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Detection of Toxigenic *Staphylococcus aureus* and *Escherichia coli* Serotypes in Egyptian Cheeses by Multiplex PCR

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Abstract

FROM WHITE soft cheese, 150 samples were gathered from various regions in Kafrelsheikh, Egypt. Samples represented as Kariesh, Damietta and feta cheese (50 each). Microbiological analysis to detect *Escherichia coli and staphylococcus aureus.* The isolated *S. aureus* samples were tested biochemically then examined for the existence of genes of staphylococcal enterotoxins (SE) by multiplex PCR. Biochemical identification of *E. coli* and serological detection of enteropathogenic types were done. Molecular diagnosis was performed to identify Shiga toxin (Stx1), Shiga toxin (Stx2) and intimin (*eaeA*) genes of enteropathogenic E. coli. Results declared that *S. aureus* was found in 62% of Kariesh, 38% of Damietta and 28% of feta cheese samples. Results of PCR displayed that 33.3 % of the isolated *S. aureus* had SEA gene, 3.33% had SEB gene, 3.33% had SEC gene, 6.67% had SED gene, 3.33% had SEC & SED genes and 3.33% possessed SEA, SEB and SEC genes. *E. coli* was found in 26% of Kariesh samples while Damietta and feta cheese samples revealed same occurrence (4%). Ten various serotypes of *E. coli* were characterized as following O26:H11(2.7%), O128:H2(2%), O111:H2 (1.3%), O124 serotype (1.3%), O91:H21(0.6%), O119:H6 (0.6%), O125:H21 (0.6%), O44:H18 (0.6%), O121:H7(0.6%) and O86 (0.6%). Regarding PCR results for *E. coli*, 23.5% of the detected serotypes carried stx1, stx2 and eaeA genes, 14.3% carried *stx2* and *eaeA* genes, 13.1% carried *stx1* plus *stx2* genes, 23.5% had *stx1* gene only and 17.6% possessed *stx2* gene only. These results concluded that white soft cheese may represent a transmission vehicle for critical pathogens.

Keywords: *S. aureus*, *E. coli*, food poisoning, Egyptian cheese, shiga toxin.

Introduction

Milk and its products form healthy and nutritious diet. However, when they are consumed raw, they act as health hazard because of possible bacterial contamination [1]. Raw unpasteurized milk is the mainly used milk in manufacturing of fresh soft cheese. In Egypt, Kariesh and Damietta cheeses are the most locally manufactured fresh soft cheeses. Kariesh cheese is famous for its elevated protein levels and affordable cost, while Damietta cheese offers a distinctively sharp and appealing flavor along with a smooth consistency [2]. Also, Feta cheese is one of fresh soft cheese which is very famous in Greece and has achieved universal commercial success [3].

Kariesh and Damietta cheese production can be done at small scale or large factories. Kariesh cheese is typically produced using a traditional technique that depends on microorganisms naturally present in the milk which make clotting of the skimmed milk. This process takes place in specific pottery pots called shalia which placed in an appropriate location to facilitate rise of fat to the surface producing cream layer. This process takes around 24-36 hours during the summer and 2-3 days in winter. Then souring and clotting of the lower layer of partially skimmed milk. After extraction of the cream layer, the curd is placed on a mat. Tying and hinging of the mat is made to allow drain of the whey. This procedure takes 2-3 days or until reaching the proper consistency. Then slicing, salting of the product and left on the mat until draining of all the whey. At this point, it becomes ready for consumption as fresh cheese [4]. Or produced by rennet addition to the milk. These preparationmodes are still unsanitary; the produced cheese is usually contaminated [5,6]. Manufacture of Damietta cheese should include the full

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pasteurization operation but, cheese production from raw milk has a long-standing tradition. The focus on minimizing heat treatment of the milk during cheese manufacturing is thought to enhance the ripening process, resulting in better ripened flavour within a shorter time and with a greater consistency [7]. Due to the use of raw milk, prophylactic measures are found to eliminate undesirable bacteria from manufactured Damietta cheese.The usual practice is to maintain the product for 60 days or more for pathogen inactivation during the ripening process. However, this approach doesn't allow decrease in all pathogens, particularly those present in high quantities, so disease outbreaks have been linked to cheese made from milk without proper pasteurization. Low salt content and pH facilitate pathogen inactivation during the 60 days of ripening [8]. With the microbial load found in raw milk, other organisms may enter Damietta cheese during processing, handling and storage processes. This contamination may cause objectionable changes leading to unmarketable product or even unfit for consumption.

Physical and chemical composition can affect the microbial contamination of cheese. Several research studies have demonstrated that protein in Kariesh cheese is the highest (19.99±1.32 g/100g) compared to other types of Egyptian cheeses, While Damietta has (7.8±0.8g/100g) and feta has (11.1±0.8g/100g). Kariesh cheese exhibits the highest moisture (68.97±1.86g/100g), along with the lowest ash[9]. The pH level of Kariesh cheese was 4.21 to 4.65 when fresh, and decreased from 3.98 to 4.35 after being stored for 15 days [10]. While Damietta recorded 62.9% ± 0.09 moisture; 6.6% ± 0.30 salt and 4.1 ± 0.05 pH [11]. Physico-chemical features of Feta cheese vary according to salt concentration affecting moisture, salt uptake and pH.A greater amount of salt in the brine make hard cheese with higher salt and higher pH [12]

Cheese can get contamination with microbes from diverse origins. The primary sources of bacterial contamination in cheese are raw milk and the methods used during cheese processing. The major method of cheese contamination during manufacture process are low acidity and high water activity of soft cheese besides imperfect decontamination [13]. European Food Safety Authority recorded that contaminated cheese consumption was responsible for about 0.4% of all European foodborne outbreaks[14]. Detection of *E.coli* or *S. aureus* in dairy produce indicates poor hygienic practices, including insufficient heat treatment, the use of low-quality components, and bad sanitation during cheese handling and storage [15]. Toxins of *S. aureus* are responsible for foodborne illness that induce serious symptoms such as excessive salivation, vomiting, abdominal pain, nausea and diarrhea. In 1884, the first case of this illness was recorded in USA, and was caused by consuming contaminated cheese [16]**.** Staphylococcal enterotoxins (SEs) are exposed by several genes; types (A-E) and these were the most widespread genes involved in statuses of food intoxication worldwide [17]. SEA is the most frequently occurring toxin in such cases followed by SED and SEB. SEA was responsible for 77.8% of *S. aureus* food intoxication flare-up in USA [18].

Traditional cheese in developing nations is often identified as a significant carrier of various types of harmful bacteria, leading to foodborne illnesses caused mainly by *E. coli* ingestion through dairy products [19]. *E. coli* presence indicates fecal contamination and bad hygiene [20]. Consumption of unpasteurized milk or its products could potentially facilitate the transmission of pathogenic *E. coli* to humans, leading to serious infections. This is due to the presence of virulent properties in these pathogens as well as their associated clinical symptoms, which have been documented globally [21]. *E. coli* strains which can produce Shiga toxins are highly pathogenic [22]. El-Baz [23] detected *E. coli* serotypes (O91: H21, O55: H7 and O26: H11) from soft cheese. Another study confirmed that enteropathogenic serogroups (O125,O114 andO18) were commonly found in Egyptian cheeses [24]. *S. aureus* was found in both Damietta cheese and Kareish cheese with a higher frequency in Kareish cheese samples which could pose a potential hazard on public health [2,25]. This paper described the process of isolating and identifying *S. aureus* and Enteropathogenic types of *E. coli* in samples of soft cheese in Kafrelsheikh governorate, to establish whether these cheese samples could potentially harbour these pathogens or not.

Experiments

Samples collection

One-hundred and fifty cheese samples were obtained from farmer market, local vendors and small scale markets from 3 cities of Kaferelsheikh governorate (Kafrelsheikh, Riyadh and Baltim) during the interval between March and August 2016. The collected cheese samples were Damietta, Feta and Kariesh (50 samples each) and the volume of each sample was about 250 g. All samples are collected under aseptic conditions in a clean, dry, and septic glass containers, preserved in ice box and sent at once to the lab.

Microbiological identification of S. aureus

Ten grams of every specimen were inoculated in nutrient broth [26] (oxoid) with addition of 6.5% NaCl and left at37° for 24hr. Baird-Parker agar was inoculated with 0.1 ml broth and then put in an incubator two days at 37°C. The grown bacteria were introduced into the slant of nutrient agar for identification. The identification process involved conducting various tests including gram staining,

catalase activity, coagulase production, Growth at 10% NaCl, oxidase test, esculin hydrolysis, arginine decarboxylase (ADH), hemolysis, mannitol fermentation and D-Nase activity.

Molecular identification of S. aureus

DNA Extraction of *S. aureus*

After incubating the culture overnight on nutrient agar, a few colonies were placed in 200 µl of sterilized filtered water. The mixture was boiled 20 minutes at100ºC followed by 20-30 min of proteinase K digestion at 56 ºC. QIA amp DNA Mini kits were used for DNA extraction based on the manufacturer's manual [27].

Identification of Staphylococcal Enterotoxins (SEs) genes by multiplex PCR according to [28]:

By PCR mechanism, identifying the genes coding for *S. aureus* enterotoxins (types A, B, C and D) was achieved with specific primers shown in table (1) (Pharmacia Biotech). For PCR, DNA specimen (10 µl) was added to nuclease free water (990 µl). The reaction mixture (25µl) had 2.5 µl 10 x buffer, 13.25 sterile dH_2O, 0.63µl 10mMNTPs, 1.25 µl of 20 pmol/ml for both primer F and primer R, 1µl 25Mm $MgCl_2$ and completed to 25 µl PCR water. The reaction was preceded on a thermocycler (Hamburg, Germany). The conditions of amplification began with 5 min at 95°C followed by30 cycles of (2 min at 94°C, 1 min at 55°C and 2 min at 72°C). After completion of the cycles, final extension step (5 min at 72 °C) was conducted. At last, analysis of the products was done by agarose gel electrophoresis (3%) (Applichem, Germany, GmbH) using ethidium bromide tincture and seen on UV trans illuminator at 254 nm.

Microbiological identification of *E. coli*:

A quantity of 0.1mL of the broth inoculated with the sample were cultured on EMB and MacConkey agar by streaking to get pure culture of *E. coli* then kept for 24h at 35°C. The culture was then identified by morphology and gram stain. The tests conducted to identify *E. coli* biochemically were: indole, motility, voges Proskuaer, methyle red, urease citrate utilization, gelatin hydrolysis, Arginine decarboxylase detection, hydrogen sulphide production, detection of ornithine decarboxylase, reduction of nitrate, oxidation–Fermentation, β-galactosidase detection, L- lysine decarboxylase detection and sugars fermentation.

Identification of *E. coli* Serologically:

Rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) were utilized to identify *E. coli* serologically [32].

Molecular identification of *E. coli* toxins

DNA Extraction of *E. coli*:

Two colonies were taken from the nutrient agar plates, incubated overnight and placed insterilized filtered water (20 ml). Then boiling for 20 minutes at 100ºC followed by 20-30 min of digestion by proteinase K at 56°C.Based on the manufacturer's manual, extraction was done by QIA amp DNA Mini kit (Qiagen) [27].

Multiplex PCR detection of *E. coli* produced shiga toxins and intimin according to [33]:

Utilizing specific primers displayed in table (2) (Pharmacia Biotech), the isolated *E. coli* was examined by PCR to recognize shiga toxins (*stx1* & *stx2*) and *intimin* (*eaeA*) genes. The amplification was carried out using a Thermo Cycler (Hamburg, Germany). The tests were conducted using DNA polymerase (4U) (Perkin-Elmer), dNTP (0.2 mM), 2 mM concentrations of each primer, 3 mM magnesium chloride, potassium chloride (10 mM), Tris-hydrochloride (10 mM, pH 8.4) and1 µl of DNA template (30 ng of DNA). The amplification conditions began with3 minutes at 95°C followed by 35 cycles of(95°Cfor 20 seconds, 40 seconds at 58°C,90 seconds at 72°C) and final extension for 5 minutes at 72°C. The reference strains utilized were *E. coli* O157:H7Sakai (posses *stx1*, *stx2* and *eaeA*) and *E.* coli K12DH5α (a non pathogenic negative control strain that were negative for all virulence genes. Analysis of the amplified DNA fragments was conducted using 2% agarose gel electrophoresis (AppliChem, Germany, GmbH) with ethidium bromide tincture and seen on UV trans illuminator.

Statistical analysis:

The univariate logistic regression was done using version 21.0. of SPSS program (IBM SPSS Inc., Armonk, NY, USA).

Ethical approval: The ethical considerations of the protocol of this study have been approved and conducted in accordance with the recommendations of the Guide Lines of Animal Care and Use of Lab Animals in Research by the Animal Ethics Committee, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.

Results

Microbiological detection of *S. aureus*. 150 cheese samples were tested and analyzed in the laboratory through miscellaneous methods including cultural, biochemical, staining and molecular tests in order to detect *S. aureus* in these samples. Table (3) shows that, 64 out of 150 samples of cheese were contaminated with *S. aureus* (42.7%). After gram staining and subsequent microscopic analysis of smears from colonies grown on Baired Parker agar, gram-positive grape-like cocci were observed. *S. aureus* isolates were further confirmed by coagulase, catalase, oxidase, Growth at 10% NaCl, arginine decarboxylase (ADH), esculin hydrolysis, hemolysis, D-Nase activity and mannitol fermentation tests

which showed positive results. Samples collected from Kafrelsheikh showed *S. aureus* contamination in 26 samples making the percentage up to 43.3 %. Samples obtained from Riyadh showed *S. aureus* contamination in 19 samples making the percentage up to 42.2 %. Samples obtained from Baltim showed *S. aureus* contamination in 19 samples making the percentage up to 42.2 %. *S. aureus* occurrence in Kariesh cheese was 62% (31/50), while in Damietta cheese was 38% (19/50) and in feta cheese samples was 28% (14/50). The likelihood of finding *S. aureus* in Kariesh cheese was 2.6 times higher than that in Damietta cheese (P = 0.02). However, the variation between the existence of *S. aureus* in Damietta and Feta cheeses is insignificant (table 4).

Identification of Staphylococcal Enterotoxins (SEs) genes by multiplex PCR

Results in (Table 5, Fig. 1&2) showed that out of 79 isolates of *S. aureus*, 16 (20%) were toxigenic. Ten enterotoxigenic strains which can produce Enterotoxin A (33.33%) were identified. Five of them were detected in kariesh cheese, three in Damietta cheese and two in feta cheese samples. The study detected also one enterotoxin B producing strain in one kariesh cheese isolate (3.33%); one Enterotoxin C producing strain in one kariesh cheese isolate (3.33%); two enterotoxin D producing strains in one Damietta cheese isolate and one feta cheese isolate (6.67%); one strain that produces C & D enterotoxins in a single feta cheese isolate (3.33%) and one strain producing A, B, and C enterotoxins (3.33%) in a single Kariesh cheese isolate.

Results of *E. coli* cultivation: In Table (6), Out of 150 samples, 17 were *E. coli* contaminated. In MacConkey agar, *E. coli* colonies appeared as bright pink colonies. In EMB agar, the colonies were metallic green sheen. After gram staining, gram-negative medium with uniform staining coccobacilli was observed. Further confirmation of *E. coli* by biochemical and serological methods revealed a total prevalence of 11.3% (17/150) among cheese samples. Samples collected from Kafrelsheikh showed *E. coli* contamination in 5 samples making the percentage up to 8.3 %. Samples obtained from Riyadh showed *E. coli* contamination in 7 samples making the percentage up to 15.6 %. Samples obtained from Baltim showed *E. coli* contamination in 5 samples making the percentage up to 11.1 %. The prevalence of *E. coli* was 26% (13/50) in Kariesh cheese; 4% (2/50) in Damietta cheese and 4% (2/50) in feta cheese. In Kariesh cheese, the likelihoods of *E. coli* was 8.4 times more than in Damietta cheese (P = 0.007) and no significant variation in *E. coli* prevalence between Damietta and Feta cheese (table 4).

Identifying of *E. coli* serologically. The recognized strains of *E. coli* were clarified as; 4 strains belonging to O26:H11 serotype (2.7%), 3 strains belonging to O128:H2 serotype (2%), 2 strains belonging to O111:H2 serotype (1.3%,), 2 strains belonging to O124 serotype (1.3%), one strain belonging to O91:H21 serotype (0.6%), one strain belonging to O119:H6 serotype (0.6%), one strain belonging to O125:H21 serotype (0.6%), one strain belonging to O44:H18 serotype (0.6%), one strain belonging to O121:H7 serotype (0.6%) and one strain belonging to O86 serotype (0.6%) as shown in Table (7).

Detecting shiga toxins and *intimin* genes. *E. coli* samples were examined using PCR to determine the existence of genes of Shiga toxins and *intimin* (eae). As shown in Table 9, 4 isolates (two O26 & two O111) carried stx1, stx2 and *eaeA* genes (23.5%), one isolate (O26) carried *stx2* and *eaeA* genes (14.3%), 3 isolates (one O26, one O91 & one O119) carried stx1 and stx2 genes (13.1%), 4 isolates (one O44 and three O128) carried stx1 gene (23.5%) and 3 isolates (one O86, one O121 & one O125) carried stx2 gene (17.6%).

Discussion

The composition and cleanliness of milk are key factors in determining its quality. Milk, being highly nutritious, provides an ideal environment for a variety of microorganisms to thrive [34]. Some authors have described cheese as one of the most safe food products. However, in 2006, contaminated cheese contributed to 0.4% of all foodborne outbreaks in Europe [35]. During the production of cheese, microbial contamination can come from a variety of sources such as curd cutting knives, cheese cloth, cheese vat, packaging materials, floors, brine and starter culture. Additionally, it can originate from the air in cold rooms and production areas. Storage containers have also been shown to be a potential source of contamination for cheese produced from pasteurized milk [36].

In this survey, *S. aureus* was determined in 42.7% of specimens. Higher occurrence (81.6%) was determined by Morar et al [37]. Lower prevalence: 26.66% [38] and 14% [39] was notified as well. *S. aureus* presence was higher in Kariesh cheese (62%) than in Damietta (38%) and feta cheese (28%). These findings agree with Hameed & El-Zamkan [2] who noted that Kareish cheese showed a higher occurrence of *S. aureus* (62%) compared to Damietta cheese (54%). Mohamed et al. [40] found *S. aureus* in 73.3% of Kariesh cheese near to our findings. Higher presence was determined in Kariesh cheese: 94% [41] and 93% [42]. Regarding the frequency in Damietta cheese, it was 38%. Higher occurrence: (58%) and (70%) were acquired Mohammed [41], respectively. Higher occurrence in Kariesh cheese may be due to it is manufactured from raw milk and the rudimentary method of cheese production [43].The variation in occurrence could be attributed

to the disparity in dairy items, hygiene standard and preparation methods [44].

Our investigation displayed that 20% of recognized *S. aureus* were toxigenic. Other studies showed that milk and its products had elevated levels of toxigenic *S. aureus*: 31.1% [45] and 41.3% [46]. The most frequently detected toxigenic gene was staphylococcal enterotoxin gene A (*se*a) (33.33%) followed by *sed (*6.67*%), seb* (3.33%) and *se*c (3.33%). Cai et al [39] detected *S. aureus* toxigenic genes in cheese in these values: 29%, 25.6%,9.8%, 8% *and*3.2% for sec, sea, see, sed and seb. Amal & Mona [25] documented that 20% of the detected *S. aureus* produce enterotoxins, with 20% produce enterotoxin A and another 20% produce enterotoxin A and C, while no enterotoxigenic genes were detected in other cheese samples. *S. aureus* recording in dairy products reflects a danger on human as even if *S. aureus* viability was lost in food, its enterotoxins can still persist [47].

Concerning the recognition of *E. coli* in cheese within this research, 11.3% (17/150) of samples exhibited this organism. Ejraes et al. [48] reported a comparable incidence of 11.54%. Higher incidence was documented: 100% of minas soft cheese [49]; 96% of Brazilian cheese samples [50] and 98.7% of Iranian soft cheese samples [51]. In Kariesh cheese, *E. coli* was revealed in 26% in our investigation. Other studies have reported a higher prevalence (75%) [52] as well as a lower prevalence (16%) [53]. Referring to *E. coli* in Damietta cheese, the prevalence was 4% similar to [3]. A higher incidence of 28% was documented [22]. Respecting the prevalence in feta cheese, *E coli* was recognized in 4% of samples. Mohammed et al. [41] recognized *E. coli* in 32% of feta cheese samples whereas Sharaf et al. [54] didn't detect *E. coli* in feta cheese. The varied *E. coli* occurrence may be originated from the difference in milk pasteurization, storage conditions, cheese type and cheese production methods. Also, it may be attributed to unsanitary environment in which cheese production occurs and persons involved in the process.*E. coli* shouldn't be present in food according to Egyptian standards, so our study's finding did not achieve the standards [55].

Serological examining of *E. coli* isolates.In table (8), EHEC strains represented 41.2% of isolates and were recognized as O26: H11, O111: H2, and O91: H21. ETEC strains represented 23.5% and were recognized as O128: H2 and O125:H21. Additionally, the EPEC strain represented 23.5% 23.5% and was recognized as O119: H6, O44:H18, O121: H7, and O86. Meanwhile, EIEC strain represented 11.8% and was recognized as O124. The most prevalent strain in this study was O26:H11, followed by O128:H2 (Table 7). Mohamed et al. [40] discovered various strains of *E. coli* in Tallaga cheese: ETEC was recognized as O125:H21 (2%); EHEC as O103:H2 (1%) and O91:H21 (4%) while EPEC as O144:H18 (2%) and O119:H6 (4%). In Feta cheese, they identified *E. coli* as O111:H2 (EHEC); O124 (EIEC) and O128:H2 (ETEC), each was found in 2% of the samples. In Baramili cheese, EIEC was recognized as O159 (2%); EHEC as O111:H2 (4%) and O26:H11 (2%);EPEC as O119:H6 (2%);ETEC as O128:H2 (2%); EIEC as O159 (2%). Furthermore, 2% of Istamboli cheese samples revealed serotypes O91:H21, O26:H2, O17:H18 (ETEC) and O121:H7 (EHEC) (one serotype of each) [40].

E. coli possess numerous genes of virulence that promote their colonization and cell invasion. Concerning the results of multiplex PCR for identification of toxigenic *E. coli*, table (9) shows that 4 isolates (two O26 & two O111) had genes of *eaeA*, *stx1* and *stx2* (23.5%), one isolate (O26) had genes of *eaeA* and *stx2* (14.3%), 3 isolates (one O26, one O91 & one O119) had *stx1* and *stx2* genes (13.1%), 4 isolates (one O44 and three O128) carried *stx1* gene singly (23.5%) and 3 isolates (one O86, one O121 & one O125) carried *stx2* gene singly (17.6%). *E. coli* O26:H11 was isolated from cattle several times [56], and its presence in dairy products was notified as well [57]. In our investigation, two O26:H11 isolates showed the presence of *eaeA*, stx1 and *stx2* genes, while one O26:H11 had *eaeA* and *stx2* genes. Five isolates of O26 STEC strains tested positive for the *eaeA* gene, which characterise typical EHEC Strains. The severity of O26: H11 positive for *stx2* may vary compared with O26:H11positive for *stx1* which appears to be linked with less severe clinical symptoms [58]. Additionally, the *stx2* subtype is recognized as the most symptomatically significant among stx variants [59].A research conducted by Kasem et al. [60] found *E. coli* strains positive singly for stx1 in 12 (70.58%), *stx2* in 10 (58.82%), and *eaeA* in 3 (17.6%) of the recognized *E. coli*. Also, *eaeA* gene was holded by only two *E. coli* strains (O111: H2 and O26: H11) and these harbored genes of *stx1* and *stx2* as well. On the contrary, gene of *stx1* was absent in O159, O146: H21, O121: H7 and O17: H18 while gene of was absent in O159, O128: H2 and O114: H4 [60].A study conducted by El-Badry and Raslan [61] showed that genes of *stx1* and *stx2* were harboured by O127:H6 strain, gene of *stx1* only was expressed by O111:H4 strain; gene of *stx2* only was carried byO55:H7 and O124:H. Other investigation recognized multiple strains in Kariesh and Ras cheeses in Egypt, which were O145: NM, O128:H2, O127:NM, O124:H7, O119:H6, O114:H2, O113:H21, O91:H10, O86:H34, O26:H11, O25: NM,O22:H5 and O15:H11[62].

Recording *E. coli* refers to low healthy habits during cheese production or fecal contamination [63]. Many strains of *E. coli* were responsible for human gastrointestinal disease such as O128, O121, O113, O91, O55, O45, O145, O111, O103, O26 and O157 strains [63]. To prevent this problem, it is recommended to make milk pasteurization during the manufacture process, as endorsed by the Egyptian Organization for Standards and Quality.

Conclusion

The findings indicate that Kariesh, Damietta, and feta cheeses in Egypt demonstrate significant levels of *E. coli* and *S. aureus*. This indicates that white soft cheese is an important carrier for transmission of well- established pathogenic bacteria. This refers to inadequate hygienic practices. Therefore, dairy plants should implement HACCP system and GMP "good manufacturing practice", as well as applying strict hygienic measures.

Author contributions

TMM-B supplied the design and concept of the work. Preparing materials, collection of data, and analytical evaluation were done by all authors. The primary draft of the manuscript was written by TMM-B, and each author corrected the manuscript. Each author read and accepted the final manuscript.

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Conflicts of interest

There are no conflicts to declare.

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TABLE I. Primers used to identify *S. aureus*:

Gene	Primer	Sequence of Oligonucleotide (5′ → 3′)	Size of the product (bp)	References
sed	sed (F)	5′ CTAGTTTGGTAATATCTCCT ′3	317	[29]
sed	sed (R)	5′ TAATGCTATATCTTATAGGG ′3		
	sec (F)	5′ GACATAAAAGCTAGGAATTT ′3	257	
	Sec (R)	5′ AAATCGGATTAACATTATCC ′3		
	seb (F)	5′ TCGCATCAAACTGACAAACG ′3	478	
	seb (R)	5′ GCGGTACTCTATAAGTGCC ′3		
	sea (F)	5′ TTGGAAACGGTTAAAACGAA′3	120	
	sea(R)	5′ GAACCTTCCCATCAAAAACA ′3		

TABLE 2. Primers used to identify *E. coli*:

Primer	Sequence (5′ → 3′)	Size of the amplified product (bp)	References
eaeA-(F)	5′ GTGGCGAATACTGGCGAGACT ′3	890	[30]
eaeA-(R)	5′ CCCCATTCTTTTTCACCGTCG ′3		
Stx1-(F)	5′ ACACTGGATGATCTCAGTGG ′3	614	[31]
Stx1-(R)	5′ CTGAATCCCCCTCCATTATG ′3		
Stx2-(F)	5′ CCATGACAACGGACAGCAGTT ′3	779	[31]
Stx2-(R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3		

TABLE 3.*S. aureus* microbiological detection in cheese samples from different areas in Kafrelsheikh Governorate:

Cheese	Positive	%	Negative	%	Total	χ2	P value
			Kafrelsheikh				
Kariesh	12	60	8	40	20	3.8 NS	0.15
Damietta	8	40	12	60	20		
Feta	6	30	14	70	20		
Total	26	43.3	34	56.7	60		
			Riyadh				
Kariesh	10	66.7	5	33.3	15	5.65 NS	0.06
Damietta	4	26.7	11	73.3	15		
Feta	5	33.3	10	66.7	15		
Total	19	42.2	34	57.8	45		
			Baltim				
Kariesh	9	60	6	40	15	5.1 NS	0.08
Damietta	7	46.7	8	53.3	15		
Feta	3	20	12	80	15		
Total	19	42.2	26	57.8	45		

TABLE 4.*S. aureus* prevalence in examined cheese samples:

Pathogen	Type of Cheese	Prevalence	OR	CI (95%)	*P* value
	Damietta	4%	-	-	-
E. coli	Feta	4%	1	0.1 – 7.4	1
	Kariesh	26%	8.4	1.8 – 39.7	0.007*
	Damietta	38%	-	-	-
S. aureus	Feta	28%	0.6	0.3 – 1.5	0.3
	Kariesh	62%	2.7	1.2 – 5.9	0.02*

TABLE 5. Recognition of enterotoxigenic *S. aureus* genes using multiplex PCR:

Cheese/ Enterotoxin	Kariesh (15)		Damietta (8)		Powdered (7)		Total (30)	
	No.	%	No.	%	No.	%	No.	%
A	5	33.33	3	37.50	2	28.57	10	33.33
B	1	6.67	0	0	0	0	1	3.33
C	1	6.67	0	0	0	0	1	3.33
D	0	0	1	12.50	1	14.28	2	6.67
A, B & C	1	6.67	0	0	0	0	1	3.33
C & D	0	0	0	0	1	14.28	1	3.33
- ve strains	7	46.67	4	50.00	3	42.85	14	46.67
Total	15		8	100	7	100	30	100

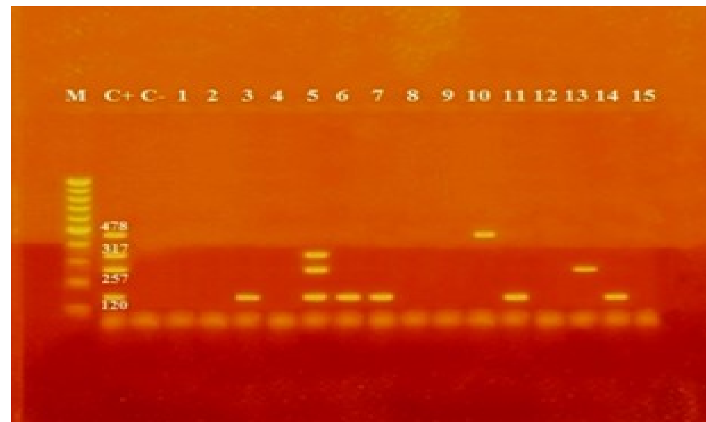

Fig.1. Multiplex PCR for genes of sed (317 bp), sec (257 bp), seb (478 bp) and sea (120 bp) to identify enterotoxigenic *S. aureus*. Lane C+: Control positive. Lane C-: Control negative. Lane M: ladder (100 bp). Lane 5: Positive strain for genes of sed, sec and sea. Lanes 3, 6, 7, 11 & 14: Positive strains for gene of sea. Lane 10: Positive for gene of seb. Lane 13: Positive for gene of sec. Lanes 1, 2, 4, 8, 9, 12 & 15: Negative for the four genes.

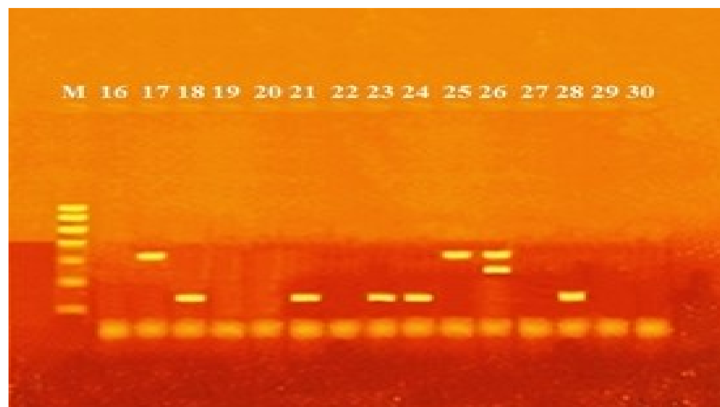

Fig. 2. Multiplex PCR for genes of sed (317 bp), sec (257 bp), seb (478 bp) and sea (120 bp) to identify enterotoxigenic *S. aureus*. Lane M: ladder (100 bp). Lane 26: Positive strain for *sed and sec* and genes. Lanes 18, 21, 23, 24 & 28: Positive strains for *sea* gene. Lane 17 & 25: Positive for *sed* gene. Lanes 16, 19, 20, 22, 27, 29 & 30: Negative for the four genes.

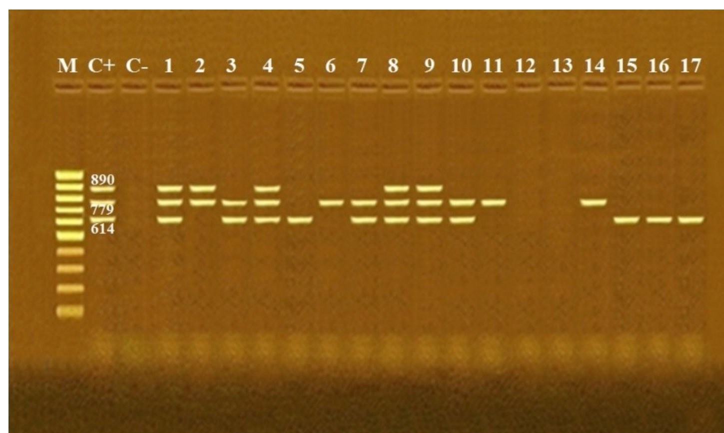

Fig. 3. Multiplex PCR of *stx1* (614 bp), *stx2* (779 bp) and *eaeA* (890 bp) genes to identify *Enteropathogenic E. coli.* Lane M: 100 bp ladder. Lane C+: Control positive *E. coli* for stx1, *stx2* and *eaeA* genes. Lane C-: Control negative..Lanes 5 (O44), 15, 16 & 17 (O128): Positive *E. coli* for stx1 gene.Lanes 6 (O86), 11 (O121) & 14 (O125): Positive *E. coli* for *stx2* gene. Lanes 1, 4 (O26), 8 & 9 (O111): Positive *E. coli* for *stx1*, *stx2* and *eaeA* genes. Lane 2 (O26): Positive *E. coli* for *stx2* and *eaeA* genes.Lanes 3 (O26), 7 (O91) & 10 (O119): Positive *E. coli* for stx1 and stx2 genes. Lanes 12 & 13 (O124): Negative *E. coli* for the three gene

TABLE 6. Microbiological detection of *E. coli* in cheese samples from different areas in Kafrelsheikh Governorate:

Cheese	Positive	%	Negative	%	Total	χ2	P value
			Kafrelsheikh				
Kariesh	4	20	16	80	20	5.67 NS	0.06
Damietta	1	5	19	95	20		
Feta	0	0	20	100	20		
Total	5	8.3	55	91.7	60		
			Riyadh				
Kariesh	6	40	9	60	15	10.5 **	0.005
Damietta	0	0	15	100	15		
Feta	1	6.7	14	93.3	15		
Total	7	15.6	38	84.4	45		
			Balteim				
Kariesh	3	20	12	80	15	1.8 NS	0.41
Damietta	1	6.7	14	93.3	15		
Feta	1	6.7	14	93.3	15		
Total	5	11.1	40	88.9	45		

TABLE 7. Detection of *E. coli* Serologically (n=50):

Strain	No. (%) of isolates	Identified serotypes
EHEC	7(4.7%)	Four O26:H11 isolates, two O111:H2 isolates and one O91:H21 isolate.
ETEC	4(2.7%)	Three O128:H2 and one O125:H21 isolate.
EPEC	4(2.7%)	O119:H6, O44:H18, O121:H7, O86
EIEC	2(1.3%)	Two O124 isolates

TABLE 8. Recognition of *E. coli* Serologically (*n* = 17).

SAMPLES	O26:H111 +ve	%	O44:H18 +ve	%	O86 +ve	%	O91:H21 +ve	%	O111:H2 +ve	%	O119:H6 +ve	%	O121:H7 +ve	%	O124 +ve	%	O125:H21 +ve	%	O128:H2 +ve	% %
KARIESH CHEESE	3	6	1	2	0	0	0	0	2	4	1	2	1	2	2	4	1	2	2	4
DOMIATTI CHEESE	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	1	2
FETA CHEESE	1	2	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	4	2.7	1	0.7	1	0.7	1	0.7	2	1.3	1	0.7	1	0.7	2	1.3	1	0.7	3	2

TABLE 9. Enterotoxigenic *E. coli* genes differentiation:

Samples	*E. coli* serovars	Toxigenic genes	
Kariesh cheese	O26: H111	STX2 & EAEA	1
		STX1 & STX2 & EAEA	2
	O44: H18	STX1	1
	O111: H2	STX1 & STX2 & EAEA	2
	O119: H6	STX1 & STX2	1
	O121: H7	STX2	1
	O124	-	0
	O125: H21	STX2	1
	O128: H2	STX1	2
Damietta cheese	O91: H21	stx1 and stx2	2
	O128: H2	STX1	1
Feta cheese	O26: H111	stx1 and stx2	1
	O86	stx2	1

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الكشف عن المكورات العنقودية السامة و الإشريشية القولونية بالجبن المصرية الطرية باستخدامتفاعل البوليمراز المتسلسل(PCR)

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الملخص

تعتبر الأجبان المصرية من أهم مشتقات الألبان وأكثرهم استهلاكا بمصر (الجبن القريش، الجبن الدمياطي وجبن الفيتا). وبسبب انتشارها بشكل كبير يهدف هذا البحث في دراسة وعزل البكتريا وسمومها التي من الممكن ان تنتقل عبر أكل الجبن ومدى انتشارها والتأكد من وجود سموم البكتريا المسببة لبعض الامراض للانسان. ويرجع السبب الرئيسي لتواجد المكورات العنقودية والاشريشية القولنية الي سوء التصنيع والتخزين للجبن خاصة الجبن القريش لعدم تعرضه للحرارة. وتم أخذ العينات من عدة محلات مختلفة بمحافظة كفر الشيخ (مركز كفر الشيخ، الرياض وبلطيم) حيث تم عزل وتصنيف البكتريا الموجودة من خلال عمل مزرعة بكتيرية ومن خلال صبغة الجرام ومن خلال التفاعلات البيوكيميائية وعن طريق البيولوجيا الجزيئية (PCR) وقد نتج عنه وجود بباكتريا المكورات العنقودية والاشريشية القولونية وبعض السموم الضارة بالإنسان.

الكلمات الدالة:الجبن المصرية، جبن قريش، جبن الدمياطي، جبن الفيتا، سموم بكتيرية