of producing STs based on targeting *stx1* and *stx2* genes for detection of *E. coli* have been done.

Antimicrobial resistance (AMR) has developed as one of the major urgent threats to public health causing serious issues to successful prevention and treatment of persistent diseases. Misusing and overusing different antibacterial agents in the health care setting as well as in the agricultural industry are considered the major reasons behind the emergence of antimicrobial resistance (Dadgostar, 2019). The threat of antimicrobial resistance is of particular importance in the category of antibiotic resistance in bacteria (Prestinaci *et al*., 2015). One of the most well-known cases of AMR, Methicillin resistance in *S. aureus* (MRSA), has been associated with high mortality rates every year across the globe (Founou, 2017). Our study aimed to determine the prevalence of *S. aureus* and *E. coli* in white soft cheese being one of the most popular dairy products in Egypt, in addition to the characterization of the isolated strains in terms of antimicrobial susceptibility and virulence traits.

MATERIALS AND METHODS

Sample Collection and Bacteriological Analysis

A total of 150 soft white cheese samples were aseptically collected in sterile containers from different artisanal markets in Delta region, Egypt during January and February 2023, and transported rapidly in a 4°C vehicle-mounted refrigerator to the Food Hygiene, Safety and Technology Laboratory, Faculty of Veterinary medicine, Zagazig university, Egypt to be investigated microbiologically within few hours.

Prior to bacteriological investigation, samples preparation and serial dilutions were made according to recommended regulations described by IDF (1992) and Wehr and Frank (2004). Each sample was aseptically added to a Whirl-Pak filter bag (Nasco, Fort Atkinson, WI, USA), and a 1:10 (w/v) dilution using PBS was performed. The samples were digested at normal speed for 60 s using a Seward Stomacher 400 Lab Blender Series (VWR International, Solon, OH, USA). The digested samples were diluted in 9 mL PBS blanks to the appropriate dilution and vortexed. The color and pH value of each cheese samples were recorded (Creamy and white, pH: 6.8-7.5).

The pH of each sample was measured by using pH meter (Crison pH meter 507) before and after sonication treatments.

Isolation and identification of S. aureus

One ml from each sample was inoculated in a tube of sterile buffered peptone water (BPW) and incubated aerobically at 37°C for 24 h. Later, one ml from incubated BPW was transferred to 10 ml BHI broth and incubated at 37°C for 24 h. Approximately 0.1 mL from the incubated broth was streaked on Baired Parker agar and incubated at 37°C for 48 h. Colonies with typical morphological characteristics (Black surrounded with halo zones) were picked up from each plate and further purified by re-streaking two successive times on fresh Baired Parker agar medium plates. All suspected isolates were maintained as frozen cultures in Tryptone Soya broth (Oxoid, UK) and 50% glycerol at -80°C.

Primary Identification of S. aureus

Primary characterization of *S. aureus* was dependent on colony characteristics, Gram staining and biochemical characteristics. According to Arora (2003), Biochemical identification included oxidase, Catalase, mannitol fermentation, Tube coagulase test and Deoxyribonuclease (D-Nase activity) tests. For performing coagulase test (CT), each suspected colony was inoculated into peptone water (Merck, Germany) at 37°C for 24 hours. Later, 0.5 mL of each tube was transferred aseptically to sterile tube contains 0.5 mL of reconstituted rabbit plasma (STAPH-ASE) (Biomérieux, France) followed by vortexing. The tubes were then incubated at 37°C for 1-3 hours to assess the coagulation activity. Additional incubation for another 24 hours was done for negative reactions. The assessment of coagulation reaction was observed by gently inclining each tube to avoid breaking of any developed clot. A negative control tube was done in parallel. The typical coagulase-positive isolates of *S. aureus* colonies were taken for further identification analysis by PCR to detect the nuc gene as a species-specific marker gene for *S. aureus*.

Confirmatory Molecular typing of S. aureus isolates based on targeting nuc gene

The *S. aureus* isolates were confirmed by PCR using species-specific thermonuclease (nuc) gene (270 bp) (Louie *et al*., 2002). Each isolate was allowed to grow in BHI broth (Merck, Germany) followed by incubation at 37ºC for 24 h. as previously described by Quintela-Baluja *et al*. (2013) and Alnakip *et al*. (2020). The bacterial pellets were obtained by centrifugation of 1 mL of overnight cultures at 5000 x g for 12 min. The bacterial cells were lysed by adding 180 µL of lysis solution (Sigma-Aldrich) and incubated for 2 hrs at 37ºC. The DNeasy Tissue Mini Kit (Qiagen, Valencia, CA) were used for extraction and purification of total DNA. PCR-amplification of a fragment of the nuc gene was done using the primer pair: nuc-F: GCGATTGATGGTGATACGGTT and nuc-R: AGCCAAGCCTTGACGAACTAAAGC according to prescribed guidelines by Louie *et al*. (2002). All PCR assays were carried out using the Thermal Cycler, as following: initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 1 min, 55°C for 30s, and 72°C for 1 min. This was followed by final extension at 72°C for 7 min. The results of PCR were visualized after agarose gel electrophoresis under UV trans-illuminator.

Isolation and identification of E. coli

One ml from each sample was inoculated in a tube of sterile buffered peptone water (BPW) and incubated aerobically at 37°C for 24 h. Later, one ml from incubated BPW was transferred to 10 ml MacConkey broth and incubated at 37°C for 24 h. Approximately 0.1 mL from the incubated broth was streaked on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. Colonies with typical morphological characteristics (green-metallic sheen colonies) were picked up from each plate and fur-

ther purified by re-streaking two successive times on fresh EMB plates. Primary characterization of *E. coli* was dependent on Gram staining and biochemical characteristics. All suspected *E. coli* isolates were maintained as frozen cultures in Tryptone Soya broth (Oxoid, UK) and 50% glycerol at -80°C.

Primary Identification of E. coli based on Biochemical characteristics

Suspected isolates were confirmed by biochemical tests as Indole production test, Voges-Proskauer test, Methyl Red Test, Citrate utilization test and Production of gas from lactose test. *E. coli* isolates were positive for Indole, Methyl red tests and negative for Vogus proskeur and citrate utilization tests.

Serological typing of E. coli isolates

Serotyping of *E. coli* was performed by slide agglutination test using polyvalent and monovalent antisera according to the instructions of the manufacturer (Bio-Rad Laboratories, Marnes-la-Coquette, France).

Detection of hemolytic activity

Production of Ehly was assayed acc. to method by Beutin *et al.* (1989) and Beutin *et al.* (1993). Different *E. coli* isolates were incubated in MacConkey broth. A loopful from each broth culture was streaked onto blood agar medium supplemented with 5% defibrinated sheep blood. The plates were incubated at 37°C for 24 hours. ß-Hemolysis was defined as a zone of complete erythrocyte lysis surrounding a bacterial colony.

Detection of stx genes using PCR

Total genomic DNA was extracted from overnight cultures of *E. coli* isolates as previously described by Quintela-Baluja *et al.* (2013) and Alnakip *et al.* (2020). The bacterial cells were lysed by the addition of 180 µL of lysis solution (Sigma-Aldrich) after incubation for 2 h at 37ºC. Total genomic DNA was extracted and purified using the DNeasy Tissue Mini Kit (Qiagen) and kept stored in a -20°C freezer. The stx genes were amplified by PCR using primer pairs illustrated in Table 1, as previously described by Paton and Paton (1998). All the PCR assays were performed using a "My Cycler" Thermal Cycler (Bio-Rad Laboratories, USA). PCR assay consisted of 35 cycles, each consisting of 1 min of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15; and 1.5 min of elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35. The results of PCR were visualized after agarose gel electrophoresis under UV trans-illuminator.

Antibiotic susceptibility testing of isolates

All nuc-positive "*S. aureus*" and *E. coli* isolates were tested for susceptibility to eleven antimicrobials using the disc diffusion method on Mueller-Hinton agar (Oxoid) and according to standard instructions (Clinical and Laboratory Standards Institute "CLSI", 2008). Zones of growth inhibition were measured, and the interpretation of results was accomplished following CLSI guidelines, whereby intermediate results were considered resistant (Tables 2 and 3).

RESULTS AND DISCUSSION

There is an increasing interest for the consumers towards artisanal dairy products due their characteristic aromatic properties coupled with beneficial effects on the human health, soft white cheese is among typical artisanal products in Egypt. Despite the interest of consumers attributed to aromatic and beneficial characteristics, dairy foods can serve as vehicles for various human food-borne pathogens.

It is well documented that the foodborne outbreaks caused by the consumption of various types of cheeses were contaminated with *S. aureus* and *E. coli* (Arau´jo *et al*., 2002; Haeghebaert *et al*., 2003; Gaulin *et al*., 2012).

Isolation of relevant bacterial pathogens and indicator or-

Table 1. PCR primers used for targeting stx genes.

Primer	Sequence (5′-3′)	Specificity	Amplicon size (bp)
*stx1*F *stx1*R	ATAAATCGCCATTCGTTGACTAC AGAACGCCCACTGAGATCATC	nt 454–633 of A subunit coding region of *stx1*	180
*stx2*F *stx2*R	GGCACTGTCTGAAACTGCTCC TCGCCAGTTATCTGACATTCTG	nt 603–857 of A subunit coding region of *stx2* (including *stx2* variants)	255

Table 2. Antibiotic susceptibility profiles of *S. aureus* isolates.

Antibiotic	Sensitive		Resistant	
	NO. of isolates	%	NO. of isolates	%
Ampicillin	24	53.34	21	46.66
Amoxicillin	26	57.77	19	42.23
Tetracycline	25	55.55	20	44.45
Gentamycin	18	40	27	60
Oxacillin	16	35.55	29	64.45
Erythromycin	27	60	18	40
Ciprofloxacin	22	48.88	23	51.12
Clindamycin	28	62.22	17	37.78
Kanamycin	25	55.55	20	44.45
Lincomycin	30	66.66	15	33.34
Vancomycin	15	33.34	30	66.66
Total number of isolates		45		

ganisms is used to evaluate microbiological safety and quality of food. The use of tests such as *S. aureus* and *E. coli* counts as an indicator of postprocess contamination by the dairy industry permits sanitary control (Flowers *et al.* 1992).

S. aureus is an important food-borne pathogen and considered to be one of the leading causes of food-borne illnesses worldwide. Additionally, *S. aureus* is the most important and predominant mastitis pathogen; being existed in several peracute, acute, subacute, and chronic forms of intramammary infections (Alnakip *et al*., 2014). The presence of *S. aureus* in cheese may cause staphylococcal food poisoning which is a major type of food intoxication (Willey *et al*., 2008).

The results showed that the *S. aureus* count was ranged from 3.00×10 to 2.30×10^3cfu/gm, with a mean value of $3.20\times10^4\pm0.69\times10^4$cfu/gm and these results do not exceed the hazardous level of 10^6 (according to ICMSF specifications). Tatini (1973) reported that the presence of *S. aureus* in a count lesser than 10^6/g food doesn't support production of enough dose of enterotoxins to cause human illness. However, the presence of *S. aureus* in foods can`t be neglected even in lower levels because it could increase as in the cases of poor storage and repeated improper heating and cooling of foods (Peles *et al*., 2007).

The examined soft cheese samples were produced under unmechanized conditions so many types of pathogens may enter the cheese during manufacture and handling (Turantas *et al*., 1989). The results of biochemical tests showed that the prevalence of *S. aureus* was 55.26% in the examined samples. While coagulase-positive *S. aureus* strains can produce an enterotoxin, coagulase-negative isolates could able to produce enterotoxin (Nunes *et al*., 2015; Yildirim *et al*., 2019). Several studies indicated the prevalence and presence of *S. aureus* strains in various cheese samples. The detection percentage were ranging from 20.2% to 92% (Yücel and Anıl, 2011; Gökmen *et al*., 2013; Bingöl and Toğay, 2017).

Detection of *S. aureus* is usually done by culture techniques that include the use of selective media, biochemical confirmation, and other parameters for bacterial identification. At recent time, molecular techniques offer the possibility of a rapid and accurate identification of *S. aureus* isolates. polymerase chain reaction (PCR) was applied to detect the *nuc* gene as a species-specific marker gene for *S. aureus*. In our study *S. aureus* isolates (*nuc* positive) was 42.85%.

The use of antibiotics in agriculture farming is mostly for therapeutic purposes, and as a preventive measure during dry cow therapy, and being used in lesser extend as growth promoters (Butaye *et al*., 2003).The overuse and misuse of antibiotics in the last two decades increased the abundance of AR bacteria, which elevated the risk of emergence of resistant zoonotic bacterial pathogens (Mevius *et al*., 2005; Haran *et al*., 2012).

Antimicrobial resistance among staphylococci of animal origin is based on a wide variety of resistance genes. These genes mediate resistance to many classes of antimicrobial agents approved for use in animals, such as penicillins, cephalosporins, tetracyclines, macrolides, lincosamides, phenicols, aminoglycosides, aminocyclitols, pleuromutilins, and diaminopyrimidines. In addition, numerous mutations have been identified that confer resistance to specific antimicrobial agents, such as ansamycins and fluoroquinolones (Stefan *et al*., 2018).

The phenotypic AR of *S. aureus* strains were checked against eleven selected antibiotics (Table 2). None of the strains was resistant to all antibiotics, but all of them were resistant to at least two antibiotics.

The AR percentage was recorded for tested antibiotics as follows: ampicillin (46.66%), amoxycillin (42.23%), tetracycline (44.45%), gentamycin (60%), oxacillin (64.45%), erythromycin (40%), ciprofloxacin (51.12%) and clindamycin, (37.78%), while no

Table 3. Antibiotic susceptibility profiles of *E. coli* isolates

Antibiotic	Sensitive		Resistant	
	No. of isolates	%	No. of isolates	%
Ampicillin	12	40	18	60
Amoxicillin	15	50	15	50
Tetracycline	17	56.66	13	43.34
Gentamycin	9	30	21	70
Oxacillin	5	16.66	25	83.34
Erythromycin	12	40	18	60
Ciprofloxacin	8	26.66	22	73.34
Clindamycin	14	46.66	16	53.34
Kanamycin	9	30	21	70
Lincomycin	16	53.34	14	46.66
Vancomycin	4	13.34	26	86.66
Total number of isolates	30			

Table 4. Serotyping of *E. coli* isolated from the examined samples and their hemolytic activity.

Serotype	No.	%	Hemolytic activity	%
O111:H4	5	16.67	1	3.33
O91:H21	7	23.33	0	0
O26:H11	4	13.33	1	3.33
O127:H6	6	20	0	0
O157:H7	5	16.67	2	6.66
Untyped	3	10	0	0
Total	30	100	4	13.32

resistance by all strains was found towards kanamycin, lincomycin and vancomycin.

High level of *E. coli* in cheese indicated unsanitary practices in the cheese making process and may sometimes give rise to early blowing or gassing of the product. It is characterized by large gas holes, a spongy texture of the cheese and generally occurs 1-2 days after manufacture (Bintsis, 2006). *E. coli* is strongly incriminated as a potential food poisoning agent and are associated with infantile diarrhea and gastroenteritis in adults (El-Diasty and El Kaseh, 2009). Our results revealed that *E. coli* counts were ranged from 8.00×10^3 cfu/g to 1.30×10^4 cfu/g with a mean value of $1.06\times10^4\pm0.50\times10^3$ cfu/g which indicate poor sanitary conditions during cheese processing. Similarly, high levels of *E. coli* were previously recorded in soft cheese (Arau´jo *et al*., 2002; Jaber, 2011; Pešić Mikulec *et al*., 2012).

Our results are not in accordance with the Egyptian Standard Guidelines in which should be no *E. coli* or *S. aureus* in cheese (EOSQ, 2005).

The results in this study, showed that 30 out of 120 examined samples were contaminated with *E. coli* (25.00 % out of all samples). Compared to our results pickled Domiati cheeses were devoid of such bacterium. Also, in a study by (Mansouri-Najand and Khalili, 2007), *E. coli* O157 and non-O157 STEC have been isolated from raw milk cheese. Additionally, STEC strains were isolated during various stages of cheese manufacture starting from curd-making process until final ready-to-consumption stage (Pradel *et al*., 2000; Rey *et al*., 2006). The absence of *E. coli* in Domiati cheese particularly could be attributed to high salt content (5-14%) incorporated during preparation of pickled Domiati cheese and the long storage period of cheese within brine solution. Taken together with the poor survivability of *E. coli* isolates for even 96 h in milk supplemented with 10-12% NaCl (El-Sharoud *et al*., 2015), this could explain the absence of *E. coli* in pickled Domiati cheese samples.

The phenotypic AR of *E. coli* strains were checked against eleven selected antibiotics (Table 3). The AR percentage was recorded for tested antibiotics as follows: ampicillin (60%), amoxycillin (50%), tetracycline (43.34%), gentamycin (70%), oxacillin (83.34%), erythromycin (60%), ciprofloxacin (73.34%), clindamycin, (53.34%), kanamycin (70%), lincomycin (46.66%) and vancomycin (86.66%).

E. coli represents a major tank of resistance genes that may be responsible for treatment failures in both human and veterinary medicine. An increasing number of resistance genes have been identified in *E. coli* isolates during the last decades, and many of these resistance genes were acquired by horizontal gene transfer. In general, antimicrobial resistance in *E. coli* is considered one of the major challenges in both humans and animals at a worldwide scale and needs to be considered as a real public health concern. *E. coli* of animal origin often also show resistances to other mostly older antimicrobial agents, including tetracyclines, phenicols, sulfonamides, trimethoprim, and fosfomycin (Laurent *et al*., 2018).

Serotype O26:H11, previously considered a classic enteropathogenic *E. coli* serotype, is now shown to be EHEC. EHEC cause hemorrhagic colitis and hemolytic uremic syndrome (Levine *et al*., 1987).

EPEC is one of the leading causes of diarrhoeal morbidity and mortality among children in developing countries (Clarke *et al*., 2002).

As shown in Table 4, 27 out of 30 *E. coli* strains recovered from examined samples, were successfully serotyped with correspondence to 5 different serogroups, mean while 3 strains were untypeable by available antisera. The predominant serotype was O91 (23.33%) followed in order by O127 (20.00%), O111 and O157 (16.67% of each) and O26 (13.33%).In a study by Rey *et al*. (2006) eight different serotypes such as O27:H18, O45:H38, O76:H19, O91:H28, O157:H7, ONT:H7, ONT:H9 and ONT:H21, have been identified in fresh cheese curds and cheeses; among the serotype O27:H18; which has not been reported previously as STEC. Also, in a study by (Pradel *et al*., 2000) other *E. coli* serotypes as OX3:H2 and O91:H21; that are known to associate HUS, have been isolated from cheeses.

Haemolytic activity is considered as an important virulence factor for some strains of *E. coli* to overcome host defense mechanism through enterohaemolysin production which is related to release of iron into the bacterial environment and cytotoxic effect towards attacking neutrophils (Cavalieri and Snyder, 1982; Schmidt *et al*., 1995). As illustrated in Table 4, only 4 out of all 30 *E. coli* isolates (13.33%) carried this feature. The four isolates corresponded to O111 (1 strain), O26 (1 strain) and O157 (2 strains).

STEC organisms of different serotypes have been increasingly isolated from humans with disease and from healthy domestic animals (Fukushima and Gomyoda, 1999). Many of these isolates were typical STEC belonging to serotypes O26, O111, and O157, yet most belonged to serotype O157:H7, which can cause severe disease in humans, such as hemorrhagic colitis and hemolytic-uremic syndrome (Karmali, 1989). To assess the STs production by our *E. coli* isolates, PCR was carried out for targeting *stx1* and *stx2* genes. The genotypic screening of isolates revealed that that the 30 strains of all *E. coli* isolates were carrying either only *stx1* (O157, O111), only *stx2* (O127) or both stx genes (O91, O26).

CONCLUSION

Results show that white soft cheese is contaminated by *S. aureus* and *E. coli* which indicates the important role of white soft cheese as vehicle for transmission of potentially pathogenic bacteria. This indicates improper hygienic measures. Therefore, establishment of GMP "good manufacturing practice" and HACCP system in dairy plants and strict hygienic measures should be applied.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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