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BACTERIOLOGICAL AND MOLECULAR CHARACTERIZATION OF SOME PATHOGENS FROM FAST FOODS

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ABSTRACT

This study was conducted to evaluate the bacteriological status of some fast food and the public health significance of some isolated pathogenic bacteria from their bacteriological and molecular point of view. Sixty random samples of chicken nuggets, beef shawerma and sausage sandwiches (20 samples for each) were collected from different fast food restaurants in Dakahlia Governorate and examined for their bacteriological status. The obtaind results indicated that the mean values of *APC* count, *coliform* and *Staph. aureus* counts of chicken nuggets, beef shawerma and sausage were $9.1x10^3 \pm 1.2x10^3$ $1.3x10^4 \pm 3.5x10^3$ and $1.2x10^4 \pm 2.2x10^3$; < 10, $1.1x10^3 \pm 1.2x10^2$ and $8.5x10^3 \pm 1.1x10^3$, $5.6x10^3 \pm 1.2x10^3$, $8.7x10^3 \pm 1.2x10^3$ and $6.3x10^3 \pm 3.1x10^3$ respectively. The bacteriological examination of these products showed the presence of one *Salmonella* isolate (*S. Typhimurium*) with a percentage of (5%) and two *E. coli* isolates with a percentage of (10%) in 20 meat shawerma samples, meanwhile nuggets and sausage were negative for both Salmonella and *E.coli* isolation. Eight coagulase positive *Staphylococcus aureus* were isolated with a percentage of (10%, 15% and 15%) from chicken nuggets, beef shawerma and sausage, respectively. PCR was applied to evaluate the presence of some virulence genes in the isolated *Salmonella*, *E. coli* and *Staphylococcus aureus*. The isolated *S. Typhimurium* harbored *inv*A and *stn* genes. The isolated *E.coli* showed absence of shiga toxin genes (*stx*1 and *stx*2). The examined coagulase positive *Staphylococcus aureus* showed the presence of different enterotoxin genes (*sea*, *seb*, *sec*, *sed* and *see*). The puplic health significance and the possible sourcs of contamination of isolated organisms as well as some recommendations to improve the quality were discussed.

Key words: *Fast Foods, Pathogens, Virulence genes.*

INTRODUCTION

Nuggets, Shawarma and Sausage are the most ready to eat sandwiches sold in fast food restaurants.

There is an increase in the consumption of ready-to-eat fast food because of a changes in social patterns characterized by increased mobility, large numbers of itinerary workers and less family centered activities. Thus, good manufacturing practices of foods taken outside the home such as good sanitation or sanitary measure and proper food handling have been transferred from individuals/families to the food vendor who rarely enforces such practice (Musa and Okande, 2002).

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Sandwiches are manipulated extensively during processing and there for have a potentiality for high bacterial contamination level on the surface and depth of meat so there is an increased risk of pathogens surviving and transferring not only by cross contamination but also through undercooking as in fast food industry (Nimri- Laila *et al*., 2014).

Microorganisms in fast foods are responsible for many human diseases. e.g Salmonella bacteria which considered a common cause of food borne illness, particularly in undercooked chicken and chicken eggs (Woodward, 1996; Kaneko *et al*., 1999; Uyttendaele *et al*., 1999 and Angelillo *et al*., 2000).

On the other hand *Escherichia coli* is common, harmless bacteria of the human intestinal flora. However, five groups of E. coli-causing diarrhea in humans and other warm-blooded animals have been identified (Brook *et al.*, 1994; Wasteson, 2001).

Staph.aureus is the most prevalent contagious pathogens, which rapidly and easily transmitted, as

well as it causes a zoonotic disease which transmitted to human being (Forbes and Weissfield, 2002).

In recent years fast food restaurants have added salads fresh vegetables e.g Onion (Some sauas such as Ketchup and Mayonise). Some foods will be cooked before consumption others will be eaten raw. Contamination of fast foods during processing, and changes in microbial growth patterns during storage, may affect the microflora of these foods quantitatively and qualitatively. The inner tissues of healthy animals are free of microorganisms. However, the surfaces of raw vegetables and meats are contaminated with a variety of microorganisms and this depends on the condition of the raw product, the method of handling, the time and conditions of storage (Wood-Ward, 1996; Odumeru *et al.*, 1997 and Pelczar *et al.*, 2006).

The prevalence of *Staphylococcus spp*., *Escherichia* coli, Salmonella spp., on meat, sea foods, vegetable ingredients, chicken shawarmas, raw and cooked foods, raw chicken, beef burger sandwiches, ready-to eat salad vegetables, commercial mayonnaise, frozen chicken, poultry products, it depends on the contamination level of food workers hands (Kaneko *et al*., 1999 and Pelczar *et al*., 2006).

The purpose of this study is to determine the bacteriologicale status of nuggets, shawarma and Sausage sandwiches, public health significance of isolated pathogenes and their impact on consumer health.

MATERIALS AND METHODS

1- Collection of samples:
A total of 60 random chicken nuggets, beef shawarma and susage sandwiches (20 of each) were collected from different fast food restaurants in Dakahlia governorate. The collected samples were directly transferred to the laboratory to carry out the following bacteriological examination.

2- Bacteriological examination:
2.1- Preparation of food homogenate: according to technique recommended by (ISO, 6887-2, 2003).

2.2- Total Bacteriological count: Total aerobic plate count: (APHA, 2001) spreading technique using standard plate count agar, incubated at 35^{o}C for 48 hr.

2.3- Total *coliform* count: (APHA, 2001) spreading technique using violet red bile (VRB) agar, incubated at 37^{o} C for 24 hr.

2.4- *Staphylococcus aureus* count: (FDA, 2002) using Baird-Parker agar plates, incubated at 35 ^{o}C for 48 hr. The suspected *Staph. aureus* colonies were isolated , purified and confirmed by coagulase test.

2.5- Isolation of *Salmonellae* (ISO, 6579, 2002): by enrichment in Tetrathionate (37 ^{o}C for 24hr) and rappaport vasiliades at 41.5 ^{0}C for 18 hr., platting on XLD, MaCconkey's and Hektone entreic agar at 37^{o}C for 24 hr. The presumptive colonies were confirmed biochemically and serologically.

2.6- Isolation of *E. coli* according to technique recommended by (ISO, 16649/2, 2001).

3- Detection of virulence genes in Salmonella, *E. coli* and *Staphylococcus aureus* using PCR.

3.1- DNA extraction:
DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

3.2- Oligonucleotide Primer:
Primers used were supplied from Metabion (Germany) are listed in table (1) and table (2).

3.3- PCR amplification:
For uniplex PCR, primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. For stx1, stx2 duplex PCR, primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 13 µl of water, and 8 µl of DNA template. The reaction was performed in an Applied biosystem 2720.

3.4- Analysis of the PCR Products:
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products and 30 µl of the duplex PCR products were loaded in each gel slot. Generuler 100 bp ladder (Fermentas, Thermo Scientific, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1: Primers sequences, target genes, amplicon sizes and cycling conditions.

target	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	94˚C 5 min.	94˚C 30 sec.	59˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Murugkar *et al.*, 2003**
invA	GTGAAATTATCGC CACGTTCGGGCAA TCATCGCACCGTCA AAGGAACC	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	72˚C 7 min	**Oliveira *et al.*, 2003**
Stx1	ACACTGGATGATC TCAGTGG CTGAATCCCCCTCC ATTATG	614	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Dipineto *et al.*, 2006**
Stx2	CCATGACAACGGA CAGCAGTT CCTGTCAACTGAG CAGCACTTTG	779						

Table 2: primer sequence for *Staph. aureus* enterotoxins genes used in multiplex PCR (Mehrotra *et al.*, 2000).

Primer pairs	Nucleotide sequence (5′→3′)	Amplicon size (bp)
sea Forward Reverse	5` GGTTATCAATGTGCGGGTGG 3` 5` CGGCACTTTTTTCTCTTCGG 3`	102 bp
seb Forward Reverse	5` GTATGGTGGTGTAACTGAGC 3` 5` CCAAATAGTGACGAGTTAGG 3`	164 bp
sec Forward Reverse	5`AGATGAAGTAGTTGATGTGTATGG 3` 5` CACACTTTTAGAATCAACCG 3`	451 bp
sed Forward Reverse	5` CCAATAATAGGAGAAAATAAAAGG 3` 5` ATTGGTATTTTTTTTCGTTC 3`	278 bp
see Forward Reverse	5`AGGTTTTTTCACAGGTCATCC 3` 5`CTTTTTTTTCTTCGGTCAATC 3`	209bp

Statistical analysis:
The results are expressed as mean ± standard Error (SE). Data were statistically analyzed using statistical analysis systems.

RESULTS

Table (1) revealed that, the total aerobic plate count (APC) for chicken nuggets ranged from $1.7x\ 10^3$ to $2x10^4$ with mean value of $9.1x10^3 \pm 1.2x10^3$, while in beef shawarma $1.2x10^3$ to $6.5x\ 10^4$ with mean value of $1.3x10^4 \pm 3.5x10^3$. APCs were ranged from $1.3x10^3$ to $3x10^4$ with mean value of $1.2x10^4 \pm 2.2x10^3$ for Sausage sample Table (2) revealed that chicken nuggets samples were free from coliform. While the coliform counts ranged from $7.0\ x10^2$ to $1.5\ x10^3$ with mean value $1.1x10^3 \pm 1.2\ x10^2$in beef shawarma and from $5.0\ x10^3$ to $1.2x10^4$ with mean value $8.5x10^3 \pm 1.1x10^3$in Sausage. From Table (3) *Staph. aureus* counts ranged from $7.0\ x10^2$ to $65\ x10^3$ with mean value of $6.3\ x10^3 \pm 3.1\ x10^3$ in chicken Nuggets ,while in beef shawarma ranged from $3.0\ x10^2$ to $19x10^3$ with mean value of $5.6x10^3 \pm 1.2x10^3$, while *Staph. aureus* counts were ranged from $1.0\ x10^3$ to $2.0\ x10^4$ with mean value $8.7x10^3 \pm 1.2\ x10^3$ in examined Sausage samples. Table (4) represent the prevalence of *Salmonella, E. coli* and *Staph. aureus* microorganisms. *Staph. aureus* isolated with 10% from chicken Nuggets sandwiches While in shawarma sandwiches as they constituted 5 % ,10% and 15 % for *salmonella, E. coli and Staph. aureus* respectively. In addition *Staph. aureus* could be isolated with 15% of examined sausage sandwiches . Moreover nuggets and sausage sandwiches were negative for *Salmonella* and *E. coli* isolation. The isolated strain of *Salmonella* from beef shawarma sandwiches was serologically identified as *S.Typhimurium*.

Table 1: Mean values of APC cfu/g count in examined fast food products.
(N=20 of each)

Type of fast food.	Min.	Max.	Mean ±SE
Nuggets	$1.7x\ 10^3$	$2.0x10^4$	$9.1x10^3 \pm 1.2x10^3$A
Shawerma	$1.2x10^3$	$6.5x\ 10^4$	$1.3x10^4 \pm 3.5x10^3$a
Sausage	$1.3x10^3$	$3.0x10^4$	$1.2x10^4 \pm 2.2x10^3$a

A & a There were significant differences between the small and capital litter (P<0.05).

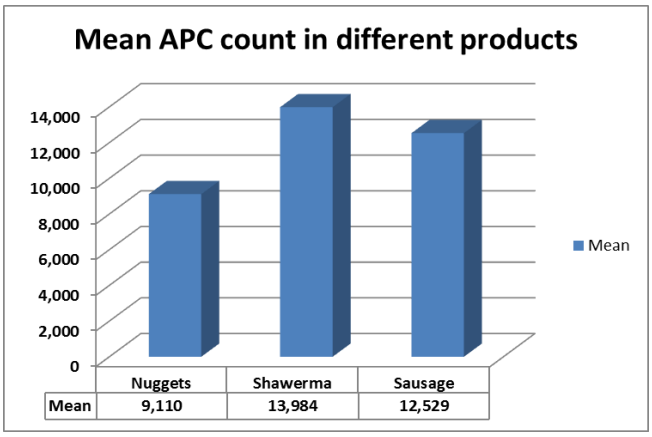

Fig. 1: Mean values of APC count in products

Table 2: Mean values of coliform count cfu/g in examined fast food products.
(N=20 of each)

Type of fast food.	Min.	Max.	Mean ±SE
Nuggets	**< 10**	**< 10**	**< 10A**
Shawerma	**$7.0x10^2$**	**$1.5x10^3$**	**$1.1x10^3 \pm 1.2x10^2$aB**
Sausage	**$5.0x10^3$**	**$1.2x10^4$**	**$8.5x10^3 \pm 1.1x10^3$ab**

There were a significance differences between capital and small letters **(P<0.05)** within the same column

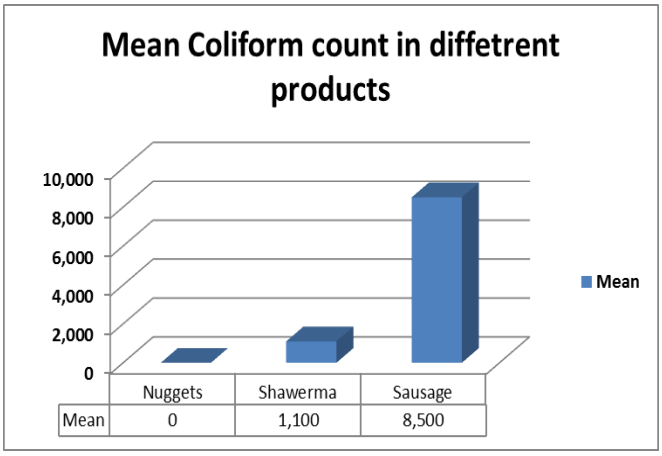

Figure 2: Mean values of coliform count in products.

Table 3: Mean values of *Staph. aureus* count cfu/g in examined fast food products. (N=20 of each).

Typeof fast food.	Min.	Max.	Mean ±SE
Nuggets	**$7.0x10^2$**	**$65x10^3$**	**$6.3x10^3 \pm 3.1x10^3$**
Shawerma	**$3.0x10^2$**	**$19x10^3$**	**$5.6x10^3 \pm 1.2x10^3$**
Saus age	**$1.0x10^3$**	**$2.0x10^4$**	**$8.7x10^3 \pm 1.2x10^3$**

There no significance difference between three examined product **(P>0.05)** regarding to *Staph. aureus* count

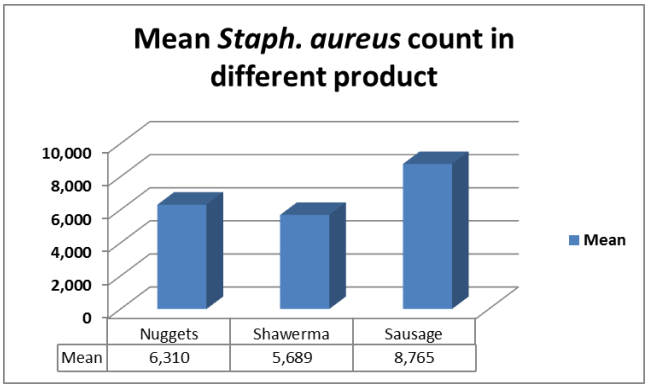

Figure 3: Mean values of *Staph. aureus* count in products.

Table 4: Incidence of *Salmonella*, *E. coli* and *Staph. Aureus* isolated from examined Nuggets, shawarma and Sausage sandwiches samples (N= 20 of each).

Type of Fast food	Isolated organisms					
	Salmonella		*E. coli*		*Staph. aureus*	
	No	%	No	%	No	%
Nuggets	-	0.0	-	0.0	2	10
Shawerma	1	5	2	10	3	15
Sausage	-	0.0	-	0.0	3	15

DISCUSSION

The present study demonstrated that fast food sandwiches samples were contaminated with *Salmonella*, *E. coli* and *Staphylococcus aureus* which indicates a potential breakdown of hygiene at various stages of the food processing and distribution chain.

The result reported in table (1) revealed that, mean value of aerobic plate count (APC) for chicken Nuggets of $9.1x10^3 \pm 1.2x10^3$, while in beef shawarma $1.3x10^4 \pm 3.5x10^3$. And $1.2x10^4 \pm 2.2x10^3$ for Sausage sample there were significant difference between chicken Nuggets and beef shawarma, Sausage sample ($P<0.005$) with regarding to *APCs*. The *APCs*. were nearly similar to that recorded by Amany *et al.* (2015) which found that the total aerobic plate count ranged from 6×10^3 to 3.4×10^5 with mean value of $4.8 \times 10^4 \pm 3.6 \times 10^3$ in beef shawarma and lower than that recorded by Nimri *et al.* (2014) and Odu and Akano, (2012) they found that *APCs* for shawarma samples were in the range of $2.0x10^3$ to $1.8x10^6$cfu/g.

Despite the use of heat in the preparation of fast food, some pathogenic organisms still present during samples anaylsis. This may be due to the fact that some of the enumerated microorganismes can survive high in cooking temperature to which Shawarma products were exposed which is not sufficient to eliminate harmful microorganisms Abdelhai *et al.* (2015).

Table (2) Fig No (2) revealed that Nuggets samples were free from coliform. While the coliform mean value $1.1x10^3 \pm 1.2 \times 10^2$in beef shawarma and $8.5x10^3 \pm 1.1x10^3$in Sausage. There were a highly significant difference between chicken Nuggets and beef shawarma, Sausage sample ($P<0.001$) While significant difference between beef shawarma and Sausage sample ($P<0.005$). These results were lower to that recorded by Odu and Akano, (2012) who found that the total coliform count ranged from $1.9x10^3$ to $9.4x10^5$, for shawarama and higher than recorded by Eman and Sherifa, (2012) who found that coliform count was 3.9×10^2 for shawarama. *El-kewaiey*, (2012) who found that mean values of APC, total coliforms and total Staphylococcus aureus in chicken nuggets were: $8.2X10^4 \pm 1.2X10^4$, $2.4X10^2 \pm 8.0X10$ and $6.0X10^3 \pm 1.5X10^3$ cfu/g, respectively.

Staphaureus is Gram positive cocci resistant to heat and drying. They produce heat stable enterotoxins that render the food dangerous (Prescott *et al.*, 2005). From table (3) Fig No (3) Mean values of *Staph. Aureus were* $6.3 \times 10^3 \pm 3.1 \times 10^3$ in chicken Nuggets, $5.6x10^3 \pm 1.2x10^3$ in beef shawarma, while $8.7x10^3 \pm 1.2 \times 10^3$ in examined Sausage samples. (EL-Mossalami *et al.*, 2009) were 92%, 80% and 88% with mean values of $3.25 \pm 6x10^3$, $2.8 \pm 1.4x10^2$ and $4.1 \pm 2x10^3$cfu/g. respectively in sausage, beefburger and shawerma and less than (Armany *et al.*, 2016) were 24% in raw sausage.

Table (4) represent the prevalence of *Salmonella, E. coli* and *Staph. aureus* microorganisms. *Staph. aureus* isolated with 10% from chicken Nuggets sandwiches While in shawarma sandwiches as they constituted 5 %, 10% and 15 % for *salmonella, E. coli and Staph. aureus* respectively. In addition *Staph. aureus* could be isolated with 15% of examined sausage sandwiches. Moreover nuggets and sausage sandwiches were negative for *Salmonella* and *E. coli*

isolation. The isolated strain of *Salmonella* from beef shawarma sandwiches was serologically identified as *S.Typhimurium*. These results were higher than that recorded by (Abdel-Rahman *et al.,* 2011 and Abdalhamid *et al*., 2013) as they couldn't detect *Salmonella* in examined sampels *on the other hands* lower result were obtained by *Shahram et al.* (2012) who found that *Escherichia coli* (40.3%) was the most prevalent food-borne pathogen isolate followed by Staphylococcus aureus (4.5%) from 134 fast food sandwiches.

PCR was applied to evaluate the presence of virulence gens in isolated *Salmonella, E. coli* and *Staphylococcus aureus*. From Photo No. (1) for isolated *Salmonella. Typhimurium were positive inv*A and *stn* virulence genes. *inv*A gene was amplified and detected at 284 bp while *stn* gene could be detected and amplified at 617 bp.

In Korea, Li *et al*. (2006) could detect 17 virulence genes from isolated *Salmonella* using PCR assays, 14 genes assayed (82.4%) out of these 17 genes included *invA* gene.

Photo No. (1) showed that neither *stx*1 nor *stx*2 could be detected in examind two *E. coli* isolates. In contrary Balague *et al*. (2006) who collected 500 food samples from shops selling ready to eat foods in Argentina and *E. coli* virulence gens were examined by multiplex PCR (*stx*1, *stx*2, *eae* A, *cnf*1, *cnf*2, *ein* v, *Lt1*, *ST1* and *ST11*), ten *E. coli* isolates showed the presence of *stx*1, *stx*2 genes while other genes were negative. Moreover, Bohaychuck *et al.* (2006) reported shiga toxin producing *E. coli* O22: H8 from beef samples in Alberta, Canada.

Staph. aureus is one the leading causes to food poisoning, its pathogenicity resulted from possession of virulence genes that able to produce different toxins which resulted in self-limiting sever illness. For this reason, the virulence genes of 8 isolated coagulase positive *Staph. aureus* were examined by PCR and the results showed the presence of enterotoxin producing genes (A,C,D and E) in *Staph. aureus* isolated strains from nuggets sandwiches, while the three *isolates of Staph. aureus* isolated from shawarma showed the presence of enterotoxin gene (A) and only one isolates of them showed presence of enterotoxin gene (D). The three *isolates of Staph. aureus* isolated from sausage the 1st isolate showed presence of enterotoxin genes (B and D), the 2nd isolate showed the presence of enterotoxin gene (D) while 3rd *Staph. aureus* isolate showed presence of enterotoxin genes (B) (Photo No. 2).

Staph. aureus enterotoxin were analyzed from ready to eat products including pork ham, chicken cold cuts, pork sausage, salami and pork luncheon meat in a study conducted by Fijalkowski *et al*. (2016), they found that that the most prevalent enterotoxin genes were sei (36%), seln (32%) and eta encoding exfoliative toxin A (37%). Another study conducted by Puah *et al*. (2016) revealed an incidence of (96.2%) virulence genes from *Staph. aureus* isolated from 200 food samples. A total of 30.8% of the isolates carried *SE* gene which cause food poisoning. Meanwhile, the most common enterotoxin genes found were *seg* (11.5%) and *egc* (5.8%).

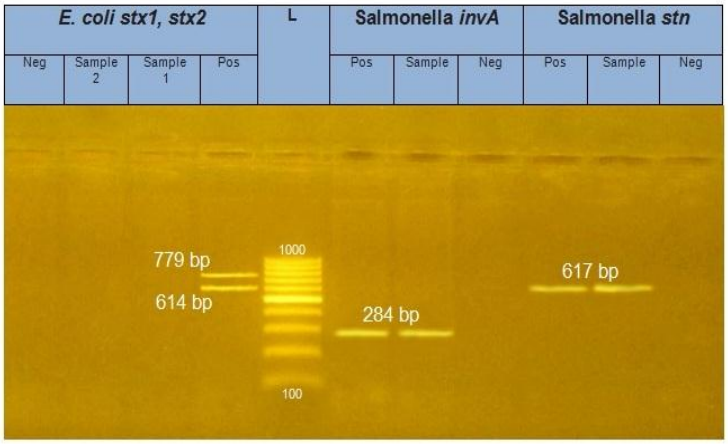

Photo No. (1): Agarose gel electrophoresis of *Salmonella* and *E. coli* PCR products using *inv*A, *stn, stx1 and stx2* primers.
L= 100 bp DNA ladder.
Neg= negative control.
Pos= positive control (give amplification at 617 pb for *stn* gene, 284 bp for *inv*A, 614 bp for *stx1* gene and 779 bp for *stx2*.
isolate (1) and isolate (2) of E. coli isolates were negative.
***Salmonella Typhimurium* isolate showed 284 bp amplification for *inv*A gene and 617 pb for *stn* gene.**

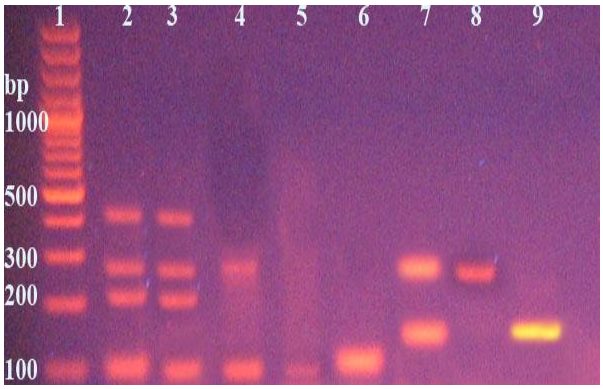

Photo No. (2): Agarose gel electrophoresis of *S. aureus* PCR products using enterotoxins Staphylococcus primer.
Lane "1": 100 bp DNA ladder
Lane "2 ": positive amplification of 102 bp for enterotoxin A, 209 bp for enterotoxin E, 278 bp for enterotoxin D and 451 bp for enterotoxin C
Lane "3": positive amplification of 102 bp for enterotoxin A, 209 bp for enterotoxin E, 278 bp for enterotoxin D and 451 bp for enterotoxin C
Lane "4": positive amplification of 102 bp for enterotoxin A and 278 bp for enterotoxin D
Lane "5": positive amplification of 102 bp for enterotoxin A
Lane "6": positive amplification of 102 bp for enterotoxin A
Lane "7": posistive amplification of 164 bp for enterotoxin B and 278 bp for enterotoxin D
Lane "8": positive amplification of 278 bp for enterotoxin D
Lane "9": positive amplification of 164 bp for enterotoxin B

CONCLUSIONS

This study confirms that fast food sandwiches may serve as a source of foodborne pathogens and a potential public health hazard. Corrective action needs to be employed to minimize the risk of consuming this type of fast food and we are in need for regular surveillance by the public health regulatory bodies with WHO and ISO standards for food safety. More attention should be given to the cleanliness of utensils used in preparing the sandwiches. In addition to the personal hygiene of the workers preparing and stuffing the sandwiches.

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التوصيف البكتريولوجى والجزيئى لبعض انواع الميكروبات المعزولة من الوجبات السريعه

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تم إجراء هذه الدراسة لعمل تقييم بكتيريولوجى وجزيئى على بعض أنواع البكتيريا الممرضه المعزولة من الوجبات السريعة. حيث تم فحص ٦٠ عينة من ناجتس الدواجن وشاورما اللحم والسجق بواقع ٢٠ عينه لكل منهما. حيث تم عمل عد بكتيري لكلا من الميكروبات الهوائية والميكروبات القولونية والميكروب العنقودي الذهبي. واظهرت النتائج انه تم عزل عترة واحدة من السالمونيلا من شاورما اللحم وبتصنيف هذه العترة المعزولة كانت سالمونيلا تيفيميوريم بنسبة ٥% وايضا تم عزل معزولتان من الميكروب القولوني من شاورما اللحم بنسبة ١٠%. وتبين من هذه الراسة ان عينات الناجتس والسجق كانت خالية من السالمونيلا والميكروب القولوني عند الفحص البكتيريولوجي. تم عزل ٨ معزولات من الميكروب العنقودى الذهبي (الموجبة لتجلط البلازما) بنسبة ١٠% و ١٥% و ١٥% من الناجتس وشاورما اللحم والسجق على التوالي. بالاضافه الى ذالك اجراء اختبار تفاعل البلمرة المتسلسل لتحديد وجود جينات الضراوة في الميكروبات المعزولة والتي من دورها تؤثر على قدرة الميكروب على احداث حالات مرضية عند تناول الاطعمة الملوثة بهذه الميكروبات. عند فحص السالمونيلا تيفيميوريم المعزولة اظهرت وجود جيني stn و invA. بينما معزولات الميكروب القولوني لم يتواجد بهم جيني stx1و stx2 وعند فحص المعزولات الخاصة لميكروب المكور العنقود الذهبى كانت النتائج الخاصة بالناجتس وجود جينات *sea* و *sed* و*sec* و*see* بينما المعزولات الخاصة بالشاورما وجد فيها جينى *sea* و *sed* فقط والمعزولات الخاصة بالسجق وجد فيها جيني *seb* و *sed* فقط. وتم مناقشة الأهمية الصحية للميكروبات المعزولة .