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ISOLATION AND IDENTIFICATION OF ENTEROTOXIGENIC HALOPHILIC BACTERIA IN SOME PICKLED DAIRY PRODUCTS IN ASSIUT GOVERNORATE, EGYPT

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ABSTRACT

A total of one hundred and twenty random samples of pickled Domiati and Kareish cheese (60 each) during the period from May 2023 to June 2024 were collected from dairy shops and markets in Assiut governorate, Egypt; for isolation and identification of enterotoxigenic halophilic bacteria in some pickled dairy products. The results showed that salt concentration in Domiati and Kareish cheese ranged from 2.6 and 2.9 to 6.8 and 12.9 with a mean value of 5.1 and 9.2, respectively. The pH values ranged from 2.7 and 3.4 to 5.1 and 7.9, with a mean value of 4.1 and 5.2, respectively. On the other hand, the count of halophilic bacteria in examined pickled Domiati and Kareish cheese samples recovered on halophilic agar containing 3% salt ranged from $<10^2$ and $<10^2$ to $1.5x10^5$ and $1.03x10^4$, with a mean value of $1.8x10^4 \pm 0.63x10^4$ and $1.1x10^3 \pm 0.42x10^3$, respectively. Corresponding counts on halophilic agar containing 10% NaCl ranged from $1.3x10^3$ and $0.23x10^2$ to $1.45x10^6$ and $2.1x10^4$, with a mean value of $0.8x10^5 \pm 0.62x10^5$ and $0.56x10^4 \pm 0.81x10^3$, respectively. The isolates were identified biochemically as *Staph. aureus, S. epidermidis, Micrococci spp., B. cereus, B. licheniformis, and B. coagulence, B. subtilis, B. mycoids, E. coli and Proteus* species, and only 31 samples (46.3 %) out of 67 examined *S. aureus* samples identified as coagulase positive. The Polymerase Chain Reaction (PCR) for coagulase positive *S. aureus and B. cereus* showed that 19 (61.3%) and 25 (30.9%) samples were enterotoxigenic halophiles, respectively. Some of these halophilic bacteria, such as *Staph. Aureus, B. cereus, E. coli* and *proteus* are of public health hazards which cause several food poisoning outbreaks. So, milk must come from dairy farms and apply HACCP system and strict hygiene during processing, storage and handling and finally periodical examination of milk, and dairy products chemically and bacteriologically must be applied.

Keywords: Pickled dairy products, halophilic, *Staph. spp., Bacillus spp*., enterotoxigenic, PCR

INTRODUCTION

Halophilic bacteria are salt-loving microorganisms that require certain minimal concentrations of sodium chloride (NaCl) for their growth. They are classified according to the concentration of saline into three classes of bacteria. The first class is slightly halophiles, which grow well in 0.5 -3 % salt. The second class is moderate halophiles, which grow in 3 -15 % salt. The third class grows in 15-30 % salt, which is called extremely halophiles. Gram-positive microorganisms like Bacilli and a few species of Coryne

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bacteria are classified as halophilic microorganisms. The species Micrococci and Bacilli are considered moderate halophiles, which can produce enzymes characterized by salt stability and in high salinity able to carry out catalysis, which are salt tolerant, tolerated and active at high pH and temperature, leading to spoilage of salted food (Osama *et al.*, 2020). *Staphylococcus spp.* and *Bacillus spp.* are considered members of the most important food-borne pathogens, which produce several enterotoxins that cause a public health hazard, such as human food poisoning characterized by abdominal cramps, nausea, diarrhea, and emesis (Logan, 2012 and Normanno *et al.*, 2005). Dairy products such as pickled soft white cheese (Domiati cheese) and Kareish cheese are mainly produced by people in small farms under incomplete hygienic measures. These manufactured dairy products are exposed to contamination with halophilic bacteria, such as *Staphylococcus spp. and Bacillus spp.*, which are widely distributed in nature. Dairy products are a dangerous source of infection and unfit for consumption among consumers, causing health hazards due to enterotoxin production (Bightwell *et al.*, 2006). Pickled Domiati and Kareish cheese are examples of moderate salted dairy products that contain 1 to 15% salt by weight. Microbial spoilage is a possibility for these products, and they are also more likely to contain viable human pathogens. So, the present work aimed to secure the numbers and types of moderate halophiles in some pickled dairy products in Assiut governorate, Egypt.

MATERIALS AND METHODS

Collection of samples:

Sixty random samples of pickled Domiati and Kareish cheese were collected in sterile jars during the period from May 2023 to June 2024 from Assiut Governorate, Upper Egypt. The samples were transferred to the laboratory, without delay, and either examined directly or kept in the (4^0C) refrigerator until examination.

part - I

I - Chemical Examination:

1- Determination of sodium chloride (A.O.A.C, 2000):

In a 200 ml Erlyn – Meyer flask, 3 grams of prepared sample, 10 ml of halogen-free nitric acid, 25 ml of N/10 silver nitrate solution and 50 ml of distilled water were added, then the mixture was boiled;15 ml of 5% potassium permanganate solution was added to a 5 ml portion during boiling until the solution becomes yellowish and clear. After the solution cooled, filtered into a volumetric flask. Distilled water was used at 20^{o}C to thoroughly wash the filtrate, which has been made up to standard volume. The excessive amount of silver nitrate was titrated in 100 ml of the clear solution against 0.1 N potassium thiocyanate solution (9.71 g/liter), using 2 ml of saturated iron alum solution as an indicator. The salt content was calculated according to the following equation

NaCl% = 2 (25-R)*X0.00584*100 weight (g)

2-Determination of pH value:

The pH value was determined using a pH meter (PROBE Benchtop pH Meter) equipped with a standard electrode.

II -Bacteriological Examination:

1-determination of total Halophilic bacterial counts (A.P.H.A.2004):

Serial dilutions were prepared by putting 10 gm of each sample in a sterile stomacher bag with 90 ml of synthetic saline solution (3% NaCl for slight or 10% NaCl for moderate halophilic counts). The contents were homogenized for 2 minutes in a stomacher lab-blender (Seward Stomacher Model 400) (2000 rpm) to make a 1:10 dilution (wt/vol). Then, decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared. From each dilution, 0.1 ml was inoculated in duplicated plates containing standard plate count agar (oxoide) followed by spread plate technique, and the plates were incubated at 37^{o}C for 48 hours, then the

total slight and moderate halophiles were counted and expressed as CFU/g.

2 - Identification of halophilic bacteria (FDA, 2001):

Suspected colonies were examined morphologically, microscopically by Gram stain and biochemically by Coagulase test, Catalase test, egg yolk lecithinase, sugar fermentation, citrate utilization, nitrate reduction test, methyl red (MR), Voges-Proskauer (VP) tests and anaerobic growth on blood agar (Aruwa and Olatope, 2015).

Part II: PCR detection of halophilic bacteria enterotoxins genes:

The identified strains of *Staph. aureus* and *B. cereus* were tested for the production of different enterotoxins.

Extraction of DNA (Mehrotra *et al.*, 2000):

The DNA extraction from samples was performed according to the recommend-dations of the QIA amp DNA Mini kit (Qiagen Germany, GmbH), with modi-fications, for Gram-positive bacteria. Briefly, at 56°C, a total of 200 ml sample suspension was used to incubate 200 ml of lysis buffer and 10 ml of proteinase K for 10 minutes. After incubation, 200 µl of ethanol 100% was added to the lysate. According to the manufacturer's recomm-endations, the mixture was washed and centrifuged, then eluted with nucleic acid 100 µl of elution buffer provided in the kit.

PCR amplification:

The PCR for the suspected colonies of *S. aureus* gene was carried out using the primers:

Target gene	primer sequences	Amplified segment (bp)
sea	GGTTATCAATGTGCGGGTGG	102
	CGGCACTTTTTTCTCTTCGG	
seb	GTATGGTGGTGTAACTGAGC	164
	CCAAATAGTGACGAGTTAGG	
sec	AGATGAAGTAGTTGATGTGTATGG	451
	CACACTTTTAGAATCAACCG	
sed	CCAATAATAGGAGAAAATAAAAG	278
	ATTGGTATTTTTTTTCGTTC	
see	AGGTTTTTTCACAGGTCATCC	209
	CTTTTTTTTCTTCGGTCAATC	

The suspected colonies of *B. cereus* were subjected to PCR-based identification, using primers; BcAPR1 (CTTCT TTGGCCTTCTTCTAA) and BcFF2 (GAGATTTAAATGAGCTGTAA)**.** All *Bacillus*-like isolates that did not yield PCR products with primers BcAPR1 and BcFF2 were subjected to 16S rRNA GS-based identification, as described by Alnakip, (2014), and quantified using the Quanti TTM. The 16S rRNA gene fragment was amplified using the universal primer pair: p8FPL (5′-AGTTTGATCCTGGCTCAG-3′) and p806R (5′-GGACTACCAGGGTATCT-AAT-3′) within a "My Cycler" Thermal Cycler (BioRad, Hercules, USA). Then direct GS was performed by the "Big Dye Terminator v3.1" Cycle Sequencing Kit and used the same primers used for PCR. The sequencing reactions were analyzed in ABI3130 automatic GS sys. (ABs, USA). The entire 16S rRNA gene sequences were analyzed using Chromas software and aligned with Clustal-X software. Next, these sequences were identified by sequence homology alignment among published reference sequences using the web tool; NCBI BLAST

Cycling conditions of the primers during cPCR:

Primary Denaturation	*Secondary Denaturation*	*Annealing*	*Extension*	*No. of cycles*	*Final extension*
94˚C/ 5 min.	94˚C /30 sec	57˚C/ 30 sec.	72˚C/ 30 sec.	35	72˚C/ 7 min.

Agarose gel electrophoresis with modification:
Computer software and gel documentation systems were used to analyze the data of the gel photographed.

RESULTS

Table 1: Statistical analytical results of sodium chloride % in pickled Domiati and Kareish cheese samples (n=60) compared with Egyptian Standards 2005.

Samples	*Min.*	*Max.*	*Mean ± SE*	*E.S 2005*
Pickled Domiati cheese	2.6	6.8	5.1	Not more than 7.0%
Pickled Kareish cheese	2.9	12.9	9.2	Not more than 15%

Table 2: Statistical analytical results of pH value in pickled Domiati and Kareish cheese samples (n=60) compared with Egyptian Standards 2005.

Samples	*Min.*	*Max.*	*Mean ± SE*	*E.S 2005*
Pickled Domiati cheese	2.7	5.1	4.1	Not more than 4.9
Pickled Kareish cheese	3.4	7.9	5.2	Not more than 4.6

Table 3: Statistical analytical results of total Halophilic bacterial count in examined samples.

Examined samples	*Examined Samples*			
		Min.	*Max.*	*Mean ± SE*
Pickled Domiati cheese	**3% Nacl**	$<10^2$	1.5×10^5	$1.8 \times 10^4 \pm 0.63 \times 10^4$
	10% Nacl	$< 10^2$	1.03×10^4	$1.1 \times 10^3 \pm 0.42 \times 10^3$
Pickled Kareish cheese	**3% Nacl**	1.3×10^3	1.45×10^6	$0.8 \times 10^5 \pm 0.62 \times 10^5$
	10% Nacl	0.23×10^2	2.1×10^4	$0.56 \times 10^4 \pm 0.81 \times 10^3$

Table 4: Frequency distribution of the isolated halophilic bacteria from the examined samples:

Isolates	*Total*	*Pickled Domiati Cheese 3% NaCl*		*Pickled Domiati Cheese 10% NaCl*		*Pickled Kareish Cheese 3% NaCl*		*Pickled Kareish cheese10% NaCl*	
	No.	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
S. aureus	67	15	22.4	12	17.9	24	35.8	16	23.9
S.epidermidis	22	6	27.3	4	18.2	9	40.9	3	13.6
Micrococci	95	28	29.5	17	17. 9	35	36.8	15	15. 8
B.cereus	81	24	29.6	14	17.3	26	32.7	17	21
B.subtilis	84	21	25	14	16.7	28	33.3	21	25
B.coagulans	59	16	27.1	12	20.3	19	32.2	12	20.3
B.mycoids	78	22	28.2	10	12.8	28	35.9	18	23.1
B.lichenformis	41	10	24.4	8	19.5	16	39	7	17
E.coli	25	7	28	4	16	9	36	5	20
Proteus spp.	14	5	35.7	3	21.4	4	28.6	2	14.3
Total	566	154	27.1	98	17.3	198	35.7	116	19.9

Table 5: PCR results for detection of enterotoxigenic coagulase positive *S. aureus* toxins from the examined samples

Types of Examined samples	*No. (%) of Examined sample*	*No. (%) of positive samples*	*No. (%) of entrotoxigenic strain*
Pickled Domiati 3% Nacl	15 (22.4)	8 (53.3)	5 (62.5)
Pickled Domiati 10% Nacl	12 (17.9)	5 (41.6)	3 (60)
Pickled Kareish 3% NaCl	24 (35.8)	13 (54.2)	7 (53.8)
Pickled Kareish 10% Nacl	16 (23.9)	5 (31.3)	4 (80)
Totals	67	31(46.3)	19(61.3)

Table 6: PCR results for detection enterotoxigenic *B. cereus* isolates isolated from the examined samples

Types of Examined samples	**No. (%) of positive samples**	**No. (%) of enterotoxigenic strain**
Pickled Domiati 3% Nacl	24 (29.6)	8 (33.3)
Pickled Domiati 10% Nacl	14 (17.3)	3 (21.4)
Pickled Kareish 3% NaCl	26 (32.1)	9 (34.6)
Pickled Kareish 10% Nacl	17 (21)	5 (29.4)
Totals	81	25 (30.9)

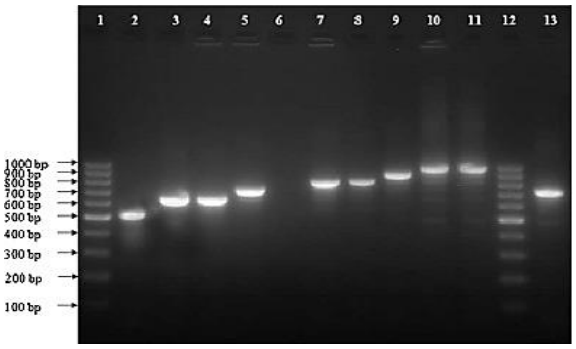

Fig. 1: PCR products of S. *aureus* coagulase genes *(coa)*.
Lanes 1 and 12, 100-bp ladder; Lane 6, *S. epidermidis* ATCC 12228 negative control. Lane 13, *S. aureus* COL positive control. Lanes 2–5, 7–11, *S. aureus* COL positive.

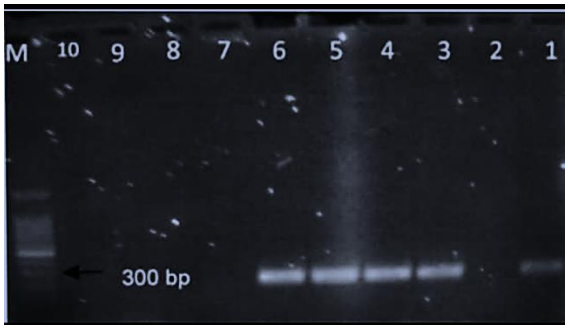

Fig. 2: PCR amplification fragments using BcAPR1 and BCFF2 primers.
Lanes 1, 3, 4, 5 and 6 are positive.
Lanes 2, 7, 8, 9 and 10 are negative.

DISCUSSION

The salt concentration (sodium chloride) % in pickled Domiati and Kareish cheese samples ranged from 2.6 to 6.8 and 3.9 to 12.9, with a mean value of 5.1 and 9.2, respectively (Table 1). All the results obtained were within the normal range of the Egyptian Standards, 2005 (not more than 7.0% and 15%) in pickled Domiati and Kareish cheese samples, respectively. Pickling or salting is one of the classical methods of preserving dairy products. These methods can extend the shelf life of food and reduce the risk of microbial contamination. Pickled products have a gradual increase in salt content during storage, due to a decrease in moisture content. Nearly similar results were reported before (Sabreen *et al.*, 1999; Ceylan *et al.*, 2003; Hassan and Afify, 2007; Nawar, 2007; El Bakry, 2012; Yasser, 2015; Saad *et al.*, 2019). On the other hand, higher results were recorded before (Mohamed, 2004; Patrick *et al.*, 2004; Hayaloglu and Kirbag, 2007; EL Zahar, 2010). But lower results were obtained by Riad, (1996) and Sadek, (2009).

The summarized results in Table (2) showed that the pH values of the examined pickled Domiati and Kareish cheese samples ranged from 2.7 and 3.4 to 5.1 and 7.9, with a mean value of 4.1 and 5.2, respectively. The pH values of the examined samples did not follow the normal range of the Egyptian Standards, 2005 (within the range of 4.9 to 4.6%) in the pickled Domiati and Kareish cheese samples, respectively. It has been proved that the pH value of these products decreased along the days of storage, which may be due to the accumulation of lactic acid produced from lactose fermentation. On the other hand, the salt contents of such pickled products increase gradually during storage (Sabreen *et al.,* 1999).

The results in Table (3) revealed that all samples were contaminated with halophilic bacteria using halophilic agar containing either 3% or 10% salt. On the other hand, the total slight and moderate halophilic count in examined pickled Domiati and Kareish cheese samples recovered on halophilic agar containing 3% salt ranged from $<10^2$ and $<10^2$ to $1.5x10^5$ and $1.03x10^4$, with a mean value of $1.8x10^4$ $\pm0.63x10^4$ and $1.1x10^3$ $\pm0.42x10^3$, respectively. Corresponding counts on halophilic agar containing 10% NaCl ranged from $1.3x10^3$ and $0.23x10^2$ to $1.45x10^6$ and $2.1x10^4$, with a mean value of $0.8x10^5$ $\pm0.62x10^5$ and $0.56x10^4$ $\pm0.81x10^3$, respectively. These results are nearly similar to (Freitas *et al.,* 1993). Higher results are obtained by (Riad 1996 , Omar *et al.,* 2007 and Saad *et al.,* 2019). All those types of halophilic bacteria, which can grow and tolerate 3-10% NaCl concentration, could reduce the risk of contamination with other types of microbes (Saad *et al.,* 2019).

The data in Table (4) revealed the frequency distribution of the isolated halophilic bacteria from the examined pickled Domiati and Kareish cheese samples on both types of media (3 and 10% salt). There were 67, 22 and 95 out of 583 isolates identified as *Staph. aureus, S. epidermidis and Micrococci*, respectively. Out of the positive 67 samples for *S. aureus*, the distribution of *Staph. aureus* was 22.4% and 17.9% in Domiati cheese and 35.8% and 23.9% in Kareish cheese, respectively. The count results were almost identically obtained by (Ewida 2009; Salama *et al.,* 2015; Meshref *et al.,* 2019), while higher results were obtained by (El-Kholy *et al.,* 1995; Kandil *et al.,* 2018), but lower results were recorded by (Moawad *et al.,* 2002; Al-Tahiri 2005; Bahout and Moustafa 2006; Aly *et al.,* 2007; Sadik 2009; Armanios 2013; Mousa 2017; Abo El Makarem and Amer 2018). There is a high prevalence of *S. aureus* in the examined Kareish cheese as it is made by farmers from raw milk, which acts as a source of *Staph. aureus* since it is not heat-treated. Improper handling during processing and distribution can act as an additional source of contamination. Street vendors put Kareish cheese in pans exposed to dust and flies (Zakary *et al.,* 2011). So, Kareish cheese is involved in food poisoning outbreaks. From a public health view, *S. aureus* has been implicated in many cases of food poisoning and gastroenteritis among consumers.

On the other hand, the distribution of 22 isolates of *Staph. epidermed epidermis* were 6 (27.3%), 4 (18.2%), 9 (40.9%) and 3 (13.6%). The distribution of 95 isolates of *Micrococci* isolates were 28 (29.47%), 17 (17.89%), 35 (36.84%) and 15 (15.78%), respectively. These results are nearly similar to those obtained by (Sabreen *et al.,* 1999). Higher results are obtained by (Sheleby, 2008 and Elshafey, 2011).

The isolates identified *as B. cereus, B. subtilis, B. coagulase, B. mycoids and B. licheniformis* were 81, 84, 59, 78 and 41. The distribution of *B. cereus* in both Domiati and Kareish cheese were 24 (29.6%), 14 (17.3%), 26 (3٢.7%) and 17 (21%), *B. subtilis* were 21 (25%), 14 (16.7%), 28 (33.3%) and 21 (25%), *B.*

coagulans were 16 (27.1%), 12 (20.3%), 19 (32.2%) and 12 (20.3%), B. mycoids were 22 (28,2%) ,10 (12.8%), 28 (35.9%) and 18 (23.1%) and *B. licheniformis* were 10 (24.4%), 8 (19.5%), 16 (39%) and 7 (17%), respectively. *Bacillus spp*., especially spore-forming *Bacillus cereus*, which widely spread in the environment, is considered a common contaminant of dairy products, such as cheese. It can contaminate milk at the time of milking and can also reach the dairy products at any stage of production, storage, and ripening (Saad *et al.,* 2019). So, the sanitation procedures applied in the production plants must be focused on two main objectives, which are the removal of the biofilm and the inactivation of spores.

Furthermore, 25 and 14 isolates out of all isolates were identified as *E. coli* and *Proteus spp*., where *E.coli* distribution was 7 (28%), 4 (16%), 9 (36%), and 5 (20%) and *Proteus spp.* distribution of were 5 (35.7%), 3 (21.4%), 4 (28.6%) and 2 (14.3%). *Escherichia coli* is commonly found in the intestinal flora of man and animals. It is a facultative anaerobic Gram-negative bacillus. It causes food poisoning and gastroenteritis and leads to severe diarrhea in infants and young children (Olsvik *et al.,* 1991). *Proteus spp*. in the examined samples is of public health importance, as they have been causing some cases of summer diarrhea in infants and some cases of urinary tract infection (Bightwell *et al.,* 2006).

The PCR for coagulase positive *S. aureus* toxins were 31 (46.3%) samples out of 67 examined samples were identified as coagulase positive and only 19 (61.3%) samples were enterotoxigenic (Table 4). The distribution in pickled Domiati cheese and pickled Kareish cheese (3% and 10% NaCl) were 5 (62.5%), 3 (60%), 7 (53.8%), and 4 (80%) respectively. Nearly similar results obtained by (Lee *et al.,* 2001; Peles *et al.,* 2007). Higher results were recorded by (Ghaleb *et al.,* 2005) (Mathenge *et al.,* 2015), but lower results were reported by (Naffa *et al.,* 2006 *and* Zouharova & Rysanek, 2008).

Data recorded in Table (5) shows 31 (46.3%) samples out of 67 examined samples were identified as coagulase positive and only 19 (61.3%) samples were enterotoxigenic

Data recorded in Table (٦) shows that 25 (30.9%) samples out of 81 examined positive samples identified as entero-toxigenic *B. cereus*. On the other hand, the distribution in pickled Domiati cheese and pickled Kareish cheese (3% and 10% NaCl) were 8 (33.3%), 3 (21.4%), 9 (34.6%) and 5 (29.4) respectively. Nearly similar results were obtained by (Sabreen *et al.,* 1999; Saad *et al.,* 2019).

Enterotoxigenic strains of *S. aureus and Bacillus cereus* were thermostable and has ability to remained in the environmental conditions, such as drying, heat and freezing (Le Loir *et al.,* 2003), causing food poisoning outbreaks due to ingestion of contaminated dairy products when contain at least 10^6 enterotoxigenic *S. aureus* CFU/g or lower in case of Enterotoxigenic spore-forming *Bacillus cereus*.

CONCLUSION

It is evident from this study that some dairy products, such as Domiati and Kareish cheese preserved by pickling in brine solution; however, they still harbor certain types of bacteria which are called halophilic or halotolerant bacteria. Some of these halophilic bacteria, such as *Staph. aureus, B. cereus, E. coli* and *proteus* are of public health hazards which cause several food poisoning outbreaks. So, milk must come from dairy farms apply HACCP system and cooling system for the milk during the production, transportation, and distribution process, particularly during the summer season. Strict hygiene during processing, storage and handling such products to destroy or even minimize the existing bacteria to negligible numbers

that represent no health hazard and finally, periodical examination of milk, and dairy products chemically and bacteriologically must be applied.

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عزل وتصنيف البكتيريا المسببة للسموم المعوية المحبة للملوحة في بعض منتجات الألبان في محافظة أسيوط، مصر

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تم جمع ١٢٠ عينة عشوائية من الجبن الدمياطي والجبن القريش بمعدل (٦٠ عينة لكل منهما) من محلات الألبان والأسواق بمحافظة أسيوط، مصر، خلال الفترة من مايو ٢٠٢٣ إلى يونيو ٢٠٢٤. وأظهرت النتائج أن تركيز ملح الطعام (كلوريد الصوديوم) يتراوح من ٢,٦ و٢,٩ إلى ٦,٨٪ و ١٢,٩ بمتوسط ٥,١ و٩,٢ بينما تراوحت قيم الأس الهيدروجيني من ٢,٧ و٣,٤ إلى ٥,١ و٧,٩ بمتوسط قيمة ٤,١ و٥,٢ على التوالي. من ناحية أخرى، تراوحت أعداد البكتيريا المحبة للملوحة في عينات الجبن المفحوصة والمستنبتة علي الأجار المحب للملوحة و يحتوي على ٣٪ ملح من $<10^2$ إلى $1,5 \times 10^5$ بمتوسط قدره $1,8 \times 10^4 \pm 0,63 \times 10^4$ في الجبن الدمياطي و$1,3 \times 10^3$ إلى $1,45 \times 10^6$ بمتوسط قدره $0,8 \times 10^5 \pm 0,62 \times 10^5$ في الجبن القريش بينما تراوحت أعداد البكتريا المستنبتة على الأجار المحب للملوحة ويحتوي على ١٠٪ كلوريد الصوديوم من$<10^2$ إلى 1.03 $\times 10^4$ بمتوسط قيمة $1.1 \times 10^3 \pm 0.42 \times 10^3$ في الجبن الدمياطي و$0,23 \times 10^2$ إلى $2,1 \times 10^4$ و بمتوسط قيمة $0,56 \times 10^4 \pm 0,81 \times 10^3$ في الجبن القريش على التوالي.

وتم تصنيف العزلات بيوكيميائيا علي أنها *Staph. aureus, Staph. epidermedis, Micrococci, B. subtilis, B. coagulase, B. mycoids and B. licheniformis, E. coli , Proteus spp.* علي التوالي.

تم تصنيف ٣١ (٤٦,٣ %) فقط من العينات من أصل ٦٧ عينة *Staph. aureus* وأظهر تفاعل البوليميراز المتسلسل أن ١٩ (٦١ ,٣ %) و٢٥ (٣٠,٩ %) عينة كانت من الميكروبات المسببة للتسمم المعوي علي التوالي. بعض هذه البكتيريا المحبة للملوحة، مثل *Staph. aureus, B. cereus, E. coli* and *proteus* ، تُشكل خطرًا على صحة مستهلكيها مسببة بعض الأمراض و حالات تسمم غذائي . لذلك يجب أن يُطبق نظام ال(HACCP) في المزارع المنتجة للألبان المستخدمة في انتاج تلك المنتجات وكذلك إجراء فحص دوري للألبان الخام وتلك المنتجات كيميائيًا وبكتريولوجيًا .