Staphylococcus aureus in some beef and chicken meat products

Aboubakr M. Edris[1], Reham A. Amin[1], Hanan T. Elawandi[2], Aboubakr A. Abdelkader[2], Amina M. El-amin[2]

[1]Department of Food Control, Faculty of Veterinary Medicine, Benha University [2]Animal Health Research Institute, Zagazig Branch

A B S T R A C T

A grand total of 120 random samples of beef and chicken meat products were collected. Beef products were represented by 60 samples of pasterma, luncheon and beef burger(20 of each) (25g of each), while chicken meat products were represented by 60 samples of luncheon, shawerma and shish tawuq (20 of each).The samples were collected from different supermarkets in Sharkia governorate .The incidence of *Staphylococcus aureus* in the examined beef samples were 5% in pasterma , 25% in beef luncheon and 15% in beef burger, while 10% , 15 % and 5% in the examined chicken meat shawerma , luncheon and shish tawouq samples, respectively. The incidence of enterotoxins (A, B, C and D) produced by *Staphylococcus aureus* were *Sea* 2 (13.3%), *Seb* 1 (6.7%) and *Sec* 1(6.7%). Also 1 strain contain Sea& *Sed* (6.7%)and 1 strain contain *Sea, Seb* and *Sed*, while *Sed* alone failed to be identified in isolated *Staphylococcus aureus* strains. *Staphylococcus aureus* isolated from the examined samples of beef and chicken meat products were very susceptible to Oxacillin (OX), then Ciprofloxacin (CP). While Neomycin (N) was the lowest susceptible antimicrobial for *Staphylococcus aureus* strains followed by Oxytetracycline (T).

Keywords: *Staphylococcus aureus*, incidence, antimicrobial sensitivity, Enterotoxins.

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1. INTRODUCTION

The increasing incidence and severity of food borne illnesses worldwide has increased public awareness about food safety and demand for safer food is growing as consumers now better understand the links between diet and health (Campos et al., 2009). Foodborne pathogens cause many acute and life threatening diseases which are highly aggravated in the developing world. *Staphylococcus aureus* commonly occurs on the skin and nasopharynx and it can survive, colonize and persist at various processing stages in plants due to the expression of various key properties including adhesion and chlorine resistance (El-Said, 2005). Moreover, *Staphylococcus aureus* food poisoning symptoms appear within 4-5 hrs. after ingestion of contaminated food with enterotoxigenic *Staphylococcus aureus* strains. The ability to produce such enterotoxin in food is more likely when competing microorganisms are absent (Argudin et al., 2010). *Staphylococcus aureus* enterotoxins are the most frequent causes of food poisoning with outbreaks caused by mishandling of foods after heat treatment and the heat destroys the vegetative bacterial in food (Al-kour, 2001). Polymerase chain reaction (PCR) based methods have been identified as a powerful diagnostic tool for detection of pathogenic microorganisms (Al- Zahrani - Salha, 2012). So, the aim of this study achieved to detect of the incidence of *Staphylococcus aureus* in beef and chicken meat products with reference to its enterotoxins.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A grand total of 120 (25 g of each), random samples of beef and chicken meat products were collected. Beef products were represented by 60 samples of pasterma, luncheon and beef burger (20 of each), while chicken meat products represented by 60 samples of luncheon, shawerma and shish tawuq (20 of each). The samples were collected from different supermarkets in Sharkia governorate. The collected samples were kept in separate plastic bags and aseptically transferred in an insulated ice box to the laboratory as rapidly as

possible for isolation and identification of *Staphylococcus aureus*.

2.2. Preparation of samples (ISO, 2003):

To 25 grams of the sample, 225 ml of sterile peptone water were added and thoroughly mixed using sterile blender for 1 – 1.5 minutes, from which tenfold serial dilutions was prepared. The prepared samples were subjected to the following examinations

2.3. Isolation of Staphylococcus aureus:

Isolation of *Staphylococcus aureus* according to International Organization of Standardization "ISO" (2003), from each of previously prepared serial dilutions, 0.1 ml was evenly spread over a dry surface of Baird Parker agar medium plates using a sterile bent glass spreader. The inoculated and control plates were incubated at 37ºC for 48 hours. Shiny black colonies were enumerated

2.4. Identification of Staphylococcus aureus: (MacFaddin, 2000)

2.4.1. Morphological examination:

Gram's stain test: Suspected colonies were stabbed into semi solid agar to a depth of 5 cm and incubated at 37ºC for 18-24 hrs. circular growth around the line of stabbing was recorded as positive result.

2.4.2. Biochemical identification

Biochemical identification according to (MacFaddin, 2000):

2.4.2.1. Coagulase test:

One ml from an overnight incubated BHI (Brain heart infusion) broth culture was transferred to Wassermann tubes containing 0.3 ml of sterile reconstituted rabbit plasma Inoculated tubes were incubated at 37ºC for 4 hours, the tubes were reexamined for clotting (fibrin clot formation). The extent of Coagulase reaction was recorded. The tubes showed no clot were further incubated and then examined every 2 hours up to 24 hours. The extent of coagulation of plasma was reported after 4 and 24 hours.

2.4.2.2. Catalase test:

A clean glass slide was divided into two sections, one labeled as test and other as control. A small drop of normal saline was placed on each half. A small amount of the tested culture was picked up with a sterilized inoculating loop; one or two colonies were emulsified on each drop to make a smooth suspension. One drop of fresh hydrogen peroxide 3% was placed over the test smear and cover by a clean over slide, the other drop was left as control. *Staphylococcus aureus* revealed catalase positive result.

2.4.2.3. Oxidase test:

Oxidase test was done by streaking of the pure culture onto filter paper moistened with oxidase reagent. The test is positive if the color turns to mauve, violet or deep purple within 10 seconds. *Staphylococcus aureus* revealed negative result.

2.4.3. Serological identification of Staphylococcus aureus (Oxoid, 1990):

Staphylococcus aureus isolates were serologically identified by using Staphylase (Oxoid Dry Spot Staphytect Plus Kit) (Dr 100M) which is a reliable latex slide agglutination test for detection a wide range of *Staphylococcus aureus* strains.

2.5. Detection of enterotoxin genes of isolated Staphylococcus aureus strains by mutiplex PCR

Application of PCR for identification of virulence factors including Enterotoxins A, B, C and D (sea, seb, sec & sed) of *Staphylococcus aureus* was performed. The clear culture supernatant fluid was tested serologically by RPLA technique using kits for the detection of staphylococcal enterotoxins A, B, C and D (SET-RPLA, Denka Sekeu LTD, Japan for Oxoid LTd) by using primers as shown in the following table (A):

Table (A): Amplification of enterotoxin genes of *Staphylococcus aureus* according to Mehrotra et al. (2000).

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)
sea (F)	5′ TTGGAAACGGTTAAAACGAA′3	
sea (R)	5′ GAACCTTCCCATCAAAAACA ′3	120
seb (F)	5′ TCGCATCAAACTGACAAACG ′3	
seb (R)	5′ GCGGTACTCTATAAGTGCC ′3	478
sec (F)	5′ GACATAAAAGCTAGGAATTT ′3	
sec (R)	5′ AAATCGGATTAACATTATCC ′3	257
sed (F)	5′ CTAGTTTGGTAATATCTCCT ′3	
sed (R)	5′ TAATGCTATATCTTATAGGG ′3	317

2.6. Antibiogram for antibiotic

Sensitivity of isolated strains of *Staphylococcus aureus* antimicrobial susceptibility was tested by the single diffusion method according to Deresse et al. (2012) for *Staphylococcus aureus*. Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated *bacterial* strains (Oxoid Limited, Basingstoke, Hampshire, UK). Agar plate method was applied by using of nutrient agar as a substrate for growth of the tested bacterium for its antibiotic sensitivity.

3. RESULTS

It is evident from the results recorded in table (1) that incidence of *Staphylococcus aureus* was 5% , 25% and 15% of the examined pasterma , beef luncheon and beef burger samples, respectively, while 10 % , 15% and 5 % of the examined shawerma, chicken meat luncheon and shish tawouq samples , respectively. Both beef luncheon and chicken meat luncheon showed the highest incidence of *Staphylococcus aureus.*

Results achieved in table (2) showed the serological identification of *Staphylococcus aureus* enterotoxins, both beef luncheon and beef burger contained one strain (6.7%) of *Staphylococcus aureus* which able to produce(*A*) toxin. Chicken meat luncheon samples contained one strain (6.7%) of *Staphylococcus aureus* which able to produce(*C*) toxin, while one strain (6.7%) of *Staphylococcus aureus* which able to produce(*A*&D) toxin in beef burger and one strain (6.7%) of *Staphylococcus aureus* which able to produce (*B*&C) toxin in shish tawouq. Results achieved in table (3) showed using PCR for identification of *Staphylococcus aureus* enterotoxins, both beef luncheon and beef burger contained one strain (6.7%) of *Staphylococcus aureus* which able to produce(A) toxin, also beef luncheon samples contained one strain (6.7%) of *Staphylococcus aureus* which able to produce(B) toxin, Chicken meat luncheon samples contained one strain (6.7%) of *Staphylococcus aureus* which able to produce(C) toxin, while one strain (6.7%) of *Staphylococcus aureus* strain (6.7%) of *Staphylococcus aureus* which able to produce(A&D) toxin in beef burger and one strain (6.7%) of *Staphylococcus aureus* which able to produce(A&C&B) toxin in shish tawouq. The results in table (4) revealed that that the isolated *Staphylococcus aureus* strains were highly sensitive to Oxacillin (OX) 86.7% then Ciprofloxacin (CP)66.7%, Cloxacillin (CL) 60% while, both Enrofloxacin (EN) and Gentamicin (G) were 46.7%. On the other hand, *Staphylococcus aureus* strains were highly resistant to Neomycin (N) 100%, Oxytetracycline (T) 93.3% then both Sulphamethoxazol (SXT) and Kanamycin (K) were 80%, followed by Erythromycin (E) 73.3% and Ampicillin (AM) 66.7%.

Table (1): Incidence of *Staphylococcus aureus* in some beef and chicken meat products (n=20):

	No of +ve samples	Percentage
1-Beef product		
• Pasterma	1	5%
• Luncheon	5	25%
• Beef burger	3	15%
2-Chicken meat product		
• Shawerma	2	10%
• Luncheon	3	15%
• Shish tawouq	1	5%

Table (2): Serological identification of *Staphylococcus Aureus* Enterotoxins:

Products	Enterotoxin production	No.	%
Beef Luncheon	A	1	6.7
Beef burger	A	1	6.7
Chicken meat Luncheon	C	1	6.7
Beef burger	A & D	1	6.7
Shish tawouq	B & C	1	6.7

N.B: % was calculated according to positive number of samples

Table (3): Occurrence of enterotoxin genes of *Staphylococcus aureus* strains isolated from the examined samples of beef and chicken meat products (n= 15 strains)

Products	*Staphylococcus aureus* enterotoxins	No.	%
Beef Luncheon	A	1	6.7
Beef burger	A	1	6.7
Beef Luncheon	B	1	6.7
Chicken meat Luncheon	C	1	6.7
Beef burger	A+D	1	6.7
Shish tawouq	A+B+C	1	6.7

N.B: % was calculated according to positive number of samples

Table (4): Percentages of Antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from the examined samples of beef and chicken meat beef products.

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Neomycin (N)	-	-	-	-	15	100
Oxytetracycline (T)	-	-	1	6.7	14	93.3
Sulphamethoxazol (SXT)	-	-	3	20.0	12	80.0
Kanamycin (K)	1	6.7	2	13.3	12	80.0
Erythromycin (E)	2	13.3	2	13.3	11	73.3
Ampicillin (AM)	4	26.7	1	6.7	10	66.7
Chloramphenicol (C)	4	26.7	3	20.0	8	53.3
Norfloxacin (NOR)	5	33.3	2	13.3	8	53.3
Cephalotin (CN)	4	26.7	4	26.7	7	46.7
Gentamicin (G)	7	46.7	2	13.3	6	40.0
Enrofloxacin (EN)	7	46.7	3	20.0	5	33.3
Cloxacillin (CL)	9	60.0	2	13.3	4	26.7
Ciprofloxacin (CP)	10	66.7	3	20.0	2	13.3
Oxacillin (OX)	13	86.7	1	6.7	1	6.7

S: sensitive I: intermediate R: resistant

N.B: % was calculated according to positive number of samples

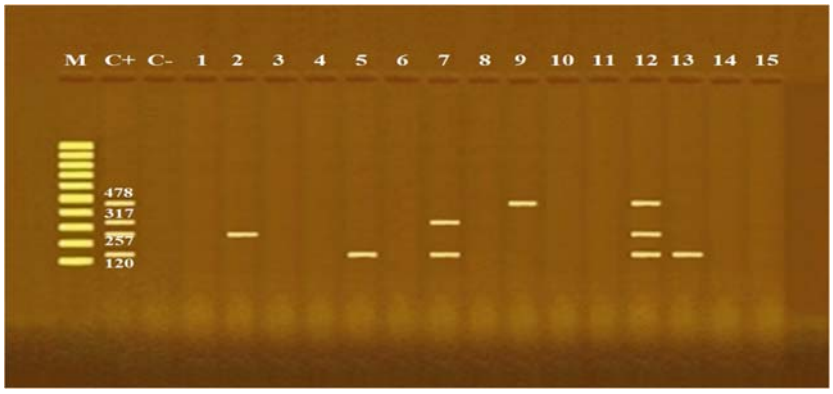

Photo (1): Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes for characterization of *Staphylococcus aureus*.

Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive for sea, seb, sec and sed genes. Lane C-: Control negative. Lane 2: Positive *Staphylococcus aureus* strain for sec gene. Lanes 5 & 13: Positive *Staphylococcus aureus* strains for sea gene. Lane 7: Positive *Staphylococcus aureus* strain for sea & sed genes. Lane 9: Positive *Staphylococcus aureus* strain for seb gene. Lane 12: Positive *Staphylococcus aureus* strain for sea, sab & sec genes. Lanes 1, 3, 4, 6, 8, 10, 11, 14 & 15: Negative *Staphylococcus aureus* strains for enterotoxins.

4. DISCUSSION

Beef and chicken meat products are liable to harbor different types of microorganisms through a long chain of handling, processing, distribution and storage as well as preparation. *Staphylococcus aureus* food poisoning is an intoxication and its symptoms appear within 4-5 hrs. after ingestion of contaminated food with enterotoxigenic *Staphylococcus aureus* strains (Al- Zahrani - Salha, 2012), the ability to produce such enterotoxin in food is more likely when competing microorganisms are absent.

The current results of the examined beef samples were agreed to some extent, with these reported by (Armany, 2016) (24% in luncheon, 4% in pasterma), while higher results were obtained by Shahraz et al. (2012) (25% in burger) and Ismail-Seham et al. (2013)(32%in luncheon). Lower results were obtained by Hassanien-Fatin (2004) (15 % of luncheon) and El-Said (2005) (4%in burger). while the results of *Staphylococcus aureus* incidence in chicken meat products agreed to some extent to those recorded by Shaltout (2002) (6% in shish tawouq), while lower results obtained by Alet (2000) (zero in shawerma) and Sharaf and Sabra (2012) (zero in chicken meat shawerma & 10% in luncheon), Higher result obtained by Rady et al. (2011) (25% in chicken meat luncheon) and Nimri et al. (2014) (30% in shawerma). Luncheon samples in both beef and chicken meat products was the highest contaminated one with *Staphylococcus aureus* than the other products and this may reflect the bad hygienic conditions during production and processing of luncheon

Pasterma as a beef product and Shish tawouq as a chicken meat product were the least products contaminated with *Staphylococcus aureus* and this due to its contamination from food handlers, inadequate cleaned equipment or post-processing contamination

Staphylococcus aureus intoxication is caused by the ingestion of food contaminated with preformed staphylococcal enterotoxins (Argudin et al., 2010). There are several different types of SE (A, B, C &D) The staphylococcal enterotoxins can withstand heating at 100ºC for 30 minutes and therefore, the absence of viable organisms in the food is not a proof of safety. Moreover, table (2) illustrated the occurrence of entertoxin genes of *Staphylococcus aureus* strains isolated from the examined samples which were Sea 2 (13.3%), Seb 1 (6.7%) and Sec 1(6.7%). Also one strain contains Sea& Sed (6.7%) and another one strain contain Sea, Seb and Sed, while Sed alone couldn't be identified. Symptoms of SFP have a rapid onset (2–8 h), and include nausea, violent vomiting and abdominal cramp with or without diarrhea. The disease is usually self-limiting and typically resolves within 24–48 h after onset (Al-kour, 2001).

These results which obtained by Photo (1) and table (3) were nearly similar those obtained by Pinto et al. (2005) and Oh et al. (2007) , who found 41% and 47% of isolates were positive for enterotoxins production , respectively. Lower incidence detected by Faiek (2015) who failed to detect any enterotoxin genes in chicken meat products samples. Higher incidence was detected by Madahi et al. (2014), who detected 83% of 27 positive examined samples were able to produce enterotoxins. When we compared serological results with PCR results in detection of *Staphylococcus aureus* strains which contain enterotoxigenic genes. PCR results were more accurate, specific and sensitive which revealed by detecting more enterotoxigenic genes, moreover PCR is a faster method for identification. False negative result may be due to low number of bacterial load which can't detected by conventional methods (Chen et al., 2015). Also inhibition of some microbes to the selective microbe's appearance on the media, so addition of selective enrichment media is also necessary to suppress the natural background microorganisms to improve detection efficacy and to avoid false result. while disadvantage of PCR that it can't allow the microorganisms to be retained for further cultivation beside that the high cost of PCR. Also it depends upon the efficient of DNA extraction, PCR method haven't the ability to distinguish between the DNA of dead and viable cells. PCR method consider as expensive technique.

Results of antimicrobial susceptibility of *Staphylococcus aureus* strains isolated from the examined samples of beef and chicken meat products revealed that Oxacillin (OX) was the most susceptible antimicrobial for *Staphylococcus aureus* strains then Ciprofloxacin (CP). While Neomycin (N) was the lowest susceptible antimicrobial for *Staphylococcus aureus* strains followed by Oxytetracycline (T). Finally, the results proved that multiple antibiotic resistances are widely spread among isolated *Staphylococcus aureus* strains and approved the fact that the food chain can be considered as the main route of transmission of antibiotic resistant bacteria between the animal and human populations. The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due to longer duration of illness, additional tests and use of more expensive drugs. This study concluded beef and chicken meat

products could be constituted as a potential hazard to human health. Vendors should receive education in food hygiene. Special attention should be given to the causes of diarrhea, the transmission of diarrheal pathogens, application of strict hygienic measures during production, processing, handling and storage of raw materials and final products.

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