ASSESSMENT OF MICROBIAL QUALITY AND CHEMICAL CONTAMINATION OF SOME MILK AND MILK PRODUCTS

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Food contamination is the presence of unwelcome pathogens, materials or chemicals that could be harmful to the general public's health. It is an issue that concerns the entire world and has a big impact on every other industry. Depending on the kind of food contamination present, the presence of undesirable substances on food might result in foodborne diseases and other harm. At any point in the food supply chain, food contamination is possible (Hussain, 2016).

Milk's unique composition and properties make it an excellent substrate for bacterial development and a source of bacterial illness. Milk-borne pathogenic bacteria cause over 90% of all dairy-related disorders, posing a severe hazard to human health. The main microbiological risks linked with raw milk consumption are *Staphylococcus aureus*, *Salmonella spp.*, *Listeria monocytogenes*, *Escherichia coli O157:H7*, and *Campylobacter*. Animal health, farming techniques, ambient cleanliness, and insufficient temperature control are all factors that influence the microbiological state of raw milk (Berhe *et al.*, 2020).

Aflatoxins are mycotoxins that are amongst the most toxic mycotoxins and are produced by certain moulds (*Aspergillus flavus* and *Aspergillus parasiticus*) which persist in soil, decaying vegetation, hay, and grains of major concern to the dairy industry. It is known to be one of the most known natural carcinogens. Com-

mercially supplied milk is tested for aflatoxin M1. When M1 aflatoxin levels of 0.5 parts per billion (ppb) or more are detected, milk is rejected because it cannot be used for commodities entering the human food supply. Milk producers sometimes use a level of less than 0.5 parts per billion or 500 parts per trillion as a guideline when choosing to allow milk in the human food supply (Muaz *et al.*, 2022 and Omar, 2016).

There are several types of detection of most of food pathogen such as: Conventional procedures include plating and culture, as well as the use of biochemical assays. Furthermore, immuno-detection has been a popular method for detecting *E. coli O157:H7* since it provides for sensitive and specific detection. In recent years, PCR has grown in popularity as a tool for detecting germs (Kim and Oh, 2020).

Prior studies found Aflatoxin M1 (AFM1) in milk and dairy products using liquid chromatography (LC) with fluorescence detection (FLD) and enzyme-linked immunosorbent assay (ELISA). Nevertheless, there are further techniques, including thin-layer chromatography (TLC), fluorometry, (UHPLCeMS/MS), lateral flow immunoassays, and gel-based immunoassays. High-performance liquid chromatography (HPLC) with fluorescence detection (FD) is commonly used and successfully for the analysis of AFM1. TLC is a very old technique for the separation, purity evaluation, and identification of organic compounds. In fact, it was one of the most widely used separation strategies in previous AF analysis. TLC has been replaced by HPLC with FD, which is now combined with other tools such as MS or GC. The main advantage of using HPLC is the ability to combine different detection systems (fluorescence and UV), allowing identification of many materials from a single sample in addition to the high quality of separation and low Limit of detection (LOD) (Pandey *et al.*, 2021).

Hence, the aim of this study is the assessment of microbial quality and chemical adulteration for some milk and milk products by A) evaluate the microbial contamination in milk and milk products and B) assessment of Aflatoxin M1 in milk and milk products.

MATERIALS AND METHODS

Media and reagents

Different culture media were purchased from Biolife (Italy), Conda (Spain) and Hi-Media (India) and were prepared according to the manufacture recommendation. Water was deionized (DW) in the laboratory using a water purification system from Millipore Milli-Q (USA). All Acetonitrile, acetone, and methanol LC-MS grade were purchased from JT Baker (USA). Aflatoxin M1 reference standards were brought from Dr. Ehrenstorfer (Germany). These solutions cover the range of 0.2–10.0 ng/mL AFM1. Construct the standard curve prior to analysis and check the plot for linearity by examining the correlation coefficient (R2 >0.99) of concentrations and responses.

Methods

Collection of dairy product samples

As shown in Table (1), 25 samples of yoghurt, milk, Karish cheese, old cheese, cheese salad, mesh cheese, Barmili cheese, light salt cheese, chili cheese, Ashura, rice with milk, pudding, and pepper cheese were collected from different markets in Giza governorate and were transferred in an ice box with a monitoring data logger to the microbiological laboratory. A sample of 10 grams was weighed and added to a sampling bag for enumerating contamination for *E. coli*, *Staphylococcus aureus*, Coliform, STEC, and *Salmonella* sp.

Sample preparation

For the pre-enrichment of samples, Buffer Peptone Water (BPW) was prepared by dissolving all components in deionized water, mixed for 10 minutes, and then sterilized. Tryptone Bile Glucuronic Agar (TBX) medium was prepared by dissolving components, adjusting pH according to the manufacture instruction, sterilizing, and cooling. Maximum Recovery Diluent (MRD) was similarly prepared for sample pre-enrichment. Dairy product samples were prepared by adding 10 grams to separate sampling bags followed by the addition of 90 ml BPW and mixing which was equivalent to 10^{-1} dilution.

Enumeration of *E. coli*

After sample preparation, inoculation involved transferring 1 ml of the test sample to Petri plates and test tubes containing MRD. Dilutions were made, followed by adding TBX medium to Petri plates. Inoculated plates were incubated at 44ºC for 24 hours according to the ISO 16649. Enumeration of *E. coli* colonies was done post-incubation, with β-glucuronidase-positive colonies counted.

Enumeration of Coliform bacteria and *Staphylococcus aureus*

Solid selective medium Violet Red Bile Lactose Agar (VRBL agar) and Baird Parker Media (BP) were prepared for coliform bacteria (ISO 4832:2006) and *Staphylococcus. aureus* enumeration (ISO 6888-1:2021) respectively. Inoculation involved transferring samples onto prepared plates, incubating, and counting colonies. Confirmation tests were performed according to what was stated in the ISO methods.

Detection of Enteroinvasive *E. coli* (*ipaH* gene) and Shiga-toxin producing *E. coli* (*stx1*, *stx2* and *eae* genes)

Further isolation, DNA extraction, PCR reaction, and gel electrophoresis were conducted to identify the ipaH gene. Extraction of bacterial DNA was performed using the extraction kit from Biotechon diagnostics (foodproof starprep one kit) ™.

PCR amplification was performed in a 25µL reaction mixture containing 200 ng of DNA template (1µL), 12µL ready to use Mastermix (Deram Taq Green PCR Master Mix, Thermo Fisher scientific), 10 µL of distilled water and 1µL of forward

and reverses primers. DNA amplification was carried out with a thermal cycler (Biorad C-1000, USA) with the following thermal cycling program: initial denaturation at 95ºC for 5min followed by 30 cycles of amplification (denaturation at 95ºC for 30 sec, annealing for 30 sec 61ºC for *ipaH* and extension at 72ºC for 30sec) ending with a final extension at 72ºC for 5 min. Sequence of the forward and reverse primers were designed according to Oscar et al, 2010 (Table 4).

Agarose gel (1%) was prepared by dissolving 1g agarose in 100 ml electrophoresis buffer (TAE, 1X) in microwave. Melted agarose was cooled to 50ºCand ethidium bromide was added at a final concentration of 0.5 mg/ml. The agarose gel was submerged in1X TAE electrophoresis buffer in a horizontal electrophoresis apparatus and DNA samples were loaded, 1 kb DNA ladder (Thermo Scientific) was used as a marker for fragment molecular size determination. Electrophoresis was performed at 80 - 90V, for 30-45 min at room temperature in Biometra power Pack P 25. The gel was visualized by U.V transilluminator (IN Geniuse 3). Additionally, steps for the detection of *Salmonella* spp (ISO 6579-1:2017) and Shiga toxin-producing *E. coli* (STEC) (ISO 13136:2012) were detailed, including pre-enrichment, selective media preparation, inoculation, incubation, and PCR amplification. Detection and interpretation of PCR products were conducted for STEC identification.

Extraction and procedure of AFM1 by Liquid Chromatography (LC)

For each sample, 8.0 g test portion was weighed into a 50 mL centrifuge tube. Then, 22 mL of methanol and 13 mL of water were added. The mixture was shaken at 400 rpm for 10 minutes and then centrifuged at 3000 rpm for 10 minutes. Following centrifugation, the upper oil layer was aspirated and discarded. Subsequently, 30 mL of supernatant was transferred to a 125 mL Erlenmeyer flask and mixed with 60 mL of water. The mixture was passed through glass microfiber paper to collect approximately 60 mL of filtrate (equivalent to about 4.6 g of the test portion) into a 100 mL graduate cylinder for further processing (Manetta, 2011).

IAC isolation

The IAC (Immunoaffinity Chromatography) column, stored at 4ºC, was equilibrated to room temperature for at least 15 minutes prior to use. Following equilibration, the top cap of the column was removed and connected to the reservoir of the column manifold. The bottom cap was also removed, and liquid in the column was allowed to pass through until it was about 2–3 mm above the column bed. Subsequently, 60 mL of the filtrate was passed into the column reservoir, allowing it to flow through the IAC by gravity. After that, 10 mL of water was added to the column reservoir when the liquid level was 2 mm above the column packing. The column was washed with an additional 10 mL of water and allowed to run dry. Then, 10 mL of air was forced

through the column with a syringe. Elution was performed with 0.5 mL of methanol, collecting AFM1 in a 4 mL vial. The column was allowed to run dry, followed by two additional elutions with 0.5 mL of methanol each, collected into the same vial. After allowing the column to run dry again, 10 mL of air was forced through the column. The eluate was evaporated to dryness under a stream of nitrogen at 40°C, followed by the addition of 0.5 mL of LC mobile phase to the residue. After vortexing for 1 minute, 0.05 mL was injected for LC analysis.

1.1.1. LC analysis and AFM1 quantitation and calculation

Prepare standard curves of AFM1 using working standard solutions containing AFM1 covering the range of 0.2–10.0 ng/mL AFM1 (Manetta, 2011). Construct the standard curve prior to analysis and check for linearity by examining the correlation coefficient (R^2 > 0.99) of concentrations and responses. If the test solution area response is outside (higher than) the standard range, dilute the purified test extract with LC mobile phase and reinject it into the LC column. Inject 0.05 mL of reagent blank, AFM1 working standards, or test solution into the LC column. Identify AFM1 peaks in the test solution by comparing the retention time with those of the standards. The retention time of AFM1 was approximately 7 min, and the peaks were baseline-resolved. Quantitate AFM1 by measuring the peak area at the AFM1 retention time and comparing it with the standard curve. Plot the peak area (response, y-axis) of AFM1 standard against the concentration (ng/mL, x-axis) and determine the slope (S) and y-intercept (a). Calculate the level of toxin in the test sample using the formula:

$$\text{Toxin, ng/kg} = ([(R - a)/S] \times V/W) \times F \times 1000$$

Where: R is the test solution peak area, V is the final volume (mL) of the injected test solution, F is the dilution factor (1 when V is 0.5 mL), and W is the weight (4.6 g) of the test sample passed through the IAC edit English if present any wrong.

RESULTS AND DISCUSSION

Microbial contamination in dairy products

Milk and dairy products serve as essential dietary components, providing vital nutrients often challenging to obtain from non-dairy sources. However, these products can also harbor various pathogens, including *E. coli*, Shiga toxin-producing *E. coli* (STEC), coliform bacteria, *Staphylococcus aureus*, and *Salmonella enterica*, which pose significant food safety concerns. In this study, we aimed to evaluate the microbial quality and chemical contamination of selected milk and dairy products obtained from local markets.

Monitoring of *E. coli* bacteria

Escherichia coli, a common indicator of fecal contamination, was detected in 14 out of 25 tested dairy product samples (sample 1, 5, 7, 8, 9, 10, 11, 12, 13, 17,

19, 21, 22, 23 and 24), representing 56% of the total samples as shown in Fig. (1). The highest counts of *E. coli* colonies were observed in Karish cheese (sample 9); while yogurt (sample 5) exhibited the lowest count. Statistical analysis revealed that 44% of the total samples did not contain *E. coli* as shown in Table (2).

Results of this work revealed that *E. coli* is present in tested dairy products (56%) of milk samples, cheese and yogurt, referring to the obtained results in comparison with previous studies, which were higher than El- Barody *et al*. (2022) *E. coli* was detected in 57 samples representing 47.5% of the total examined samples 120 (El-Barody *et al.*, 2022), but they were less than the results of ElMalt *et al*. (2013) *E. coli* was detected in 63 samples representing 63% of the total examined samples 100 (El-Malt *et al.*, 2013), Karish cheese which results the highest amount of *E. coli* agree with El- Barody *et al*. (2022), on the other hand, *E. coli* could not be detected in old cheese samples and Mesh cheese, this was due to the high amount of salts added which acted as natural preservative agent inhibiting pathogenic bacterial growth (Henney *et al*., 2010). The presence of *E. coli* in dairy products was utilized as an indicator of manufacturing environment cleanliness, water quality used in milk product handling and processing, and food handler personal hygiene. In the case of heat-treated dairy products, the pasteurization process can easily kill *E. coli*; thus, the presence of the bacteria in heat-treated dairy products implies that some level of contamination occurred after pasteurization during production and/or packaging (Bagel and Sergentet, 2022). All isolated *E. coli* was identified by PCR, in which all were negative for *ipaH* gene.

Monitoring of Coliform bacteria

Coliform bacteria, another indicator of fecal contamination and overall microbial quality, were identified in 19 out of 25 samples (76%). Among the samples, milk (sample 9) exhibited the highest count of coliform colonies, while Ashura (sample 18) showed the lowest. Confirmation tests showed gas formation in the Durham tube and turbidity in 18 samples, except for sample 12, mesh cheese. Statistical analysis indicated that 72% of the total samples contained coliform bacteria as shown in Table (2).

Coliform count is a traditional indicator of possible fecal contamination, microbial quality, and wholesomeness and reflects the hygienic standards adopted in the food operation. Because coliform organisms are easily killed by heat, these bacteria can also be used as an indicator of heat treatment failure as well as post heat treatment contamination. The presence of coliforms in the analyzed samples indicated a lack of hygienic procedures, incorrect heat processing, or post-pasteurization contamination by handlers (Trmčić *et al.*, 2016). The results showed that 72% of the analyzed samples contained coliform, with 60% of samples having high coliform skipped the Egyptian regulation (> 120 CFU/g).

Monitoring of *S. aureus* and *Salmonella* spp.

After testing *S. aureus* and *Salmonella* in 25 samples of different dairy products, all the samples were not detected as *S. aureus* and *Salmonella* as shown in Fig. (2).

Detection of Shiga Toxin-producing *E. coli* (STEC)

STEC, known for causing severe gastrointestinal illnesses such as hemorrhagic colitis and hemolytic-uremic syndrome, was detected in 12% of the analyzed samples as shown in Fig. (3).

Real-time PCR analysis identified the presence of virulent genes (*stx1*, *stx2*, IAC, and *eaeA*) in three samples: milk (sample 1), Karish cheese (sample 9), and Barmili cheese (sample 13) as represented in Fig. (4). These findings underscore the importance of stringent food safety measures to prevent the transmission of pathogenic bacteria through dairy products

Figure (4). Represent the amplification of *STEC* virulent genes in sample 9 Karish cheese, FAM fluorescence detection of *stx1* gene, VIC fluorescence detection of *stx2* gene, ROX fluorescence detection of *eaeA* gene and cy5 fluorescence are internal amplification control (IAC).

Shiga toxin-producing E. coli (STEC) are thought to be the primary cause of hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS) (Liao *et al*., 2019). Undercooked meat, unpasteurized dairy products and vegetables, and feces-contaminated water are all plausible routes for STEC human exposure (Dias *et al*., 2022). STEC was identified using a real-time PCR method. The quantitative polymerase chain reaction (qPCR) methodologies are frequently applied in microbiological research to identify the amount and expression of a given target gene, which in this case are the target genes (stx1, stx2, IPC and eaeA). In other words, it is an effective method for measuring gene expression levels. Furthermore, real-time PCR has greater precision, sensitivity, dynamic range, and resolution than classical PCR (Li *et al*., 2017). The current study work agrees with another study reported from Iran by Mohammadi *et al*. (2013), who used PCR to target stx1 and stx2 and then eaeA. This investigation included 206 raw milk samples, 36 of which were determined to be infected with STEC (17%) (Mohammadi *et al*., 2013).

`Detection of Aflatoxin M1

Aflatoxin M1 (AFM1), a mycotoxin commonly found in milk and dairy products. As shown in Table (3), after detection of aflatoxin AFM1using LC, sample 8 represents the highest amount ofAFM1, there are two samples 10 and 16 have low amount of AFM1low of detection by LC and sample 25 not detected as shown in Fig. (5).

The result showed that AFM1 was detected in all samples, with varying concentrations exceeding regulatory limits. While 88% of the samples exceeded Egyptian regulations (0 µg/kg), 52% sur-

passed EU regulations (0.05 µg/kg). These results highlight the need for comprehensive monitoring and control strategies to mitigate the health risks associated with mycotoxin contamination in dairy products.

As milk is used on a big scale by the people, there is an increase in manufacturing of Egyptian raw milk free of mycotoxin. Egypt uses a different standard than other countries such as the USA. The maximum residue limit for AFM1 in raw milk in Egypt is zero, 0.05 µg\L in the EU, and 0.5 µg\L in the US. It was also discovered that an acceptable threshold of risk for AFM1 in fresh raw milk was 0.05 µg/kg , in accordance with Codex Alimentarius and the Joint Expert Committee on Food Additives regulations (JECFA) The current study discovered that all samples under investigation contained aflatoxin M1 at varying concentrations ranging from high to medium to low in all species with 92% of samples detected AFM1, while 8% Exceeding US Limit (0.5 µg\L), and 56% Exceeding EU Limit. According to a different revelation by Anonymous, the European Commission's maximum permitted amount of AFM1 in milk is 0.05 µg\L, (Bakirci, 2001) analyzed 90 raw milk samples for AFM1 and discovered that 87.77% of the positive samples contained aflatoxin M1 and 44.30% of the positive samples above the maximum tolerance limit (0.05 ppb) Approved European Union (EU) (Bakirci, 2001) and ElSayed, *et al*. (2000) who investigated 15 Egyptian cow's milk samples and discovered that three were positive for AFM1 with a mean value of 6.3 ppb (El-Sayed *et al*., 2000). In the current study, we discovered that AFM1 was present in most of samples, although at varying levels ranging from high to medium to low in raw milk and across dairy species. Even at a low level, AFM1 surpasses Egyptian regulations, which state that it should be zero. A similar record explains elevated levels of AFM1 in raw milk because of dairy cows' diets consisting primarily of silage or contaminated feed items. Furthermore, it was discovered that there is a seasonal effect on AFM1 levels, with summer being lower than winter, or that there is a distribution effect due to the long distance between producer and consumer. While low AFM1 concentrations in raw milk in some tests were explained because of mixing and dilution of contaminated milk with less contaminated or non-contaminated milk from various sources. While storage, processing, and fabrication had no effect on AFM1 level (Mahmoudi, 2014).

This study concluded that after testing for the presence of *E. coli*, coliform, *Salmonella* and STEC using selective media, followed by confirmation tests. The tested milk and dairy products collected from local markets in Giza were discovered contaminated with pathogenic bacteria that cause a variety of diseases that impair human health, particularly immunocompromised individuals. Therefore, the high degree of contamination was most likely caused by poor hygiene and the use of unpasteurized milk in dairy product manufacture. Also, Control tech-

niques approved by each country may be able to minimize the limit of aflatoxin in milk and reduce the risk of mycotoxin's influence on human health. So, in Egypt, quality assurance regulations should have been implemented to reduce the quantity of mycotoxins and their negative consequences. The limitations included detecting the presence of more pathogenic bacteria in milk and dairy products, doing PCR with more E. coli genes to identify the pathogenic group that return to, and having limited funding and resources. Furthermore, the experimental time was quite brief, necessitating additional confirmatory studies.

Therefore, it is recommended to conduct further studies to know the types of *E. coli* that are found in milk and cheese and find solutions to get rid of them and do some studies on antibiotics associated with these microbes that are found in milk and cheese.

Conclusion: This study revealed concerning levels of microbial contamination and chemical adulteration in milk and dairy products obtained from local markets. The presence of pathogenic bacteria and mycotoxins underscores the need for stringent food safety protocols, effective regulatory enforcement, and continuous monitoring to safeguard public health. Future research should focus on identifying specific sources of contamination and developing targeted interventions to mitigate risks associated with dairy product consumption. Additionally, efforts should be directed towards enhancing public awareness, improving hygiene practices, and strengthening regulatory frameworks to ensure the safety and quality of dairy products.

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SUMMARY

Food contamination poses a significant threat to public health, potentially causing illnesses due to the presence of infectious organisms like fungi, bacteria, viruses, parasites, or their toxins. Notable bacteria contributing to contamination include *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus*, each capable of causing various infections. Additionally, chemical contaminants like Aflatoxin M1 further compound the risks, being known carcinogens. This study aimed to evaluate the microbial quality and chemical contamination of selected milk and milk products. Twenty-five samples were weighed and subjected to enrichment media, followed by culturing on specific selective media for identification of bacteria such as *E. coli*, *Staphylococcus. aureus*, coliforms, and *Salmonella* spp., further confirmed through confirmatory tests. Shiga-toxin producing *E. coli* (STEC) was identified using Real-time PCR, while

PCR and Gel Electrophoresis were utilized to determine the pathogenic group of *E. coli*. Furthermore, liquid chromatography (LC) analysis was conducted to quantify Aflatoxin M1 levels in the samples. Analysis revealed that 56% of tested samples were positive for *E. coli*, while 76% contained suspected coliform colonies, with 72% confirmed. Additionally, 12% of samples harbored STEC, while none contained *Salmonella* or *S. aureus*. Alarmingly, 88% of samples exceeded Egyptian regulations for Aflatoxin M1 (> 0 ug/Kg), with 52% surpassing EU regulations (> 0.05 ug/Kg). These findings underscore significant contamination of milk and dairy products by various bacteria and the presence of excessive Aflatoxin M1 levels, highlighting the urgent need for regulatory measures and quality control in the food industry.

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Table (1): Samples collection from market in Egypt (One sample from each category).

Sample number	Product	Source
1	Milk	Market at Hadayek El Ahram
2	Cheese	Market at Hadayek El Ahram
3	Old cheese	Market at Hadayek El Ahram
4	Salad cheese	Market at Hadayek El Ahram
5	Yoghurt	Market at Hadayek El Ahram
6	Karish cheese	Market at Hadayek El Ahram
7	Milk	Market at Hadayek El Ahram
8	Old cheese	Local market at Giza, (Faisal)
9	Karish cheese	Local market at Giza, (Faisal)
10	Milk	Local market at Giza, (Faisal)
11	Karish cheese	Local market at Giza, (Talbiya)
12	Mesh cheese	Local market at Giza, (Talbiya)
13	Barmili cheese	Local market at Giza, (Talbiya)
14	Light salt cheese	Local market at Giza, (Talbiya)
15	Old cheese	Local market at Giza, (Talbiya)
16	Chili cheese	Local market at Giza, (Dokki)
17	Yogurt	Local market at Giza, (Dokki)
18	Ashura	Local market at Giza, (Dokki)
19	Rice with milk	Local market at Giza, (Dokki)
20	Pudding	Local market at Giza, (Dokki)
21	Barmili cheese	Local market at Giza, (Hadayek el Ahram)
22	Istanbuli Cheese	Local market at Giza, (Hadayek el Ahram)
23	Chili cheese	Local market at Giza, (Hadayek el Ahram)
24	Karish cheese	Local market at Giza, (Hadayek el Ahram)
25	Mesh cheese	Local market at Giza, (Hadayek el Ahram)

Table (2): Enumeration of positive Suspected colonies of *E. coli* and coliform by measuring colony forming unit per gram (CFU/g), in 25 different yogurt and dairy products samples the highest E. coli colonies samples 9 and the lowest colonies sample 5, and the highest coliform colonies sample 9 and the lowest colonies sample 18.

Sample Number	E. coli (CFU/g)	Coliform (CFU/g)	Confirmation
1	88	140	Positive
2	0	0	Negative
3	0	0	Negative
4	0	15	Positive
5	5	20	Positive
6	0	0	Negative
7	100	135	Positive
8	134	28	Positive
9	180	101	Positive
10	15	47	Positive
11	208	304	Positive
12	100	0	Negative
13	205	100	Positive
14	0	45	Positive
15	0	131	Positive
16	8	4	Negative
17	98	98	Positive
18	18	2	Positive
19	0	311	Positive
20	118	0	Negative
21	32	74	Positive
22	99	33	Positive
23	59	105	Positive
24	0	53	Positive
25	0	0	Negative

Table (3): Aflatoxin M1 (AFM1) Levels Detected in Dairy Product Samples Using Liquid Chromatography (LC).

Sample Number	Amount of AFM1 (ppb)
1	0.0563
2	0.1964
3	0.0015
4	0.0289
5	0.2889
6	0.0159
7	0.0222
8	0.6726
9	0.1926
10	< LOQ
11	0.6649
12	0.0138
13	0.0214
14	0.1926
15	0.0230
16	< LOQ
17	0.2132
18	0.1082
19	0.1396
20	0.1142
21	0.0142
22	0.0676
23	0.0939
24	0.4563
25	ND

"< LOQ" stands for "below the limit of quantification" and "ND" stands for "not detected"

Table (4): List of oligonucleotide primers sequences.

target gene name	Forward primer, reverse primer and probe sequences (5 → 3')	Amplicon size (bp)	reference
Stx1	Forward- TTTGTYACTGTSACAGCWGAAGCYTTACG Reverse- CCCCAGTTCARWGTRAGRTCMACRTC Probe- CTGGATGATCTCAGTGGGCGTTCTTATGTAA	131	Perelle *et al*. 2004
Stx2	Forward- TTTGTYACTGTSACAGCWGAAGCYTTACG Reverse- CCCCAGTTCARWGTRAGRTCMACRTC Probe-TCGTCAGGCACTGTCTGAAACTGCTCC	128	Perelle *et al*. 2004
ipah	Forward- CTCGGCACGTTTTAATAGTCTGG Reverse- GTGGAGAGCTGAAGTTTCTCTGC	933	Oscar *et al*. 2010

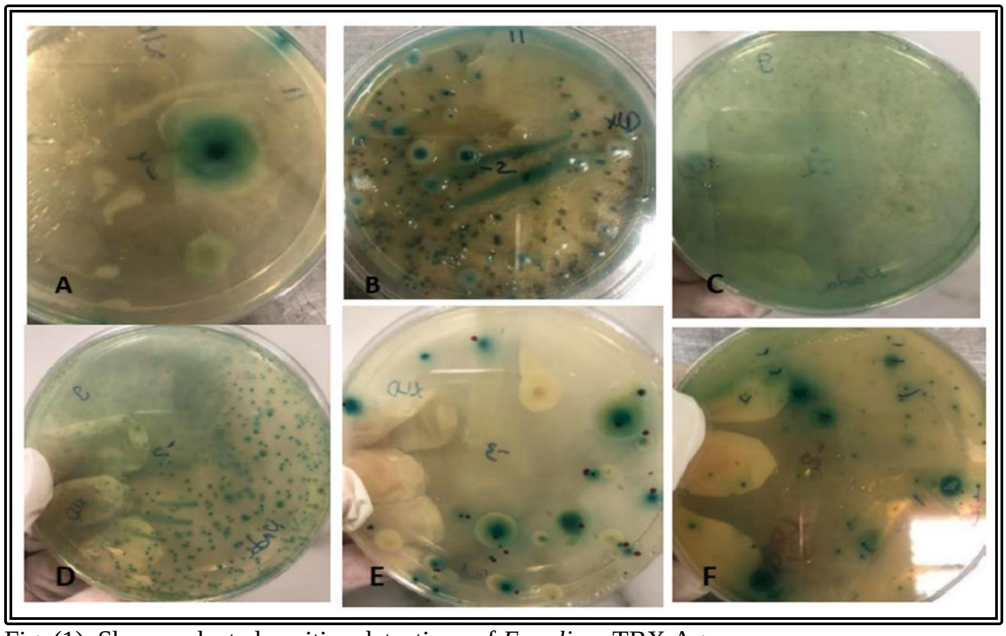

Fig. (1). Shows selected positive detections of *E. coli* on TBX Agar.

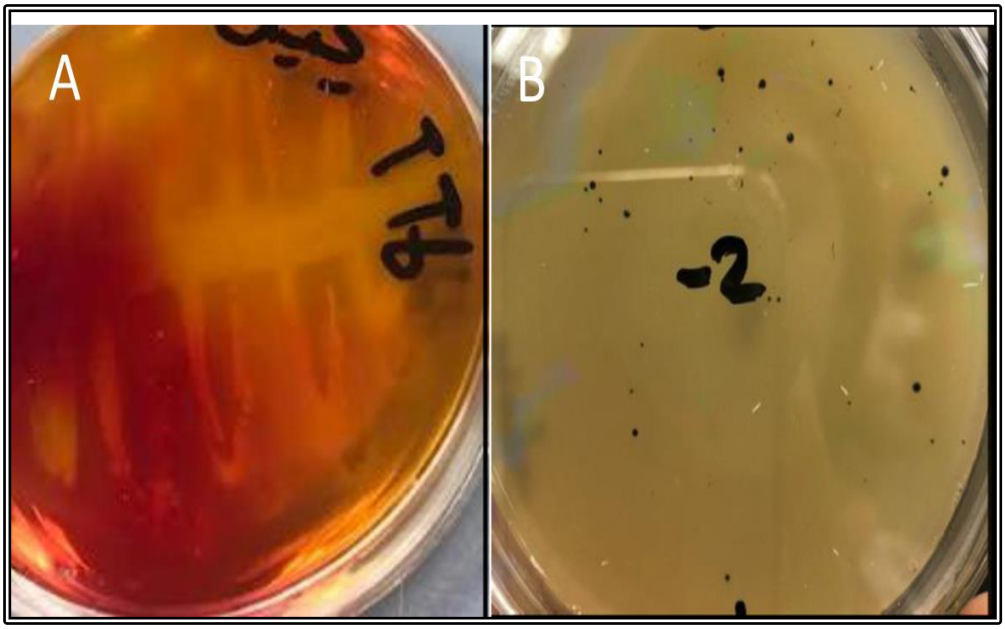

Fig. (2). (A) Shows negative result of *Staphylococcus. aureus* on BP culturing agar and (B) Shows negative detection of suspected colonies of Salmonella spp. on XLD agar.

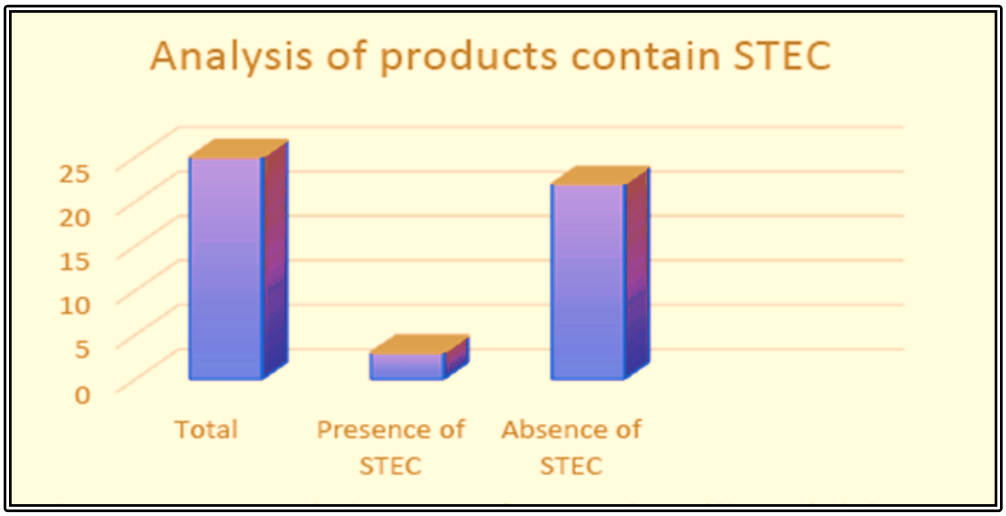

Fig. (3).The presence and absence of STEC in milk and dairy products.

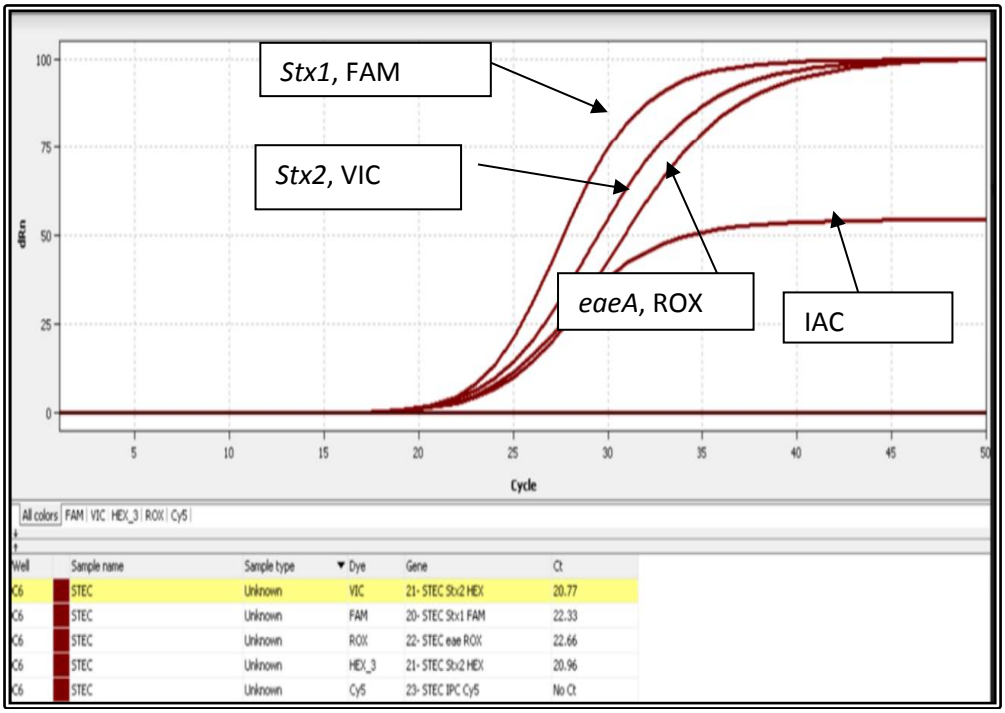

Fig. (4). Represent the amplification of *STEC* virulent genes in sample 9 Karish cheese, FAM fluorescence detection of *stx1* gene, VIC fluorescence detection of *stx2* gene, ROX fluorescence detection of *eaeA* gene and cy5 fluorescence are internal amplification control (IAC).

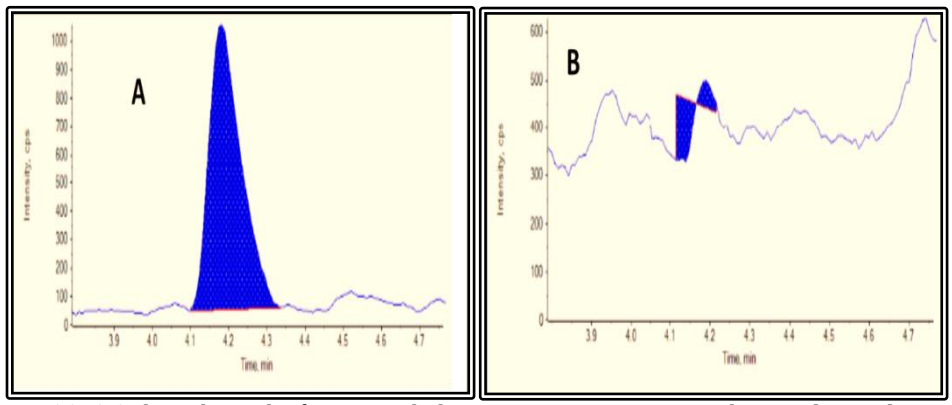

Fig. (5). (A) show the peck of AFM1with the amount 0.6726 ug/Kg in the sample 8 and (B) Show there is no detection of AFM1 in the sample 25.