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Assessment of some food poisoning bacteria in ready-to-eat meals

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ABSTRACT

A total of 100 random of ready to eat Shish tawook, Chicken fajitas, Beef fajitas and Hotdog (25 of each) were collected from different restaurants in Menoufia governorate. The obtained results revealed that the mean values of aerobic plate count (cfu/g)were$1.37\times10^6\pm1.74\times10^6$in Shish tawook, $3.24\times10^6\pm3.69\times10^6$in Chicken fajitas, $1.48\times10^5\pm1.92\times10^5$ in Beef fajitas and $1.94\times10^5 \pm 2.65\times10^5$ in Hotdog. The mean values of coliform count (cfu/g) were $4.54\times10^4\pm8.66\times10^4$, $9.33\times10^4\pm1.49\times10^5$, $2.42\times10^4\pm1.82\times10^4$ and $1.63\times10^4\pm2.64\times10^4$ in Shish tawook, Chicken fajitas, Beef fajitas and Hotdog, respectively. Also, these values of *Staph aureus* count (cfu/g) were $6.33\times10^3\pm7.06\times10^3$, $1.60\times10^4\pm1.13\times10^4$, $1.91\times10^4\pm1.28\times10^4$ and $1.18\times10^4\pm8.91\times10^3$ in the same products. The highest incidences of *Staph aureus*, *E.coli* and *Bacillus cereus* were 40%, 32% and 28%in Chicken fajitas respectively. The incidences of *Clostridium perfringens* were higher in Shish tawook (24%) and chicken fajitas (24%). In findings of multiplex PCR of *Bacillus cereus* showed that the *nhe* (non-hemolytic enterotoxin) and *CytK* (cytotoxin K)genes were positive for all isolated strains yielded a consistent fragment at 766 bp and 421bp, respectively.

1. INTRODUCTION

Street-vended foods (SVFs) are ready-made instant meals at relatively inexpensive prices and generally considered as potential vehicles for micronutrient fortification (Alimi et al., 2014). Street foods or fast foods have been defined by FAO as "Ready-to-eat (RTE) foods & beverages prepared and/or sold by vendors especially in streets & other public places for immediate consumption". These foods are well appreciated by consumers, mostly by urban workers because of their taste, low cost, nutrient value & ready availability for immediate consumption. It includes fast foods, junk foods, snacks, beverages, meals, salads, sliced fruits & drinks for a wide variety of people (FAO/WHO, 2009).

The intact tissues of healthy slaughtered animals and birds are mostly sterile, but the meat may be contaminated during processing from the hands, knives, worker's clothes, the gut, the hide or from the environment resulting in an inferior or even unfit quality for human consumption. Contaminated chicken, beef and meat products may constitute a public health hazard (Ahmed and Ismail, 2010 and Datta et al., 2012).

The following bacteria are the major foodborne pathogens: *Clostridium botulinum, Escherichia coli, Staphylococcus aureus, and Bacillus cereus*. (Gaia et al., 2017). The main sources of pathogenic bacteria in food are contaminated raw food, food handlers, dust, water, utensils & insects (Ray, 1996). RTE food has been implicated in cases of food poisoning or gastroenteritis in human beings (Eley, 1996). Hot foods have been the source of outbreaks of *Staphylococcus aureus* and *Clostridium perfringens* food poisoning (Hatakka, 1998).

Staphylococcus aureus plays a great role in bacterial contamination of fast foods, because workers during preparation and processing may touch fast foods which are usually eaten without sufficient cooking or heating (Soliman, 1988).

Escherichia coli infection is highly prevalent and poses a major threat to human health in underdeveloped communities (Abu El naga- Azza *et al*., 2014). Meanwhile, it is commonly non-virulent, but some strains have adopted pathogenic or toxigenic virulence factors that make them virulent to human and animals (Gi et al., 2009 and Datta et al., 2012). And also, it is commonly used as surrogate indicator; its presence in food generally indicates direct and indirect fecal contamination (Clarence *et al*., 2009).

Bacillus cereus, a foodborne pathogen, causes two forms of foodborne diseases: emetic food poisoning which occurs when the pathogen produces cereulide, a heat resistant toxin, during vegetative cell growth in foods; and diarrheal food poisoning, which occurs when ingestion of high numbers of viable cells in food results in heat labile enterotoxin production in the small intestine of the host (Soni et al., 2016).

Clostridium perfringens is sometimes called the "cafeteria germ" because it can be found in foods served in large amounts and kept at room temperature on poorly

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maintained steam tables or food warmers. *Clostridium perfringens* thrives in high-protein foods of animal origin, such as meat and meat products, these protein-containing foods, when kept at improper storage temperatures, between 54 ºF (12 ºC) and 140 ºF (60 ºC), provide the greatest risk of infection and disease from *C. perfringens*. This is because spores present after cooking can germinate and potentially grow to high, dangerous numbers. The danger zone exists between 109 ºF (43 ºC) and 117 ºF (47 ºC) (CDC, 2017). So, the aim of the current research was to investigate the bacterial profile of RTE beef and chicken products dishes these were Shish tawook, chicken fajitas, Beef fajitas and Hotdog samples after cooking and before serving.

2. MATERIAL AND METHODS

2.1. Collection of samples:
A total of one hundred random samples of beef and chicken meat products including Shish tawook, Chicken fajitas, Beef fajitas, and Hotdog (25 of each) were collected from different restaurants in Menoufia governorate, Egypt. All samples were transported in cold bags and transported immediately to the laboratory under possible aseptic conditions without undue delay and examined as quickly as possible.

2.2.Preparation of samples: were done as described in (APHA, 2001):
Twenty-five grams from each sample were homogenized with 225 ml sterile 0.1 % peptone solution in a sterile polyethylene bag for 1.5 minutes using stomacher (Lab-blender 400). One ml from the sample original homogenate was added to a test tube containing 9 ml 0.1% sterile peptone water to provide a dilution of 10^2. Similarly, a tenfold serial dilution was prepared. And the following bacteriological investigations were performed.

2.3. Aerobic Plate Count (APC) (cfu/gm):
It was carried out according to (ISO, 2013).

2.4. Coliform count(cfu/gm):
It was carried out according to FDA (2002).

2.5.Staphylococcus aureus count:
It was carried according to FDA (2001)

2.6.Identification of Staph. aureus:
It was carried out according to Mac-Faddin (2000).

2.7. Isolation and identification of E.coli:
The method described by ISO (2017)

2.8. Isolation of Bacillus Cereus:
The technique used was recommended by ISO (2004a).

2.9. Isolation and identification of Clostridium perfringens
It was carried according to ISO (2004b).

2.10. Lecithinase activity of C. perfringens
It was recovered on egg yolk agar media (Nagler' s reaction) according to Murrey et al. (2003).

2.11. Demonstration of C. perfringens toxins
It was adopted by application of dermonecrotic reaction in Guinea pig (Sterne and Batty, 1975).

2.12. Polymerase Chain Reaction (PCR) of Bacillus cereus.

2.12.1. Extraction of DNA
It was carried cording to QIAamp DNA mini kit instructions.

2.12.2. Preparation of PCR Master Mix

2.12.2.1. Preparation of PCR Master Mix
It was carried according to Emerald Amp GT PCR master mix (Takara) Code No.RR310Akit.

2.12.2.2. Cycling conditions of the primers during cPCR.

2.12.3. DNA Molecular weight marker.

2.12.4.Agarose gel electrophoreses
It was carried according to Sambrooket al. (1989) with modification.

3. RESULTS

It is evident from the results recorded in tables (1&2), that the mean value of aerobic plate and coliform counts (cfu/g) of examined RTE beef and chicken product samples were $1.37\times10^6\pm1.74\times10^6$& $4.54\times10^4\pm8.66\times10^4$ in Shish tawook, $3.24\times10^6\pm3.69\times10^6$& $9.33\times10^4\pm1.49\times10^5$ in Chicken fajitas, $1.48\times10^5\pm1.92\times10^5$& $2.42\times10^4\pm1.82\times10^4$ in Beef fajitas and $1.94\times10^5\pm2.65\times10^5$& $1.63\times10^4\pm2.64\times10^4$ in Hotdog. In other words, there were significant differences ($P \leq 0.05$) between the examined samples.

Table 1 Statistical analytical results of aerobic plate count (cfu/g) in the examined RTE beef and chicken meat product samples (n=25).

Samples	Max.	Min.	Mean±S.D
Shish tawook	4.5×106	2.3×104	1.37×106±1.74×106
Chicken fajitas	8.5×106	5.6×104	3.24×106±3.69×106
Beef fajitas	4.7×105	2.6×103	1.48×105±1.92×105
Hot dog	8.3×10^5	3.6×10^3	$1.94\times10^5\pm2.65\times10^5$

S.D = Standard Deviation. There significant difference between products ($P\leq0.05$).

Table 2 Statistically analytical results of coliform count (cfu/g) in the examined RTE beef and chicken meat product samples (n=25).

Samples	Max.	Min.	Mean±S.D
Shish tawook	3.5×10^5	3.1×10^2	$4.54\times10^4\pm8.66\times10^4$
Chicken fajitas	5.6×10^5	3.5×10^3	$9.33\times10^4\pm1.49\times10^5$
Beef fajitas	5.6×10^4	3.3×10^2	$2.42\times10^4\pm1.82\times10^4$
Hot dog	7.9×10^4	2.5×10^2	$1.63\times10^4\pm2.64\times10^4$

S.D = Standard Deviation. There significant difference between products ($P \leq0.05$).

As shown in table (3) results illustrated that the highest incidence for isolation of *Staphylococcus aureus* in the examined samples of street vended meat products was recorded in the examined Chicken fajitas samples at percentage of 40% with the mean value of $1.60\times10^4\pm1.13\times10^4$ cfu/g. While the lowest incidence was recorded in Hotdog samples at percentage of 20% with the

mean value of $1.18\times10^4\pm8.91\times10^3$ cfu/g. The obtained results specify that there were significant differences between samples ($P \leq 0.05$).

Table 3 Incidence and count of *Staphylococcus aureus* (cfu/g) of the examined RTE beef and chicken meat product samples (n=25).

Samples	Incidence		Count		
	No.	%	Max.	Min.	Mean±S.D
Shish tawook	8	32	2.1×104	2.5×102	$6.33\times10^3\pm7.06\times10^3$
Chicken fajitas	10	40	3.3×104	2.1×103	$1.60\times10^4\pm1.13\times10^4$
Beef fajitas	6	24	3.6×104	1.5×103	$1.91\times10^4\pm1.28\times10^4$
Hot dog	5	20	2.3×104	1.5×103	$1.18\times10^4\pm8.91\times10^3$

S.D = Standard Deviation. There significant difference between products ($P \leq 0.05$).

Moreover, results obtained in table (4) illustrated that, the highest incidence of *E. coli* was recorded in Chicken fajitas (32%), while lower incidence was in Hotdog (16%).
Serotypes isolated from the examined RTE Shish tawook, Chicken fajitas, Beef fajitas and Hotdog samples were O_{111}:H_2 (4%) EHEC, O_{113}:H_4 (4%) EPEC, O_{26}:H_{11} (4%) EHEC, O_{127}:H_6 (4%) ETEC and O_{103} (4%) EHEC in Shish tawook, O_{111}:H_2 (4%) EHEC, O_{91}:H_{21} (4%) EPEC, O_{127}:H_6 (4%) ETEC, O_{119}:H_6 (8%) EPEC, O_{113}:H_4 (4%) EPEC, O_{26}:H_{11} (4%) EHEC and O_{124} (4%) EIEC in RTE Chicken fajitas,O_{111}:H_2 (4%) EHEC, O_{113}:H_4 (8%) EPEC, O_{26}:H_{11} (4%) EHEC, O_{55}:H_7 (4%) EPEC and O_{124} (4%) EIEC in Beef fajitas and O_{127}:H_6 (4%) ETEC, O_{119}:H_6 (4%) EPEC, O_{55}:H_7 (4%) EPEC and O_{124} (4%) EIEC in RTE Hotdog.
Also, results in tables (5) showed that the incidences of *Bacillus cereus* and *Clostridium perfringens*were16%& 24% in Shish tawook, 28%& 24% in Chicken fajitas, 24%& 16% in Beef fajitas and the same incidence (20%) in Hotdog.

Table 4 Serotyping of Enteropathogenic *E. coli* isolated from the examined samples of RTE beef and chicken meat product samples (n=25).

E. coli strains	Type	Shish tawook		Hotdog		Chicken fajitas		Beef fajitas	
		No.	%	No.	%	No.	%	No.	%
O_{111}:H_2	EHEC	1	4	-	-	1	4	1	4
O_{91}:H_{21}	EPEC	-	-	-	-	1	4	-	-
O_{127}:H_6	ETEC	1	4	1	4	1	4	-	-
O_{119}:H_6	EPEC	-	-	1	4	2	8	-	-
O_{113}:H_4	EPEC	1	4	-	-	1	4	2	8
O_{26}:H_{11}	EHEC	1	4	-	-	1	4	1	4
O_{55}:H7	EPEC	-	-	1	4	-	-	1	4
O_{103}	EHEC	1	4	-	-	-	-	-	-
O_{124}	EIEC	-	-	1	4	1	4	1	4
Total		5	20	4	16	8	32	6	24

EHEC=Enteroheamorragic *E.coli*, EIEC=Enteroinvasive *E.coli*. ETEC=Enterotoxigenic *E.coli*, EPEC=Enteropathogenic *E.coli*

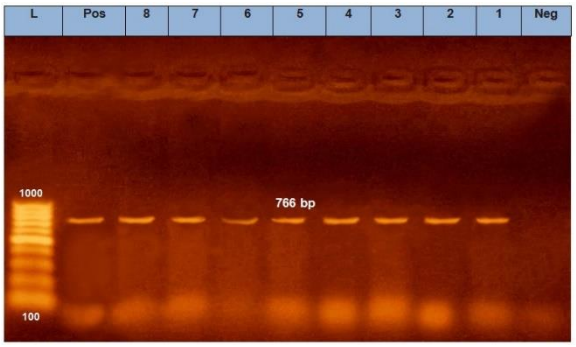
Table 5 incidence of *Bacillus cereus* and *Clostridium perfringens* isolated from the examined samples of RTE beef and chicken meat product samples (n=25).

Samples	==== *Bacillus cereus* ===		=== *Clostridium perfringens*==	
	Positive samples		Positive cases	
	No.	%	No.	%
Shish tawook	4	16	6	24
Chicken fajitas	7	28	6	24
Beef fajitas	6	24	4	16
Hot dog	5	20	5	20
Total	22	88	21	84

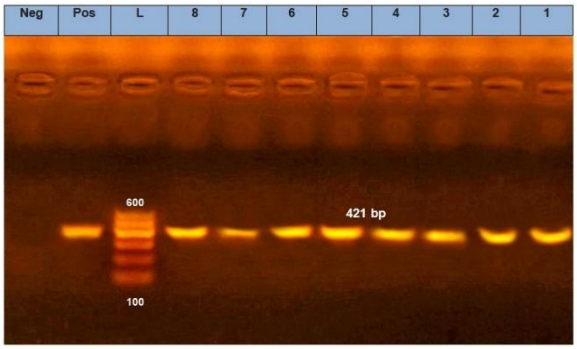
Table 6 the results of PCR amplifications of different used genes of *Bacillus cereus* (n=2).

Samples	No. of sample	*cytK*	*nhe*
Shish tawook	1	+	+
	2	+	+
Chicken fajitas	1	+	+
	2	+	+
Beef fajitas	1	+	+
	2	+	+
Hotdog	1	+	+
	2	+	+

Identification of *Bacillus cereus* isolated from samples by PCR of *Bacillus cereus* showed that the *nhe* (nonhemolytic enterotoxin) and *CytK* (cytotoxin K)genes were positive for all isolated strains (table, 6) yielded a consistent fragment at 766 bp (Photograph 1) and 421 bp (Photograph 2), respectively.



Photograph (1) Agarose gel electrophoresis of PCR amplification productsusing specific primers of (*nhe*) gene of *Bacillus cereus*.* *nhe (non-haemolytic enterotoxin gene)*. Lane L: 100-1000bp DNA Ladder. Pos.: control Positive at (766 bp). Neg.: control Negative. Lane 1, 2, 3, 4, 5, 6, 7, 8 (Positive).



Photograph (2) Agarose gel electrophoresis of PCR amplification products using specific primers of (CytK) gene of Bacillus cereus. * cytk: cytotoxin gene. Pos.: control Positive (at 421bp). Lane L: 100-600 bp DNA Ladder. Neg.: control Negative. Lane: 1, 2, 3, 4, 5, 6, 7, 8 (Positive).

4. DISCUSSION

Various factors contribute to the outbreaks of the food borne illness. The main ones are inadequate food manipulation, improper holding temperatures (failing to properly refrigerate food), inadequate cooking, contaminated equipment (failure to clean and disinfect kitchen or processing plant equipment) and poor personal hygiene. Other factors that may contribute to the food borne illness include preparing food a day or more before

serving with improper holding and reheating, cross contamination (from raw to cooked products) and adding contaminated ingredients to the previously cooked food.

Aerobic plate count can provide useful information about the remaining shelf-life of the food in question, and thus highlight potential problems of storage and handling since production (HPA, 2009) and a general indication of the microbiological quality of food not safety. So, High aerobic plate count may indicate unhygienic preparation; inappropriate storage conditions or suggests possible poor temperature control (NSW/FA, 2009).

It is evident from the obtained result in table (1) that the examined samples of chicken fajitas were the most contaminated ones followed by Shish tawook, Hotdog and beef fajitas. This could be attributed to the fact that Chicken fajitas may receive more handling during preparation as well as addition of spices and vegetables which may be contaminated with larger number of microorganisms.

Such variations may be attributed to difference in quality of meat from the sanitary point of view or ingredients added to meat as vegetables and cheese, the hygienic standard during processing or time and temperature of storage and retailing of product may play a role.

Lower results of APC in Chicken fajitas were recorded by Hamedy-Taghreed (2016) ($2.22\times10^5\pm8.4\times10^4$cfu/g) and Shaltout et al. (2019) ($4.9\times10^4\pm 1.5\times10^4$cfu/g), In Shish tawook lower results of APC were recorded byHussein et al.(2018) ($7\times10^4 \pm 2.8\times10^4$). While higher results of APC in Beef fajitas were recorded by Salem-Nehad et al. (2016) ($2.7\times10^7\pm1.5\times10^7$). While, nearly similar results of APC in Hotdog were reported by Oranusi et al. (2011) (2.0×10^2 to 5.4×10^2) and higher results recorded by Oluwafemi and Simisaya (2006) (2.06-2.80 x 10^6cfu/g).

Coliforms are used as indicator of water pollution or as a general indicator of sanitary condition in in the food processing environment (Feng et al., 2002). Also, high Coliforms count indicates poor hygienic quality of meat and is significant as indicator of fecal contamination. Coliforms also have the ability to grow well over wide range of temperature below 10^oC up to 46^0C (Gill et al., 1996).

Accordingly, the examined samples of Chicken fajitas were the most contaminated ones. This could be attributed to the neglected sanitary measures during their processing, handling and serving of such products

Lower results of coliform count were recorded by Hamedy-Taghreed (2016) (31.90±21.1MPN/g) in Chicken fajitas. In Shish tawook lower coliform count were recorded by Eid-Amal et al. (2014) ($5.1\times10^3\pm6.6\times10^4$), USDA-FSIS, (2012) ($2.5\times10^3$) and Hussein et al.(2018) ($0.15\times10^2\pm 0.04\times10^2$).While higher coliform count in Shish tawook was reported by Sampers *et al.* (2010) (2.51×10^5). In Beef fajitas lower coliform results were recorded by Salem-Nehad et al. (2016) ($5.0\times10^2\pm2.1\times10^2$), and in Hotdog recorded by Lopasovsky *et al*. (2016) (less than 10 to 1.4×10^3) and Oranusi *et al*. (2011) (1.0×10^2 to 5.0×10^2).

The presence of *Staph. aureus* in heat treated food is a pointer to largely poor personal hygiene, improper storage facilities, and unhygienic environment (Achi and Madubuike, 2007). Total *S. aureus* count can be taken as index of sanitary conditions under which the meat and its products are manufactured and handled(Potter, 2001).The presence of *S.aureus*or its enterotoxins in processed food is generally an indication of poor sanitation and poor personal hygiene (Reginald et al., 2001).

The obtained results showed that the examined samples of Chicken fajitas were the most contaminated one. Presence of Staphylococci in Chicken fajitas samples may be attributed to inadequate heat treatment, unhygienic handling practices through the workers who can transfer staphylococci on their hands and using dirty equipment's for slicing.

The lower results were recorded by Mohamed-Eman and El Zahaby- Dina (2015) ($3x10^2 \pm 0.18x10^2$) with incidence of 13.33% and Hamedy-Taghreed (2016) (90.0±64)with incidence of20%in Chicken fajitas. Also, In Shish tawook lower *Staph. aureus* was reported by Eid-Amal *et al.* (2014)($2.6x10^2 \pm 7.4x10^2$)&Mousa *et al*. (2019) (25%).While, higher mean value of *Staph. aureus* was reported by Sampers *et al.* (2010) ($7.9 x10^3$).

In Beef fajitas lower incidence were reported by Salem-Nehad *et al.* (2016) (14%). In Hotdog lower results of *Staph. aureus* was obtained by Lopasovsky *et al.* (2016)(3%). On the other hand, higher results obtained by Kothe *et al.* (2016) (25%) and Oranusi *et al.* (2011) (35%).

Table (4) revealed that the highest incidence of *Enteropathogenic E. coli* was represented in Chicken fajitas while the lowest incidence was in Hotdog.

The obtained results in RTE Shish tawook was nearly similar to Naglaa *et al.* (2009) (20%), While lower results were reported by Elbayoumi *et al*, (2018) (14.3%), Hussein *et al.* (2018) (6.7%). and Mousa *et al*. (2019) (16.7%).

Lower results of examined RTE Chicken fajitas were reported by Mohamed-Eman and El Zahaby-Dina (2015) (20%).While higher results were recorded by Alaa Eldin *et al.* (2018) (42%).

Lower results for examined RTE Hotdog were obtained by Oranusi *et al.* (2011) (11%) and Al-Mutairi (2011)(12%).On the other hand higher results obtained by Maarouf and Nassif-Marionette (2008) (29.2%), and Mansour-Amal (2013) (40%).

Lower incidence of isolated *E.coli* for examined RTE Meat fajitas was recorded by Hassanien-Fatin *et al.* (2015) (13.33%).

The variation in the results between different authors may be due to the differences in the effectiveness of hygienic measures applied during cooking, handling and serving of food.

In RTE Shish tawook Elbayoumi *et al.* (2018) could isolate serovarsO_1:H_7 (5.7%), O_{127}:H_4 (5.7%) andO_{103}:H_2 (5.7%) EHEC. and Hussein *et al.*,(2018) isolate O_{78} (5.3%). while, Mousa *et al*. (2019) isolate EPEC O_{86}:K_{61} (50%), EIEC O_{124}:K_7 (25%) and ETEC O_{127}:K_6 (25%).

In RTE Chicken fajitas Mohamed-Eman and El Zahaby-Dina (2015) could isolate one strain of O_2:H_6(EPEC), tow strain of O_{78}(EPEC) and three strain of O_{26}:H_{11} (EHEC).While Alaa Eldin *et al.* (2018) could isolate O_{111}:H_2 (4%), O_{91}:H_{21} (2%), O_{127}:H_6 (6%), O_{119}:H_6 (10%), O_{26}:H_{11} (16%) and O_{55}:H_7 (4%).

While in RTE Hotdog Darwish *et al.* (1991) could isolate serovars (O_{124}: K_{72}, O_{78}: K_{80}, O_{86}: K_{61} and O_{111}: K_{58}) and Nashed-Heba (1993) (O_{86}: K_{6I} (B7)), while El-sherif-Amal (2009) could isolate O8:H6(one strain) and O125:H7(two strains), also Al-Mutairi (2011)O119 (one strain) and O25:k11 (two strains).

Transmission of *C. perfringens* may occur via foodborne, water borne, animal contact, person-to-person, and others, at the point of consumption. *C. perfringens* transmission was referred primarily to food through unhygienic food handling and cross-contamination in the processing pathway (Butler *et al.,* 2015). Because of its ability to form a spore, this microorganism is able to survive adverse conditions such as aerobic and food processing procedures. Its spores may contaminate meat and meat products either before processing and survive cooking or after processing due to unhygienic handling of prepared food (Santos *et al.*, 2002 and Potter, 2001).

As shown in table (5) the incidence of *Bacillus cereus* and *Clostridium perfringens* were higher in RTE Chicken fajitas

In RTE Beef fajitas Salem-Nehad *et al. (*2016) isolate *bacillus cereus* in the incidence of (12%) and *Clostridium perfringens* in the incidence of (9%). In RTE Chicken fajitas Shehata-Esraa (2018) could isolates *bacillus cereus* in the same incidence of (28%) and Samaha *et al.* (2012) (8%).

In Hotdog Oranusi *et al.* (2011) isolate Bacillus cereus in higher incidence of (25%). Also, Oluwafemi and Simisaye (2006) could isolate *bacillus cereus* and *Clostridium perfringens* from hot dog.

Chicken fajitas is the most contaminated product, the high prevalence rates reported here might be due to poor manufacturing processes (poor temperature control) during processing and storage, inadequate cooking of product and bad cleaning and disinfection of both equipment and surfaces like floors and tables or poor personal hygiene and use of untrained personnel.

PCR amplification of *nhe* gene in isolated *Bacillus cereus* strains showed that the genes encoding the enterotoxin *nhe* are detectable in all isolates these results can be nearly similar to that obtained by (Anderson-Borge *et al.* (2001), Hansen and Hendriksen (2001) and Wijnands *et al.* (2006).

Also, the result of PCR amplification of *cytk* gene substantiated what has been reported by Zhang *et al.* (2016) and Rosenquist *et al.* (2005). As, they can detect genes encoding the enterotoxin *cytk* in isolated strains of *Bacillus cereus* from RTE food.

Nonhemolytic enterotoxin typically gives relatively mild and short lived diarrhoeal syndrome. cytotoxin K enterotoxin, the single-component cytotoxin K (*cytk*) has so far only been reported to be involved in a single case of severe food poisoning outbreak including the death of three persons Lund *et al.* (2000).

All isolates appear to have two enterotoxin genes (combinations of virulence factors are found), which mean that all these *B. cereus* strains are potentially pathogenic. These findings were nearly agreed with Wijnands *et al.* (2006) found the combination of virulence factors *nhe* gene and *cytk* gene in isolated strain of *Bacillus cereus* from RTE food.

5. CONCULSIONS

The obtained results in the present study concluded that the examined Chicken fajitas samples showed higher contamination than other products. While *Staph. aureus* is the most microorganisms isolated from all examined samples. So, it has further evidence that the undesirable level of contamination which might have acquired from the environment and to obtain wholesome, safe and sound meat, the Hazard Analysis and Critical Control Point system (HACCP) must be adopted.

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