**Original Paper****Enterotoxigenic Staphylococci as potential hazards in meat meals prepared at restaurant level**Marwa Gewely¹, Faten Hasanine¹, Amani Salem¹, Nahla Shawky²¹Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Egypt²Department of Food Hygiene, Animal Health Research Institute, shebin El –Kom branch, Egypt**ARTICLE INFO****ABSTRACT****Keywords**

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Enterotoxigenic staphylococci are associated with food poisoning infection especially in ready to eat meals ingestion causing serious health hazards. A total of 150 random samples of prepared meat meals represented by beef kofta, hawawshi, chicken shawarma, chicken pane, fish fillet and fried tilapia (25 of each) were gathered from several restaurants in Egypt's Menoufia governorate and analyzed bacteriologically for detection of enterotoxigenic staphylococci. The obtained results showed that, the mean values of staphylococci count (CFU/g) were $2.3 \times 10^3 \pm 0.35 \times 10^3$ in beef kofta, $2.5 \times 10^3 \pm 0.1 \times 10^3$ in hawawshi, $8.1 \times 10^3 \pm 0.21 \times 10^4$ in chicken shawarma, $2.1 \times 10^3 \pm 0.45 \times 10^3$ in chicken pane, $3.8 \times 10^3 \pm 0.06 \times 10^3$ in fish fillet, $7.6 \times 10^3 \pm 0.13 \times 10^3$ in fried tilapia. Also, *Staphylococcus aureus* had mean values of $1.3 \times 10^3 \pm 0.23 \times 10^3$, $1.5 \times 10^3 \pm 0.21 \times 10^3$, $4.5 \times 10^3 \pm 0.01 \times 10^3$, $1.5 \times 10^3 \pm 0.31 \times 10^3$, $2.2 \times 10^3 \pm 0.05 \times 10^3$, $3.0 \times 10^3 \pm 0.03 \times 10^3$ (CFU/g) and with incidences of 24%, 20%, 24%, 12%, 16% and 12% in the examined samples of beef kofta, hawawshi, chicken shawarma, chicken pane, fish fillet and fried tilapia, respectively. Restrict hygienic measures should be applied to prevent food contamination.

1. INTRODUCTION

Food either raw or cooked, hot or chilled that are ready for immediate consumption at the point of sale without further treatment are generally described as "ready-to-eat" (Tsang, 2002). Due to their biological value, affordable price, and acceptable flavor, ready-to-eat meat products are in high demand; they also represent quick, easy-to-prepare meals and alleviate the problem of fresh meat shortages that are out of reach for many low-income households (Samapundo *et al.*, 2015). Ready-to-eat meat and meat products are of special concern since they may be ingested without further cooking and are known to be a good growth medium for certain microorganism. Furthermore, lack of knowledge about foodborne illnesses is a major risk factor for food contamination and the presence of germs that can cause significant health issues in human (Derbew *et al.*, 2013). In Egypt, street-vended meat products may pose a health risk due to poor hygienic conditions such as using low-quality raw materials, poor personal hygiene, and post-cooking holding for long periods of time, which encourages heavy bacterial loads in foods containing pathogenic microorganism; such contamination may render the product of inferior quality or unfit for human consumption (El-Ziqaty *et al.*, 2016). Staphylococcal food poisoning is one of the most common food-borne diseases worldwide resulting from contamination of food with *S. aureus* enterotoxins causing economic losses and losses in human productivity (Kadariya *et al.*, 2014). Staphylococci are responsible for a wide range of tissue infections and

illnesses. More than 30 different kinds of staphylococci strains have been confirmed to be human-infectious, with symptoms and disorders ranging from mild to severe. *Staphylococcus aureus*, a prominent human and zoonotic pathogen implicated in both clinical care and food safety, is responsible for the majority of these infections (Xu *et al.*, 2011). Because of its pathogenicity and zoonotic significance, the presence of *S. aureus* in ready-to-eat meat poses a public health risk and raises food safety issues (Razmyar *et al.*, 2017). *Staphylococcus aureus* poisoning usually occurs after eating various foods, especially processed meat due to poor handling and subsequent storage at a high temperature (Argudin *et al.*, 2010). The contamination of food by *S. aureus* may directly occur due to skin lesions of food handlers or sneezing and coughing (Hanson *et al.*, 2011). It can grow and produce SEs under a wide range of conditions, including temperature, pH, sodium chloride concentration as well as water activity (Adams and Moss, 2008). Accordingly, the number of *S. aureus* bacteria can be used as an indicator of the hygienic circumstances under which meat and its products are cooked and handled (Potter, 2001). Therefore, this study was performed for assessment of staphylococci and *S. aureus* prevalence in various ready to eat beef, chicken and fish meals.

2. MATERIAL AND METHODS**2.1. Collection of Samples:**

One hundred and fifty random samples of ready to eat meat meals of beef kofta, hawawshi, chicken shawarma, chicken

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pane, fish fillet and fried tilapia (25 of each) were collected from several restaurants in Menoufia governorate, Egypt. All samples were transferred in ice box as rapidly as possible to the laboratory under possible aseptic conditions without undue delay and examined as quickly as possible.

2.2. Preparation of samples (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. Staphylococci and *S. aureus* counts (FDA, 2001).

2.4. Identification of Staphylococci:

2.4.1. Morphological and cultures character (Cruickshank et al. 1975)

2.4.2. Motility test (ICMSF, 1996)

2.4.3. Biochemical identification (MacFaddin, 2000) :

2.4.3.1. Catalase activity test

2.4.3.2. Oxidase test

2.4.3.3. Growth at 10% NaCl

2.4.3.4. Detection of Arginine decarboxylase (ADH)

2.4.3.5. Bile esculin test

2.4.3.6. Mannitol test

2.4.3.7. Detection of hemolysis

2.4.3.8. Coagulase test

2.4.3.9. Thermostable nuclease test "D-Nase activity"

(Lachia et al. 1971)

2.4.3.10. Fermentation of sugars.

2.5. The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

From the results given in Table (1) it was obvious that, staphylococci count of the examined meat samples (CFU/g) was varied from 6.2×10^2 to 8.9×10^3 with mean of $8.1 \times 10^3 \pm 0.21 \times 10^4$ in chicken shawarma, 2.7×10^3 to 5.1×10^4 with mean of $7.6 \times 10^3 \pm 0.13 \times 10^3$ in fried tilapia, 5.3×10^2 to 6.7×10^3 with mean of $3.8 \times 10^3 \pm 0.06 \times 10^3$ in fish fillet, 3.2×10^2 to 4.7×10^3 with mean of $2.5 \times 10^3 \pm 0.1 \times 10^3$ in hawawshi, 4.5×10^2 to 5.8×10^3 with mean of $2.3 \times 10^3 \pm 0.35 \times 10^3$ in beef kofta and 4.5×10^2 to 3.5×10^3 with mean of $2.1 \times 10^3 \pm 0.45 \times 10^3$ in chicken pane.

Although chicken shawarma exposed to heat treatment the statistical analysis of variance of staphylococci counts among the examined meat product samples indicated a significant difference ($P < 0.0001$) between examined chicken shawarma samples and the other product samples. Moreover, the average of staphylococci count in case of beef kofta samples was significantly different ($P < 0.0001$) compared to chicken pane samples and fried tilapia samples). However, it was not significantly different compared to hawawshi, and fillet samples. There was no significance difference between chicken pane and fried tilapia samples.

Table 1 Statistical analytical results of staphylococci count (CFU/g) in the examined samples of ready to eat meat meals (n=25)

Product	Positive samples				
	No.	%	Min.	Max.	Mean values
Beef kofta	15	6 0	4.5×10^2	5.8×10^3	$2.3 \times 10^3 \pm 0.35 \times 10^{3b}$
Hawawshi	16	6 4	3.2×10^2	4.7×10^3	$2.5 \times 10^3 \pm 0.1 \times 10^{3bc}$
Chicken shawarma	17	6 8	6.2×10^2	8.9×10^3	$8.1 \times 10^3 \pm 0.21 \times 10^{4a}$
Chicken pane	12	4 8	4.5×10^2	3.5×10^3	$2.1 \times 10^3 \pm 0.45 \times 10^{3c}$
Fish fillet	14	5 6	5.3×10^2	6.7×10^3	$3.8 \times 10^3 \pm 0.06 \times 10^{3bc}$
Fried Tilapia	13	5 2	7.2×10^3	5.1×10^4	$7.6 \times 10^3 \pm 0.13 \times 10^{3c}$

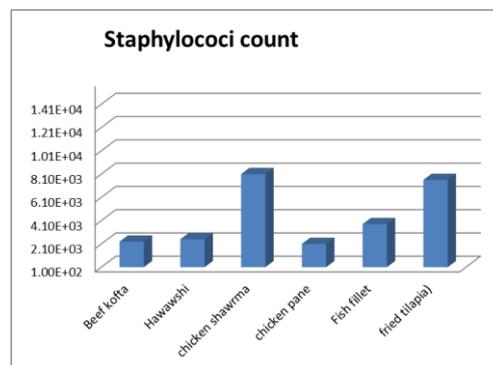


Figure 1 Mean values of Staphylococci count (CFU/g) in the examined samples (n=25).

Tables (2 and 3) indicated that *S. aureus* count (CFU/g) of the examined meat samples was 2.5×10^2 to 3.8×10^3 with mean of $1.3 \times 10^3 \pm 0.23 \times 10^3$ for beef kofta, 2.6×10^2 to 3.7×10^3 with mean of $1.5 \times 10^3 \pm 0.21 \times 10^3$ for hawawshi, 3.5×10^2 to 6.2×10^3 with mean of $4.5 \times 10^3 \pm 0.01 \times 10^3$ for chicken shawarma, 3.1×10^2 to 2.8×10^3 with mean of $1.5 \times 10^3 \pm 0.31 \times 10^3$ for Chicken pane, 2.4×10^2 to 4.5×10^3 with mean of $2.2 \times 10^3 \pm 0.05 \times 10^3$ for fish fillet and 2.6×10^3 to 3.1×10^4 with mean of $3.0 \times 10^3 \pm 0.03 \times 10^3$ for fried tilapia. The incidence of *Staphylococcus aureus* isolated from the examined product samples was 24%, 20%, 24%, 12%, 16% and 12% in beef kofta, hawawshi, chicken shawarma, chicken pane, Fish fillet and fried tilapia, respectively. There is a significant difference ($P < 0.0001$) between examined shawarma samples and other examined samples. However, there is no significant difference between the other examined samples (beef kofta, hawawshi, chicken pane, fish fillet and fried tilapia).

Table 2 Statistical analytical results of *S. aureus* count (CFU/g) in the examined samples of ready to eat meat meals (n=25)

Product	No. of examined samples	Suspected <i>S. aureus</i> positive samples				
		No.	%	Min.	Max.	Mean values
Beef kofta	25	6	2 4	2.5×10^2	3.8×10^3	$1.3 \times 10^3 \pm 0.23 \times 10^3b$
Hawawshi	25	5	2 0	2.6×10^2	3.7×10^3	$1.5 \times 10^3 \pm 0.21 \times 10^3b$
Chicken shawarma	25	6	2 4	3.5×10^2	6.2×10^3	$4.5 \times 10^3 \pm 0.01 \times 10^{3a}$
Chicken pane	25	3	1 2	3.1×10^2	2.8×10^3	$1.5 \times 10^3 \pm 0.31 \times 10^{3bc}$
Fish fillet	25	4	1 6	2.4×10^2	4.5×10^3	$2.2 \times 10^3 \pm 0.05 \times 10^{3bc}$
fried tilapia	25	3	1 2	2.6×10^2	3.1×10^4	$3.0 \times 10^3 \pm 0.03 \times 10^{3bc}$

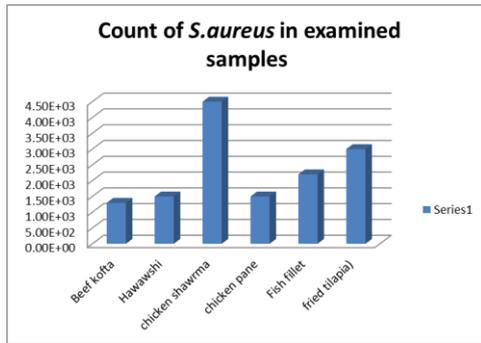


Figure 2 Mean values of *S. aureus* count (CFU/g) in the examined samples.

Results obtained in Table (3) and Fig. (3) revealed that 24%, 20%, 24%, 12%, 16% and 12% of the examined samples of beef kofta, hawawshi, chicken shawarma, chicken pane, fish fillet and fried tilapia were unacceptable where they exceeded the safe permissible limit recommended by Egyptian Organization for Standardization and Quality "EOSQ" (2005) which stated that *S. aureus* count /g should be free. Consequently, the samples exceeding the permissible limit represent a potential health hazard if stored under unfavorable condition where *S. aureus* can proliferate and produce enterotoxin causing SFP.

Table 3 Acceptability of the examined samples of ready to eat meat meals based on their *S. aureus* counts (n=25)

Type of the sample	Accepted samples		Unaccepted samples	
	No	%	No	%
Beef kofta	19	76	6	24
Hawawshi	20	80	5	20
Chicken shawarma	19	76	6	24
Chicken pane	22	88	3	12
Fish fillet	21	84	4	16
Fried Tilapia	22	88	3	12

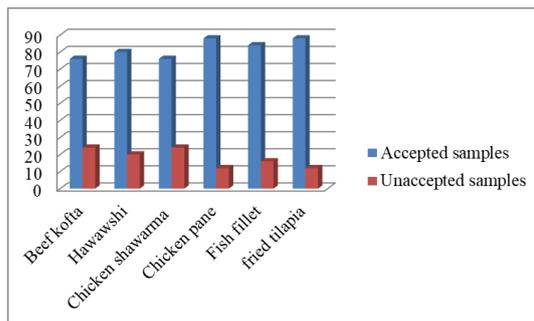


Figure 3 Acceptability of the examined samples based on their *S. aureus* counts.

Results given in Table (4) declared that the incidence of *S. aureus*, *S. epidermidis*, *S. intermedius*, *S. saprophyticus* and *S. capitis* were 24%, 16%, 8%, 8% and 4% from beef kofta, 20%, 12%, 16%, 8%, and 8% from hawawshi, 24%, 6%, 16%, 16% and 4% from chicken shawarma and 12%, 8%, 12%, 16 %and 4% from fried tilapia samples. While *S. aureus*, *S. epidermidis* *S. intermedius* and *S. saprophyticus* were isolated as 12%, 16%, 12% and 8% from chicken pane and 16%, 12%, 16% and 12% from examined fish fillet samples.

Table 4 Serotyping of Staphylococci isolated from the examined samples (n=25)

Staphylococci	Beef kofta		Hawawshi		Chicken shawarma		Chicken pane		Fish fillet		fried tilapia	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>S. aureus</i>	6	24	5	20	6	24	3	12	4	16	3	12
<i>S. epidermidis</i>	4	16	3	12	2	8	4	16	3	12	2	8
<i>S. intermedius</i>	2	8	4	16	4	16	3	12	4	16	3	12
<i>S. saprophyticus</i>	2	8	2	8	4	16	2	8	3	12	4	16
<i>S. capitis</i>	1	4	2	8	1	4	-	-	-	-	1	4
Total	15	60	16	64	17	68	12	48	14	56	13	52

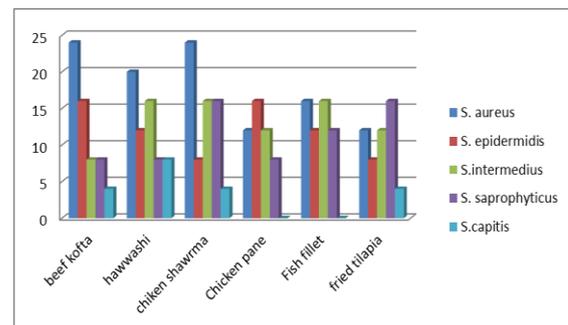


Figure 4 Serotyping of staphylococci in examined samples.

4. DISCUSSION

Over the past decade ready to eat meat meals have gained wide popularity due to their low price compared to fresh meat and their delicious taste. On the other hand, ready to eat meat products can be contaminated by food poisoning microorganisms and become unacceptable for human consumption. The majority of foodborne disease outbreaks are caused by food handler contamination and the development of heat stable toxins in food (FSIS, 2003). Food storage at potentially unsafe temperatures, unsanitary food handling practices and food contamination with uncooked meat were the main sources of contamination of street vended food (Burt *et al.*, 2003).

A high staphylococci count indicates poor sanitation and processing, as well as the presence of enterotoxin-producing strains such as *S. aureus* (ICMSF, 1996). Therefore, staphylococcus remains an important problem for food processors, food service companies and consumers. The most common way for food contamination with staphylococcus is through contact with food workers who carry the bacteria (CDC, 2006).

The results showed that the highest incidence of staphylococci in the examined samples were recorded in chicken shawarma followed by hawawshi, beef kofta and fish fillet. However, the lowest incidence of such bacteria was recorded in chicken pane and fried tilapia samples.

High incidence of staphylococci in examined chicken shawarma samples may be due to inadequate heat treatment, unhygienic handling practices through the workers who can transfer staphylococci on their hands, using dirty equipment for slicing and leaving the skewer of shawarma in unsuitable temperature along the day. In restaurants and food outlets, food handlers are a major

source of staphylococcal food contamination (Colombari *et al.*, 2007).

Although chicken shawarma exposed to heat treatment, the statistical analysis of variance of staphylococci count among the examined meat product samples indicated a significant difference ($P < 0.0001$) between examined Chicken shawarma samples and the other product samples and this may be attributed to inadequate heat treatment, unhygienic handling practices through the workers who can transfer staphylococci on their hands, leaving the skewer of shawarma in unsuitable temperature along the day and using dirty knives for slicing.

Moreover, the average of Staphylococci count in case of beef kofta samples was significantly different ($P < 0.0001$) compared to chicken pane samples and fried tilapia samples). However, it was not significantly different compared to hawawshi, and fillet samples. There was no significance difference between chicken pane and fried tilapia samples.

According to the results recorded for beef kofta they came in agreement with those obtained by Hassanien *et al.* (2015); EL- Hanafy (2016); Badr (2018) and EL-Maghraby (2018) but lower results were recorded by Shafik (2013); Sobieh (2014); Ragab *et al.* (2016) and Saad *et al.* (2018) but higher results recorded by Mohammed (2020) who found the average count of staphylococci 10.3×10^4 CFU/g in samples of beef kofta.

Meanwhile, the present results of hawawshi samples were similar to those obtained by Saad *et al.* (2018) but higher results were recorded by Ahmed - Wafaa (2015).

Moreover, the obtained results of examined chicken shawarma samples are relatively agree, to some extent, with those obtained by Hassanien *et al.* (2015) who found the mean count $9.42 \times 10^3 \pm 2.23 \times 10^3$, while lower incidence recorded by Salem-Nehad *et al.* (2016) (68%).

The obtained results of examined fish fillet are lower than those recorded by mohammed-Rehab (2020) who found the mean count of staphylococci 9.6×10^4 CFU/g.

Intoxication with *Staphylococcus aureus* is a worldwide concern, with many food poisoning outbreaks linked to the eating of infected meat and meat products. As a result, the total number of *S. aureus* can be used as a measure of the hygienic circumstances under which meat and its products are produced and handled (Potter, 2001).

The results indicated that the highest incidence of *S. aureus* in the examined samples were recorded in the examined chicken shawarma and beef kofta, hawawshi, fish fillet While, the lowest incidence was recorded in both chicken pane and fried tilapia.

The presence of *S. aureus* in heat treated food is a pointer to largely poor personal hygiene, improper storage facilities, and unhygienic environment (Achi and Madubuike, 2007). *Staphylococcus aureus* is regarded as a good sign of inefficient thermal processing, poor sanitary conditions during food manufacturing, preparation, or chilling. (Melheiros *et al.*, 2010). The presence of *S. aureus* in meat products might be due to direct contact with employees who have *S. aureus*-related hand or arm sores, or coughing and sneezing, both of which are prevalent during respiratory illnesses. In staphylococcal outbreaks, food workers are usually the cause of food contamination (Jennifer Hait, 2012). As it may be found on the skin and nose, it can be spread to foods as a result of human contamination, either through filthy hands or coughing or sneezing into RTE meals (Koo, 2008).

The results of beef kofta were relatively similar to some extent, with those obtained by Hassanein - Fatin (2004) who found the mean value of *S. aureus* $2.51 \times 10^3 \pm 0.31 \times 10^3$ (CFU/g). While higher results were recorded by Baz - Amany (2016) who found mean value $1.63 \times 10^4 \pm 0.60 \times 10^4$ (CFU/g) and an incidence of 46.7%; Nadim-Samaa (2016) (28%); EL-Maghraby- Marwa (2018) who found mean value $3.51 \times 10^3 \pm 4.6 \times 10^2$ (CFU/g) and an incidence 40% and Sabry *et al.* (2019) who found mean value $4.59 \times 10^3 \pm 0.73 \times 10^3$ (CFU/g) and an incidence 46.67%.

In hawawshi, higher incidence was recorded by Sabry *et al.* (2019) (60%); Morshdy *et al.* (2018) (65%) and Hassan *et al.* (2016) (31.4%).

Meanwhile, lower results of isolated *S. aureus* in chicken shawarma were reported by Samir *et al.* (2019) (5%). On the other hand, higher results obtained by Salem-Nehad *et al.* (2016) (26%) and Hassanien *et al.* (2015) (53.33%).

Higher results of isolated *S. aureus* in chicken pane were recorded by Shaltout-Fahim (2020) (26.6%) and Eman *et al.* (2013) (25%).

Moreover, higher results of isolated *S. aureus* in fish fillet were recorded by mohammed-Rehab (2020) (33.3%), but lower results recorded by Daniel *et al.* (2012) who found the incidence of *S. aureus* in ready to eat fish products were 10%.

Abdominal pains, nausea, vomiting, and diarrhea are all symptoms of *S. aureus* food poisoning. Shijia *et al.* (2016), which occur 2-6 hours from eating contaminated food. The severity of the disease is determined by the victim's susceptibility to infection, the amount of contaminated consumed food, the amount of toxin present in the food consumed, and the victim's overall health (U.S.FDA, 2012).

The statistical analysis of variance of *S. aureus* count among shawarma samples and other examined samples and this could be attributed to the same reasons for significant increase in staphylococci in shawarma samples compared to other product samples in addition coughing and sneezing of workers or vendors, because *S. aureus* is frequently found in the nose and respiratory tract.

The most accepted examined samples were chicken pane and fried tilapia followed by fish fillet, hawawshi then beef kofta and chicken shawarma according to the safe permissible limit recommended by Egyptian Organization for Standardization and Quality (EOSQ., 2005) which stated that *S. aureus* count /g should be free. So, samples that were exceeding the permissible limit represent a potential health hazard.

Finally, it was obvious that Incidence of Gram-positive cocci isolated from the examined samples was highest at chicken shawarma followed by hawawshi beef kofta, Fish fillet, fried tilapia and chicken pane.

5. CONCLUSION

The obtained results indicated that the beef, chicken and fish meat meals were prepared and handled under poor sanitary and hygienic conditions which were the main cause of high count of Staphylococci. It was considered that the most contaminated meat meals were chicken shawarma and beef kofta followed by hawawshi and fish fillet then finally chicken pane and fried tilapia. Finally, RTE meat products still represent health issues for consumers so more restrict hygienic measures should be applied over ready to eat food restaurants.

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