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Evaluation Of Immunological And Bacteriological Patterns Of Some Food Poisoning Micro-Organisms

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ABSTRACT

Staphylococcus aureus and *Clostridium perfringens* are important causes of food poisoning in milk and meat products due to their ability to produce one or more enterotoxins.

In this study, the bacteriological examination of 200 samples of milk and meat products (100 of each) revealed *S. aureus* in 40% and 30% of milk and meat product samples, respectively, while *C. perfringens* was detected in 18% and 23% of milk and meat product samples, respectively.

Immunological detection of Staphylococcal enterotoxins (SE: A, B, C, D) by reverse passive latex agglutination (RPLA) revealed that from 40 *S. aureus* strains isolated from milk products samples only 13 (32.5%) were toxigenic such as, enterotoxin A (22.5%), enterotoxin B (7.5%) and enterotoxin C (2.5%). Whereas, isolates didn't produce enterotoxin (D). Regarding to meat products only 17 (56.67%) out of 30 *S. aureus* isolates were toxigenic, and the identified enterotoxins were enterotoxin D (26.67%), enterotoxin A (20%) and enterotoxin B (10%). The other isolates didn't produce enterotoxin (C).

The toxicity of *C. perfringens* isolates obtained from milk and meat products were 72.2% and 65.22%, respectively. All toxigenic isolates from milk and meat products were type A measured by dermonecrotic reaction in albino guinea pigs.

As macrophages perform a variety of functions other than phagocytosis, it produces nitric oxide, also secrete many different proteins such as lysosomal enzymes. This study demonstrates that *C. perfringens* alpha toxin has a suppressive effect on phagocytic percentage and index. Also, there was significant decrease in both serum lysozyme activity and serum nitric oxide in mice intoxicated with *C. perfringens* alpha toxin.

Multiplex PCR was carried out as a confirmatory test for toxin genes for Staphylococcal enterotoxins (A, B, C, D and E). Also, conventional PCR was applied to detect the genes encoding alpha and enterotoxins of *C.perfringens* strains recovered from milk and meat products as a recent confirmatory technique for the traditional methods of identification.

INTRODUCTION

Staphylococcus aureus is considered to be one of the leading causes of food-borne illnesses. Milk, dairy products and meats are often contaminated with enterotoxigenic strains of this bacterium. Foodstuff contamination may occur directly from infected food-producing animals or may result from poor hygiene during production processes, or the retail and storage of foods, since humans may carry the microorganism *(1)*. *S. aureus* food poisoning is caused by ingestion of food containing performed enterotoxins (SEs). Symptoms have a rapid onset and may include nausea, vomiting and diarrhea *(2)*.

SET-RPLA (staphylococcal enterotoxins test - reversed-passive latex agglutination) was an immunological technique used for typing of classical enterotoxins produced by *S. aureus* (SE: A, B, C, D) *(3)*.

Clostridium perfringens are common contaminants of food and a frequent cause of food borne illness due to the production of enterotoxin *(4)*.

The species of *C. perfringens* are divided into five types from A to E on the bases of production of four major toxins namely alpha (α), beta (β), epsilon (ε) and iota (ι), each of these types has been linked to specific diseases *(5)*.

Alpha toxin is the principle lethal toxin of *C. perfringens*, and a multifunctional phospholipase that produced mainly by all types of the species *(6)*. Certain strains of *C. perfringens* type A produce an endotoxic component known as enterotoxin which served as an additional virulence factor in causing enteritis in domestic animals *(7)*.

Dermonecrotic test in albino guinea pig is a helpful method for typing *C. perfringens* isolates *(8)*.

Macrophages are a part of non specific first line of defense mechanism because of their ability to engulf and degrade invading microorganisms. Macrophages perform a variety of functions other than phagocytosis, act as secretary cells; produce nitric oxide that kill intracellular microorganisms, also secrete many different proteins such as lysosomal enzymes and cytokines that play a key role in regulating immunity *(9)*.

A multiplex PCR assay for detection of genes encoding staphylococcal enterotoxins A to E was performed *(10)*. Also, conventional PCR has been applied for detection of the genes encoding major toxins of *C. perfringens*. This method is more accurate and faster than seroneutralization with mice or guinea pigs *(11)*.

The purpose of this study was the evaluation of immunological and bacteriological patterns of some food poisoning micro-organisms, to achieve that, the following was done:

1- Collection of random milk and meat products samples.
2- Cultivation of samples on specific media.
3- Identification of recovered isolates by biochemical tests.
4- Extraction and purification of enterotoxins of *S. aureus*.
5- Immunological detection of *S. aureus* enterotoxin by Reversed Passive Latex Agglutination (RPLA).
6- Typing of *C. perfringens* using albino guinea pig as a model.
7- Serological identification of *C. perfringens* strains by toxin-antitoxin neutralization test.
8- Determination of minimum lethal dose (MLD) of *C. perfringens* alpha toxin.
9- Studying the effect of *C. perfringens* alpha toxin when injected intra peritoneal in mice on some immunological parameters involved:
   a. Peritoneal macrophages (PM).
   b. Measurement of lysosyme activity.
   c. Determination of Nitric oxide (NO).
10- Detection of gene responsible for toxin production for *S. aureus* and *C.perfringens* by polymerase chain reaction (PCR).

MATERIAL AND METHODS

1. Samples A total of 200 of different meat and milk products were included:

a. One hundred of milk products (kareish and hard cheese, 50 of each).

b. One hundred of meat products (luncheon and sausage, 50 of each).

2. Preparation of the samples

Eleven grams from each milk and meat products samples were taken and mixed with 99 ml of sterile warmed peptone water (40°C). The contents were emulsified using a stomacher *(12)*.

3. Isolation of *S. aureus*

One ml from each previously prepared homogenate was transferred onto dry surface of the following media: Mannitol Salt agar, Baird Parker agar and Blood agar medium. The inoculated plates were incubated for 24-48 hours at 37°C. Suspected colonies were picked up and examined for their morphological and cultural characters *(13)*.

4. Isolation of *C. perfringens*

Two grams of samples were inoculated into 15 to 20 ml of previously boiled and cooled cooked meat broth for enrichment. The inoculated tubes were incubated anaerobically at 35-37°C for 20 to 24 hours. Positive tubes were subcultured by breaking one loopful on the surface of 10% sheep blood agar with neomycin sulphate. Inoculated plates were incubated anaerobically at 37°C for 24 hours.

Suspected colonies were picked up and examined for their morphological and cultural characters *(14)*.

5. Identification of *Staphylococcus aureus*

The pure colonies of the isolates were identified biochemically using catalase test, coagulase test, mannitol fermentation activity, DNase activity and Gelatin Liquefaction *(15,16)*.

6. Identification of *C. perfringens*

The pure colonies of the isolates were identified biochemically using catalase test, sugar fermentation test, indole test, litmus milk medium, gelatin liquefaction test and Lecithinase activity *(17,18)*.

7.Immunological detection of Staphylococcal enterotoxin by SET RPLA kit

I. Enterotoxin production of isolated *S. aureus* by sac cultural method

Dialysis tube filled with double strength brain heart infusion broth and placed in the Erlenmeyer flask in a U shape. The growth was harvested of each strain with sterile saline in flasks containing the sacs. The flasks were incubated in electric shaker bath at 37°C for 48 hs. The culture fluid was removed from the flasks and centrifuged to obtain a clear culture supernatant fluid *(19)*.

II. Enterotoxin detection

The clear culture supernatant fluids were tested by reversed passive Latex agglutination technique using SET- RPLA for the detection of Staphylococcal enterotoxins A, B, C and D *(20)*.

8.Typing of C.perfringens toxins by dermonecrotic test in albino guinea pigs

I. Preparation of toxins and their treatment

One ml of 60% glucose was added to 50 ml of toxin production medium then inoculated 5 ml of 24hours cooked meat cultures of toxigenic strains of *C.perfringens* and incubated in water bath at 37° C for 6 hours, pH was adjusted each hour to 7.2. After 6 hours of incubation, half of the culture was syphonized and centrifuged. The clear supernatant fluid was divided into 4 portions, 3 portions was neutralized with diagnostic antiserum A,B,C in ratio (3:1) toxin antitoxin respectively and the fourth portion as control. The other half of was incubated for other 48 hours anaerobically, pH was adjusted to 7.5 twice daily then centrifuged, the supernatant was trypsinized to a final concentration of 0.1% then incubated at 37°C for an hour and neutralized by type D and E diagnostic antisera in the same ratio (3:1) *(21)*.

II. Application of dermonecrotic test

On the right side albino guinea pigs, 0.2 ml of 6 hours or trypsinized 48 hours supernatant of each culture was injected intradermally and the neutralized one was injected in the left side by the same manner. The injected guinea pigs were kept under observation for 24 hours *(22)*.

9. Toxin antitoxin neutralization test

It was performed by addition of 0.1 ml of specific antisera to 0.3 ml of the centrifuged supernatant cooked meat culture of each type (A, B, C, D and E) of *C.perfringens* (type D supernatant culture fluid was treated with 0.1% trypsin).

Injection of 0.5 ml of the previously prepared mixture into two mice intraperitoneally and kept under observation for 1-3 days. The supernatant fluid of the cooked meat culture without adding any antiserum was used as control positive while sterile saline solution was used as control negative *(23)*.

10. Experimental design of animal intoxication

1.Determination of MLD of alpha toxin: Serial dilutions of toxins were made and 0.1 ml of each dilution was injected I/V in 3 swiss mice. Mice were kept 24-72 hrs under observation. The highest dilution of toxin causing death of the injected mice was recorded, multiplied by 10 to calculate MLD/ml *(24)*.

2.Animal intoxication each mouse from toxicated group injected I/P with 0.1ml of *C. perfringens* alpha toxin at 80/ml (MLD) dilution, control group injected I/P with 0.1 ml saline. Peritoneal macrophages were collected from peritoneal cavity from each group at 3 and 6 days to assess phagocytic activity. Blood samples were taken without anticoagulant via retro orbital plexus for at 1, 3 and 6 days post injection for estimation of serum lysozyme activity and nitric oxide.

I. Peritoneal macrophages (PM)

Five ml of RPMI 1640 medium was injected I/P of each mice, then peritoneal cells were collected via needle inserted into inguinal region. After washing the collected cells 2 times, they were suspended in RPMI media with 10% FCS. Then incubated for 1 hour at 37°C in 5% CO_2 and 90% humidity *(25)*.

Assay of phagocytosis

Phagocytic activities of PM were estimated using *Candida albicans* following the method of *(26)*. After washing the monolayer of macrophage 3 times it was incubated with 1 ml *Candida albicans* (10^6/ml RPMI with 15% FCS) for 1 hour at 37°C in 5% CO_2 and 90% humidity hen it was washed three times, air dried, fixed, stained with Giemsa stain and finally, counted 100 PM cell under oil immersion lens to determine phagocytic % of PM, and phagocytic index as the mean of engulfed *Candida* spores per macrophage.

Phagocytic %=

$$\frac{\text{No. of macrophages ingested } C.\ albicans}{\text{Total No. of macrophage}}$$

Phagocytic index =

$$\frac{\text{Total No. of ingested } C.\ albicans \text{ in 100 macrophages}}{\text{100 macrophages}}$$

II. Measurement of lysozyme activity

Lysozyme diffuse through the agarose gel containing suspension of *Micrococcus lysodeikticus,* producing a clear zone ring of lysis in the initially translucent agarose gel. *(29)*.

III. Determination of Nitric oxide

One hundred µl of serum samples was transferred into flat-bottom 96 well ELISA plate and 100µl of Griess reagent were added to each well. The optical density was determined at 570nm with an ELISA plate reader. Absorbance of test samples was converted to 10 um of nitrite by comparison with absorbance values of sodium nitrite standard curve within linear curve fit *(30)*.

Detection of toxin genes by PCR

1. Extraction of DNA *(31)*.
2. Oligonucleotide primers for five toxin genes of *S. aureus* enterotoxins Table (1) *(32)* and *C. perfringens* alpha and enterotoxin genes *(33,34)* Table (2).

Table 1. Oligonucleotide primers for five *S. aureus* enterotoxins genes *(32)*

Gene	Oligonucleotide sequence (5′–3′)	Size of PCR product (bp)
sea	GGTTATCAATGTGCGGGTGG CGGCACTTTTTTCTCTTCGG	102
Seb	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164
sec	AGATGAAGTAGTTGATGTGTATGG CACACTTTTAGAATCAACCG	451
sed	CCAATAATAGGAGAAAATAAAAGG ATTGGTATTTTTTTTCGTTC	278
see	AGGTTTTTTCACAGGTCATCC CTTTTTTTTCTTCGGTCAATC	209

Table 2. Oligonucleotide primers for *C. perfringens* alpha toxin and enterotoxin genes *(33,34)*

Gene	Oligonucleotide sequence (5′–3′)	Size of PCR product (bp)
Alpha toxin	AAG ATT TGT AAG GCG CTT ATT TCC TGA AAT CCA CTC	**1167**
Enterotoxin	GGA GAT GGT TGG ATA TTA GG GGA CCA GCA GTT GTA GAT A	**233**

3. PCR amplification and cycling protocol:

Table 3. PCR cycling protocol for five enterotoxins genes of *S. aureus* using a multiplex PCR *(32)*

Amplified DNA	Initial denaturation	Actual cycles	Final extension
S. aureus enterotoxins genes (A, B, C, D, E)	94°C for 5 minutes	35cycles of : Denaturation: 94°C for 2 minutes Annealing: 57°C for 2 minutes Extension: 72°C for 1 minutes	72°C for 7 minutes

Table 4. PCR cycling protocol for alpha exotoxin and enterotoxin genes of *C. perfringens* *(33,34)*

Amplified DNA	Initial denaturation	Actual cycles	Final extension
C.perfringens alpha toxin	94°C for 5 minutes	35 cycles of : Denaturation: 94°C for 1 minute Annealing: 56°C for 1 minute Extension: 72°C for 2 minutes	72°C for 5minutes
C.perfringens enterotoxin		30cycles of : Denaturation: 94°C for 1 minute Annealing: 55°C for 2 minutes Extension: 73°C for 3 minutes	

4.Electrophoresis: PCR products were detected by agarose gel electrophoresis incorporating appropriate size ladder.

5.Specific amplicons were observed under UV light, compared with the marker and photographed by a digital camera.

Statistical analysis

Data obtained were statistically analyzed using analysis of variance and comparing between groups were performed using least significant difference (LSD) at $P < 0.05$ according to *(27)* and computerized using *(28)*.

RESULTS

It is clear from the results presented in Table (5), that *S. aureus* could be isolated from hard cheese and kareish cheese samples with percentage of 52% and 28%, respectively, and isolated from luncheon and sausage samples with percentage of 24% and 36%, respectively. Concerning to *C. perfringens* it was isolated from hard cheese and kareish cheese samples with percentage of 16% and 20%, respectively, and isolated from luncheon and sausage samples with percentage of 22% and 24%, respectively.

Table 5. Prevalence of *S. aureus* and *C. perfringens* in milk and meat products:

Type of samples	No. of examined samples	*S. aureus*		*C. perfringens*	
		No.	%	No.	%
1. Milk products	(100)	(40)	(40)	(18)	(18)
a. Hard cheese	50	26	52	8	16
b. Kareish cheese	50	14	28	10	20
2. Meat products	(100)	(30)	(30)	(23)	(23)
a. Luncheon	50	12	24	11	22
b. Sausage	50	18	36	12	24
Total	200	70	35	41	20.5

Production of enterotoxins (SEA, SEB, SEC and SED) by *S. aureus* isolates were detected by the reversed passive latex agglutination (RPLA) method. Regarding to milk products, only 8 out of 26 *S. aureus* isolates from hard cheese were toxigenic strains with perecentage of 30.8% and enterotoxins were distributed as follow: enterotoxin A was the highest among the other types of enterotoxins 5 (19.2%) followed by enterotoxin B and C whose numbers of isolates were 2 (7.7%), 1 (3.85%), all isolates didn't produce enterotoxin (D). On other hand, only 5 out of 14 *S. aureus* isolates from kariesh cheese were found to be toxigenic with an incidence of 35.7% and distribution of enterotoxin types A, B, C and D were reported in 4 (28.6%), 1 (7. 1%), zero and zero respectively Table (6).

Table 6. Prevalence of toxigenic *S. aureus* isolated from milk products using RPLA test

Type of samples	No. of *S.aureus* isolates	Toxigenic isolates		Types of toxins							
				A		B		C		D	
		No.	%	No.	%	No.	%	No.	%	No.	%
Hard cheese	26	8	30.8	5	19.2	2	7.7	1	3.85	-	-
Kariesh cheese	14	5	35.7	4	28.6	1	7. 1	-	-	-	-
Total	40	13	32.5	9	22.5	3	7.5	1	2.5	-	-

Regarding to meat products, only 7 out of 12 *S. aureus* isolates from luncheon were toxigenic strains with percentage of 58.3% and enterotoxins were distributed as follow: enterotoxin D was the highest among the other types of enterotoxins 3 (25%) followed by enterotoxins A, B both were 2 (16.7%), while enterotoxin C was zero. On other hand, only 10 out of 18 *S.aureus* isolates from sausage were found to be toxigenic with an incidence of 55.5% and distribution of enterotoxins were D was the highest 5 (27.8%) followed by enterotoxin A was 4 (22.2%), then enterotoxin B was 1 (5.6), while enterotoxin C was zero Table (7).

Table 7. Prevalence of toxigenic *S. aureus* isolated from meat products using RPLA test

Type of samples	No. of *S.aureus* isolates	Toxigenic Isolates		Types of toxins							
				A		B		C		D	
		No.	%	No.	%	No.	%	No.	%	No.	%
Luncheon	12	7	58.3	2	16.7	2	16.7	-	-	3	25
Sausage	18	10	55.5	4	22.2	1	5.6	-	-	5	27.8
Total	30	17	56.7	6	20	3	10	-	-	8	26.7

The toxicity of *C. perfringens* isolated from hard cheese and kareish cheese samples were 75% (6) and 70% (7), respectively while from luncheon and sausage samples were 63.64% (7) and 66.67% (8), respectively. Typing of toxigenic *C. perfringens* strains were applied by dermonecrotic test in albino guinea pigs. All isolated strains from milk and meat products were type A. Action of *C. perfringens* type "A" (alpha toxin) appeared as an irregular area of yellowish necrosis tended to spread downward.

Regarding to the effects of *C. perfringens* toxin on mice peritoneal macrophage there were significant decrease in phagocytic percent and index in mice intoxicated with *C. perfringens* alpha toxin at 3 and 6 days post injection compared with control. Also, there was significant decrease in both serum lysozyme activity and serum Nitric oxide in mice intoxicated with *C.perfringens* alpha toxin at 1 and 6 days post injection compared with control.

Multiplex PCR was applied to 10 isolates of *S. aureus* previously tested by RPLA and the results were confirmed by multiplex PCR by using sets of primers for enterotoxins (A,B,C,D,E). The results obtained by multiplex PCR showed that the enterotoxin A produced by 5 isolates which gave characteristic band at 102 bp, enterotoxin B produced by 3 isolates and gave characteristic band at 164 bp, enterotoxin C produced by 1 isolates only which gave characteristic band at 451 bp, enterotoxin D produced by 3 isolates gave characteristic band at 278 bp and enterotoxin E produced by 3 isolates which gave characteristic band at 209 bp (Photo 1).

Seven randomly selected *C. perfringens* isolates from milk and meat products previously typed by dermonecrotic reaction (for alpha toxin) and confirmed by conventional PCR technique, also used for detection of *C.perfringens* types "A" enterotoxin. Results of conventional PCR revealed that the 7 isolates of *C. perfringens* were positive for alpha toxin gene which gave characteristic bands at 1167 bp (Photo 2), also positive for enterotoxin gene which gave characteristic bands at 233 bp (Photo 3).

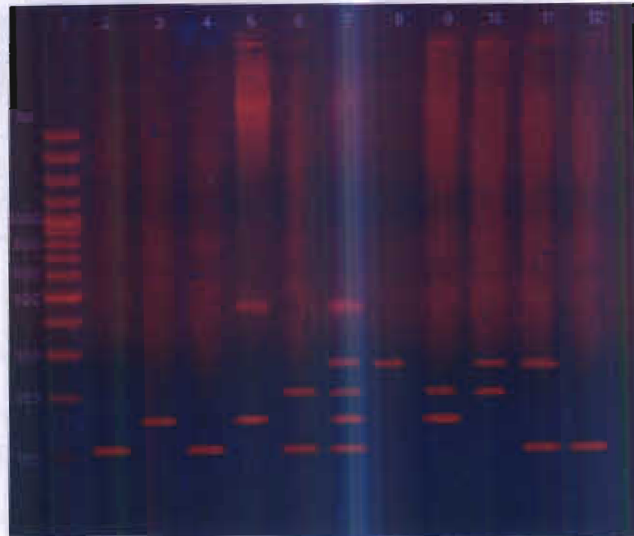

Photo 1. Agarose gel electrophoresis showing the result of multiplex PCR for detection of enterotoxin genes from *S. aureus*

Lane "1": 100 bp DNA ladder (Pharmacia).
Lane "2": positive amplification of 102 bp for enterotoxin A in milk products
Lane "3": positive amplification of 164 bp for enterotoxin B in milk products
Lane "4": positive amplification of 102 bp for enterotoxin A in milk products
Lane "5": positive amplification of 164 bp for enterotoxin B and 451 bp for enterotoxin C in milk products
Lane "6": positive amplification of 102 bp for enterotoxin A and 209 bp for enterotoxin E in milk products
Lane "7": positive control
Lane "8": positive amplification of 278 bp for enterotoxin D in meat products
Lane "9": positive amplification of 164 bp for enterotoxin B and 209 bp for enterotoxin E in meat products
Lane "10": positive amplification of 278 bp for enterotoxin D and 209 bp for enterotoxin E in meat products
Lane "11": positive amplification of 102 bp for enterotoxin A and 278 bp for enterotoxin D in meat products
Lane "12": positive amplification of 102 bp for enterotoxin A in meat products

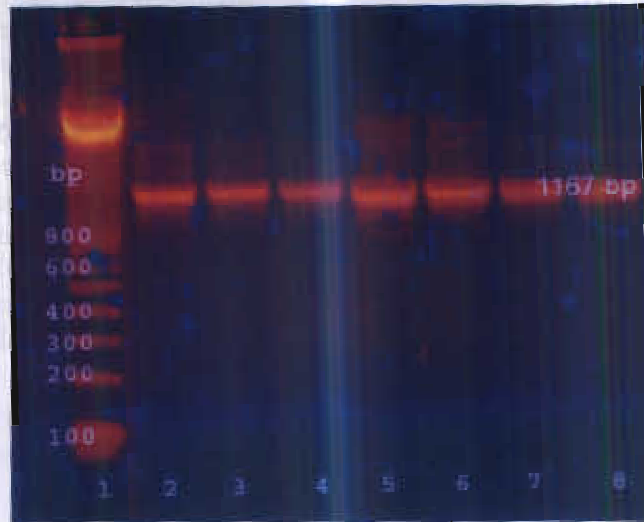

Photo 2. Agarose gel electrophoresis of *C. perfringens* type A "alpha toxin gene"
Lane "1": 100 bp DNA ladder (Pharmacia)
Lane "2" to "8": positive amplification of 1167 bp for *C.perfringens* type "A"

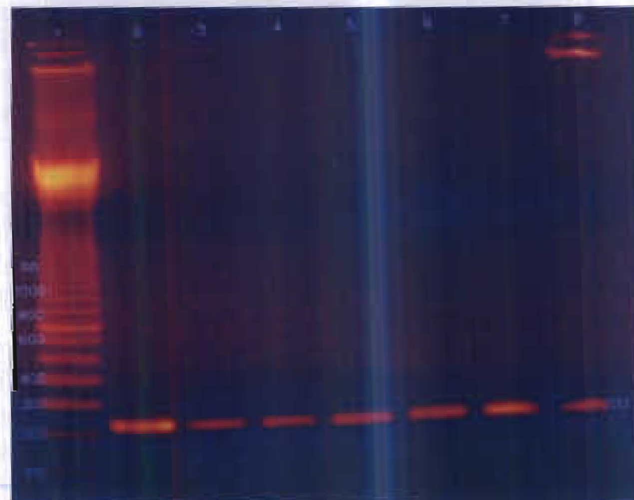

Photo 3. Agarose gel electrophoresis of *C. perfringens* type A enterotoxin genes
Lane "1": 100 bp DNA ladder (Pharmacia).
Lane "2" to "8": positive amplification of 233 bp for *C.perfringens* enterotoxin.

DISCUSSION

Staphylococcus aureus is a major cause of food borne intoxication and its presence in food constitutes an important problem for food processors, food service workers and consumers ***(35).***

In this study *S. aureus* was isolated from 52% of hard cheese samples and 28% of kareish cheese samples. Nearly similar finding reported previously ***(36).***

Concerning to meat products *S. aureus* isolated from 24% of luncheon samples and 36% of sausage. Lower incidence was achieved ***(37)***.

Clostridium perfringens are common contaminants of food and a frequent cause of food borne illness due to the production of enterotoxin ***(4)***.

In this study *C. perfringens* was isolated from 16% of hard cheese and 20% of kareish cheese samples. These results are in reasonable agreement with ***(38).***

Regarding to meat products *C.perfringens* was isolated from 22% of luncheon and 24% of sausage samples. Similar results were reported in milk and meat product ***(39).***

RPLA test was used in this study as an immunological technique used for typing of classical enterotoxins produced by *S. aureus* (SE: A, B, C, D). Regarding to milk products, only 30.8% of *S. aureus* isolates from hard cheese were toxigenic strains and enterotoxins were distributed as follow: enterotoxin A was the highest among the other types of enterotoxins (19.2%) followed by enterotoxin B (7.7%) and enterotoxin C (3.85%). All isolates didn't produce enterotoxin D. On other hand, only 35.7% of *S. aureus* isolates from kariesh cheese were found to be toxigenic, enterotoxin A (28.6%), enterotoxin B (7.1%). All didn't produce enterotoxin C and D. These results are in accordance with previous study ***(40)***.

Regarding to meat products, only 58.3% of *S. aureus* isolates from luncheon were toxigenic strains and enterotoxins were distributed as: enterotoxin D was the highest among other types of enterotoxins (25%) followed by enterotoxins A, B both were (16.7%), while enterotoxin C was zero. On other hand, only 55.5% of *S.aureus* isolates from sausage were toxigenic and distribution of enterotoxins were: D as the highest among other types of enterotoxins (27.8%) followed by enterotoxin A (22.2%), then enterotoxin B (5.6), while enterotoxin C was zero. These results nearly similar to that carried out in Korea ***(37).***

The toxicity of *C. perfringens* isolated from hard cheese and kareish cheese were 75% and 70%, respectively while from luncheon and sausage were 63.64% and 66.67%, respectively. Typing of toxigenic *C.perfringens* strains were applied by dermonecrotic test in albino guinea pigs. All isolated strains from milk and meat products were type A ***(41).***

Toxin antitoxin neutralization test on the skin of albino guinae pigs revealed that all twenty eight toxigenic *C. perfringens* isolates from milk and meat products were type A. These results are in accordance with reported in claves ***(42).***

The highest dilution of toxin causing death of the injected mice and multiplied by 10 to calculate the MLD/ml, revealed that MLD of type A was 80/ml ***(43)***.

Mice injected with toxin exhibited significant decrease in phagocytic percentage and index. These results agree with those previously recorded ***(44)***.

Significant decrease in serum lysozyme activity in mice intoxicated with *C.perfringens* alpha toxin was detected. This decrease in serum lysozyme could be attributed to the suppressive effect of *C. perfringens* toxin on phagocytic activity of macrophage; macrophage play an important role in production of lysozyme, subsequently the adverse effect of toxin reflected on lysozyme production. In contrast to our results that obtained in diary buffaloe and cow in Egypt ***(45).*** The differences in our results of lysozyme and previous studies may be due to

extraneous factors related to species, type of samples and examination time.

Also, there was significant decrease in serum Nitric oxide production in mice intoxicated with *C. perfringens* alpha toxin at 1 and 6 days post injection compared with control, these results are in agreement with the results of previous study ***(46)***.

Investigation of *S. aureus* isolates for toxin genes using multiplex PCR revealed the presence of toxin genes encoding for SEA, SEB, SED and SEE. These results have been described by several authors ***(13, 47)***.

A correlation was found between PCR results and the detection of SEs when using commercial reverse passive latex agglutination assay ***(13,40)***.

The discrepancies between RPLA and PCR may have been due to the fact that RPLA kit detects the enterotoxin itself, whereas the PCR method detects the toxin genes. Under certain circumstances, SEs may not be produced or produced to a certain limit below the detection level of RPLA kit ***(47)***.

Conventional PCR was applied to detect the genes encoding alpha and enterotoxins of *C. perfringens* strains recovered from milk and meat products as a recent confirmatory technique for the traditional methods of identification. The results revealed that *C. perfringens* type A contained alpha toxin gene which gave a characteristic band at 1167bp when visualized under UV light. Similar results were recorded ***(48)***.

PCR amplification of *C.perfringens* type A enterotoxin gave a characteristic band at 233 bp. These results are in line with previous study ***(48)***.

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الملخص العربى

تقييم الخصائص المناعية والبكتريولوجية لبعض مسببات التسمم الغذائى

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تتعرض منتجات الألبان واللحوم للتلوث بالكثير من الميكروبات وخاصة بالمكور الذهبى العنقودى و الكوليستريديم بيرفرنجينز، يحدث ذلك خلال الأنتاج والتصنيع والمعالجة، حيث أن الألبان واللحوم ومنتجاتهم وسط جيد للنمو والتكاثر وإفراز السموم أيضاً، وبذلك تصبح مصدر للتسمم الغذائى للأنسان.

فى هذه الدراسة تم فحص عدد ٢٠٠ عينة من منتجات الألبان واللحوم (عدد ١٠٠ من كل نوع) وكانت نسبة عزل المكور الذهبى العنقودى٤٠% و٣٠% من منتجات الألبان واللحوم على التولى؛ بينما كانت نسبة عزل الكوليستريديم بيرفرنجينز١٨% و٢٣% من منتجات الألبان واللحوم على التولى.

تم أستخدام الربلا(RPLA) للكشف عن أنواع السموم المعوية التى تفرزها عترات المكور الذهبى العنقودى من السموم A , B, C وD. وجد من معزولات المكور الذهبى العنقودى من منتجات الألبان أن نوع السم (A) هو الأكثر انتشاراً بنسبة (٢٢,٥%) يليه السم (B) بنسبه (٧,٥%) ثم السم (C) بنسبة (٢,٥%). أما السم (D) لم يفرز بواسطة العترات.

أما فى حالة العترات المعزوله من منتجات اللحوم فقد وجد أن (D) هو الأكثر شيوعاً وكان بنسبة (٢٦,٦٧%) يليه السم (A) بنسبه (20%) ثم السم (B) بنسبة(١٠%). أما السم (C) لم يفرز بواسطة العترات.

تم تصنيف العترات السامة بإستخدام تفاعلات تنكرز الجلد و تفاعلات معادلة السموم بمضادات السموم (بالحقن داخل الجلد فى خنازير غينيا الألبينو) وقد وجد أن كلهم من النوع A.

تم تحديد أقل جرعه مميته وحقنها فى فئران التجارب، ثم قياس النشاط التبلعمى للخلايا البريتونيه متعددة النواه و كذلك كلاً من تركيز الليزوزيم وأوكسيد النيتريك فى السيرم. وقد وجدأن هناك أنخفاض ملحوظ فى كلاً منهم فى اليوم الثالث و السادس بالمقارنه بالمجموعة المراقبه.

تم أستخدام تفاعل البلمرة المتسلسل المتعدد للكشف عن الجينات المسئولة عن إفراز السموم المعويه A C , B,, D و E لعشـــرة عترات مصنفه مسبقاً بإستخدام الربلا. واكدت النتائج وجود السم نوع (A) فى ٥ عترات (٣ فى منتجات الألبان، ٢ فى منتجات الحوم) والذى أعطى منطقة مميزة عند الوزن الجزيئى ١٠٢ قاعدة مزدوجة، وجود السم نوع (B) فى عترتين (فى كلاً من منتجات الألبان واللحوم) واعطى منطقة مميزة عند الوزن الجزيئى ١٦٤ قاعدة مزدوجة، أيضا وجود السم نوع (C) فى عترتين (من منتجات الألبان فقط) والذى أعطى منطقة مميزة عند الوزن الجزيئى٤٥١ قاعدة مزدوجة، كذلك وجود السم نوع (D) فى ٣عترات (فى منتجات اللحوم فقط) واعطى منطقة مميزة عند الوزن الجزيئى ٢٧٨ قاعدة مزدوجة وأخيراً وجود السم نوع (E)فى ٥عترات (٢ من منتجاتالألبان و٣ من منتجات اللحوم) واعطى منطقة مميزة عند الوزن الجزيئى ٢٠٩ قاعدة مزدوجة.وبهذا فقد اثبتت هذه الدراسة الحساسية العاليه لأختبار تفاعل إنزيم البلمرة المتسلسل مقارنة بالربلا. ربما يرجع ذلك إما لوجود الجين مع عدم إفراز السموم لظروف بيئيه معينه، أو لوجود السموم المعويه لكن بنسبه أقل من مستوى حساسية الربلا فلا تستطيع إكتشافها.

تم اجراء تفاعل البلمرة المتسلسل التقليدى على ٧ عترات كوليستريديم بيرفرنجينز مصنفة مسبقاً واكدت النتائج وجود سم الالفا والسم المعوى بهم حيث أعطوا منطقة مميزة عند الوزن الجزيئى ١١٦٧ و٢٣٣ قاعدة مزدوجة، على التوالى.