

## FOOD POISONING ABILITY OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM MEAT PRODUCTS AND POULTRY MEAT

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### ABSTRACT

A total of 200 samples including 100 samples from fresh and frozen chicken meat (breast and thigh muscles) and 100 samples from meat products (Minced meat, Burger, Luncheon and Sausages) were collected from Aswan markets. All samples were bacteriologically and biochemically examined for isolation and identification of *Staph aureus*, PCR detection of *sea*, *seb*, *sec* genes specific for staphylococcal enterotoxins (SEs). Prevalence of *Staph aureus* in fresh chicken meat were 72% and 68% in breast and thigh muscles, respectively, while in frozen chicken meat were 52% and 40% in breast and thigh muscles, respectively. Prevalence of *Staph aureus* in meat products were 20%, 24%, 0%, 0% in minced meat, luncheon, burger and sausage, respectively. Genetic investigation of toxigenic genes showed presence of *sea* and *sec* in all positive isolates and absence of *seb* gene. Ability of *Staph aureus* to cause food poisoning to the consumer depends mainly on the presence or absence of enterotoxins-forming genes in the isolated bacterium and PCR is a sensitive and reliable method for detection of virulent strains of *Staph aureus* in meat and poultry.

**Key words:** *Staphylococcus aureus*, meat, poultry, products, prevalence, enterotoxins, PCR.

### INTRODUCTION

*Staphylococcus aureus* (*Staph aureus*) is a gram positive bacterium secret enterotoxins which responsible for food poisoning in human through consumption of meat and poultry (Genigeorgis, 1989, Wieneke *et al.*, 1993), which containing enough dose of pre-prepared toxins (Dinges *et al.*, 2000, Le Loir *et al.*, 2003). The most characteristic feature of staphylococcal food poisoning (SFP) is the rapid onset through 3-8 hours as well as nausea, vomiting, abdominal cramps and rarely diarrhea and lasts for 1-2 days. Sever course can occur in children and elderly who get infection through consumption of processed meat and dairy products. The implicated foods are prepared and handled in unhygienic manner and stored in higher temperatures. Degree of contamination and the environmental conditions may favor the growth and multiplication of the microorganism and subsequently the formation of large amounts of enterotoxins (Downs and Ito, 2001). *Staph aureus* is the second food borne pathogen causing food poisoning in the world after Salmonella (Atanassova *et al.*, 2001). *S. aureus* conforms a large proportions of nosocomial infections especially methicillin – resistant *Staph aureus* (MRSA) which

resistant to several antibiotics such as quinolones (Ortega *et al.*, 2010). *Staph aureus* normally inhabit about 20% of people and 60% are carriers (Kluytmans *et al.*, 1997). It is found normally in the nasal cavity of most people which causing infection in people with lowered immunity especially in carrier persons while non-carrier persons can get infection through consumption of contaminated foods (Von Eiff *et al.*, 2001). Enterotoxin type A is the most common cause of food poisoning everywhere beside other types (Argudin *et al.*, 2010). Staphylococcal enterotoxins are resistant to heat, acidity and proteolytic enzymes and preserve activity after processing and digestion, it is active in low micrograms (Schantz *et al.*, 1965). *Staph aureus* is a very serious pathogen in food poisoning and in hospital infections and characterized by production of virulent heat stable toxins which are responsible for toxic shock like syndrome and food poisoning as well as induction of T cells proliferation as superantigen (Harris *et al.*, 1993, Martin *et al.*, 2003, K erouanton *et al.*, 2007). Hemolysin, thermonuclease, lipases and hyaluronidase are virulence factors responsible for the pathogenesis of *Staph aureus* (Sandel and McKillip, 2004, Normanno *et al.*, 2007, Kuroda *et al.*, 2007). Enterotoxins type A, D and B are the most risky and notable ones in food poisoning. Type A is more common then type B which used as inhalational bioweapon (Ler *et al.*, 2006). Type D is the second common cause of food poisoning and in a very small dose (Bergdoll *et al.*, 1981). Type E has the ability to

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cause food poisoning while type F causes toxic shock syndrome (Morris *et al.*, 1972). Type G, H and I are not common in food poisoning although they implicated in outbreak in Taiwan (Chen *et al.*, 2004). Type H also was isolated as a causative agent of massive food poisoning from reconstituted milk in Osaka, Japan in 2000 (Ikeda *et al.*, 2005). Therefore, this work was planned to study the prevalence of *Staph aureus* in meat products and poultry meat samples as well as PCR detection of staphylococcal enterotoxins (SEs)-forming genes in isolated bacteria,

## MATERIALS AND METHODS

### Samples

A total of 200 samples including 100 samples from chicken meat; 50 fresh and 50 frozen (breast and thigh muscles) and 100 samples from meat products (minced meat, burger, luncheon and sausages) were collected from Aswan markets. Sampling box containing ice pads was used for carrying the samples from market to laboratory maintaining low temperature. Samples were preserved in sterile polyethylene bags in the refrigerator. Twenty five grams of each chicken meat or meat product sample were aseptically transferred to sterile stomacher bag, homogenized by using Stomacher® 400 Circulator (Seward Ltd., UK) and mixed in 225 ml Buffered Peptone Water (BPW).

### Isolation and Identification

The mixture samples were incubated at 37 °C for 18-24 hours. Pre-incubated samples (0.1 ml) in BPW were spread on the surface of Baird-Parker agar (BPA) medium supplemented with Egg-Yolk Tellurite Emulsion and Mannitol salt Agar (MSA) (Oxoid Limited, Hampshire, England), a selective media for *Staph aureus* and incubated at 37 °C for 24-48 hours. Black colonies surrounded by whitish halo zone formation on BPA and yellow colonies on MSA were considered presumptive *Staph aureus*, confirmed with the help of Gram's staining, coagulase, catalase and other biochemical tests (Szabo, 2000, Bennett and Lancette, 2001).

### Genetic Detection

DNA extraction was carried out by using boiling method; DNA was prepared by the whole-cell

procedure. Each DNA template was prepared by using approximately half a loopful of culture transferred to 1 ml of TE buffer (pH 8) and mixed in suspension. The resulting suspension was heated at 100°C for 10 minutes and centrifuged at 13000 rpm for 5 minutes. A five µl aliquot was directly used as a template for PCR amplification (Lawrynowicz-Paciorek *et al.*, 2007). Primer sequences were used as forward and reverse for *sea*, *seb* and *sec* genes used for identification of toxin genes (Table 1). Multiplex PCR amplification of toxigenic genes was carried out, each reaction consists of 2.5 µl of 10x buffer, 10 µl master mix, 0.5 µl each primer, 3 µl DNA template and nuclease free water till 25 µl volume (Rahimi, 2013). Thermacycler (Eppendorf, Germany) was used with initial denaturation step at 94°C for 5 minutes followed by 30 cycles (denaturation at 94°C for 0.5 minute, annealing at 59°C for 0.5 minute and extension at 72°C for 0.5 minute for toxin genes) (Akbar and Anal, 2013), ending with final extension at 72°C for 10 minutes. Amplified products were analyzed by 1.5% agarose gel electrophoresis stained with ethidium bromide and visualized on UV transilluminator (Martineau *et al.*, 1998, Rall *et al.*, 2008, Zouharova and Rysanek, 2008).

## RESULTS

Overall prevalence of pathogenic coagulase-positive *Staph aureus* was 70%, 46% and 11% in fresh chicken meat, frozen chicken meat and meat products samples, respectively. Prevalence of *Staph aureus* in breast and thigh muscles of fresh chicken was 72% and 68% while in breast and thigh muscles of frozen chicken was 52% and 40%, respectively (Table 2). In meat products, prevalence of *Staph aureus* were 20% and 24% in minced meat and luncheon, respectively, while burger and sausage were free from contamination by *Staph aureus*. Genetic investigation of toxigenic genes showed presence of *sea* gene in 51% of fresh chicken meat, 35% of frozen chicken meat, 100% of minced meat and luncheon positive - *Staph aureus* isolates while *seb* gene was failed to be detected in examined samples while *sec* gene was found in 37% of fresh chicken meat, 17% of frozen chicken meat and 100% of minced meat and luncheon positive *Staph aureus* isolated (Table 3).

**Table 1:** Primer sequences and expected size of PCR amplified gene targets of *Staph aureus* (Gayathri and Prakash, 2014).

Gene	Primer	Oligonucleotide sequence (5' → 3')	Product size(bp)
sea	sea (F)	TTGCAGGGAACAGCTTTAGG	247
	sea (R)	TACCACCCGCACATTGATAA	
seb	seb (F)	CGCATCAAACACTGACAAACGA	243
	seb (R)	CCGTTTCATAAAGGCGAGTTG	
sec	sec (F)	TCCGTTGGCTTTTCACTTTT	163
	sec (R)	GTAAATCGGGTGGTGCAAT	

**Table 2:** Prevalence of pathogenic *Staph aureus* in fresh chicken meat, frozen chicken meat and meat products.

Type of sample	Number examined	Positive number	Samples Percentage	
Fresh chicken meat	Breast	25	18	72
	Thigh	25	17	68
	Total	50	35	70
Frozen chicken meat	Breast	25	13	52
	Thigh	25	10	40
	Total	50	23	46
Meat products	Minced	25	5	20
	Luncheon	25	6	24
	Burger	25	0	0
	Sausage	25	0	0
	Total	100	11	11

**Table 3:** Prevalence of toxigenic genes (*sea*, *seb*, *sec*) in *Staph aureus* isolated from fresh chicken meat, frozen chicken meat and meat products.

Sample examined	Number	<i>sea</i>		<i>seb</i>		<i>sec</i>	
		+ve	%	+ve	%	+ve	%
Fresh chicken meat	35	18	51	0	0	13	37
Frozen chicken meat	23	8	35	0	0	4	17
Minced meat	5	5	100	0	0	5	100
Luncheon	6	6	100	0	0	6	100
Total	69	37	53.6	0	0	28	40.6

## DISCUSSION

In this study, the overall prevalence of pathogenic coagulase-positive *Staph aureus* was 70%, 46% and 11% in fresh chicken meat, frozen chicken meat and meat products samples, respectively. It is noticed that highest rate of contamination was in fresh chicken meat (70%) then in frozen meat chicken (46%) and lower rates in meat products (20% in minced meat and 24% in luncheon). Comparison of these results with results of other authors revealed that overall prevalence of *Staph aureus* in chicken meat (70%, 46%) was in agreement with results such as: 85.71% (Capita *et al.*, 2001), 68.53% (Islam *et al.*, 2014), 67.5% (Gundogan *et al.*, 2005), 57.1% (Kitai *et al.*, 2005), 50.98% (Rasheed, 2011), 41% (Waters *et al.*, 2011) and higher than other results of 25% (Bhargava *et al.*, 2011), 18.18% (Akbar and Anal, 2013), 17.8% (Hanson *et al.*, 2011), 12.8% (Heo *et al.*, 2008), 7.8% (Lin *et al.*, 2009). Prevalence of *Staph aureus* in meat products (20% in minced meat and 24% in luncheon) was in agreement with results such as: 20.5% (Bhargava *et al.*, 2011), lower than results such as: 63% (Jackson *et al.*, 2013), 45.6% (van Loo *et al.*, 2007), 50% (Abdallahman *et al.*, 2015), 37% (Waters *et al.*, 2011) and higher than results such as: 10% (Heo *et al.*, 2008), 6.9% (Hanson *et al.*, 2011), 6.1% (Gutierrez *et al.*, 2012). Results revealed that 51% of fresh chicken meat, 35% of frozen chicken meat, 100% of minced meat and luncheon having

enterotoxin A-producing gene (*sea*) which is responsible for occurrence of staphylococcal food poisoning in consumers and considered the main cause of emetic symptoms. About 37% of fresh chicken, 17% of frozen chicken and 100% of minced meat and luncheon having enterotoxin C - producing gene (*sec*) while enterotoxin B-producing gene (*seb*) was absent in all samples. These results indicated that isolated strains of *Staph aureus* having ability to produce one or more than one enterotoxins which responsible for food poisoning in consumers and appearance of their symptoms.

Contamination of meat by *Staph aureus*, which may due to unsanitary conditions during processing and preparation, conform a high risk to the consumers (Gundogan *et al.*, 2005). Certain steps in poultry slaughter such as scalding, defeathering, washing and chilling may increase the incidence of contamination of poultry meat by *S. aureus* (Hansson, 2001, Spescha *et al.*, 2006). Large proportions of meat and poultry get contaminated by *Staph aureus* which reached 37-77% and about 52% of the isolates are resistant to multiple antibiotics (Waters *et al.*, 2011). *Staph aureus* produce several exotoxins such as toxic shock syndrome toxins, coagulase, hemolysin, exfoliative toxin and about fifteen types of enterotoxins (Mehrotra *et al.*, 2000). Shock syndrome toxin and enterotoxins type A and B are more dangerous toxins and superantigens (Gunaydin *et al.*, 2011). The higher

contamination of retail meat by *Staph aureus* is the higher potential of MRSA transfer to the consumers (Shukla *et al.*, 2010). It is found that incidence of *Staph aureus* in poultry meat (65.8%) was higher than that of beef (20-25%) or pork (26-29%) (Shimizu *et al.*, 1991, Jiang *et al.*, 2001). Poultry meat get contaminated by *Staph aureus* through two main sources; the first one is by poultry types which found in the nasal cavity and intestines of the birds and contaminate the meat during slaughter steps and the second one is the human types which found on the skin and hands of workers who handle and prepare the poultry meat (Shimizu *et al.*, 1986, Saitoh *et al.*, 1994, Arai *et al.*, 2004). The food poisoning ability and the pathogenicity of *Staph aureus* are attributed mainly to formation of exo- and endotoxins (Sandel and McKillip, 2004).

About fifteen enterotoxins produced by *Staph aureus* (Atichou *et al.*, 2004), types A, B, C, D and E are the more common and considered the main cause of food poisoning while the other types such as G and O may play minor role in food poisoning (Jay *et al.*, 2005). The role of the novel discovered types of enterotoxins in the food poisoning or other affections is still not clear enough and need more research. The use of innovative genetic characterization of enterotoxin forming genes may be useful in the study of these toxins. The majority of *Staph aureus* strains isolated from food produce enterotoxins type A, C and D (Rahimi, 2013). The enterotoxins are heat stable and resist denaturation to still intact in the food and subsequently induce food poisoning. Also, the major problem is the occurrence of multidrug resistant strains of *Staph aureus* which causing antibiotic-associated diarrhea in the hospitals (Pinchuk, *et al.*, 2010).

## CONCLUSION

Meat products and poultry meat products are considered important sources for pathogenic *Staph aureus* which get contaminated either from animal origin or from human during processing and preparation. All *Staph aureus* isolates contained one or more enterotoxin-forming genes which give the bacteria the ability to cause food poisoning to the consumers. Enterotoxin type A and type C were considered the main enterotoxins and may be responsible for occurrence of food poisoning outbreaks.

## AUTHORS' CONTRIBUTIONS

Author performs collection, preparation, processing, and analysis of samples, isolation of bacteria, data acquisition, writing, preparation and revision of manuscript.

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## قدرة ميكروب المكور العنقودي الذهبى المعزول من اللحوم و الدواجن على إحداث التسمم الغذائى

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تم إجراء هذا البحث لدراسة معدل انتشار ميكروب المكور العنقودي الذهبى فى منتجات اللحوم ولحوم الدواجن وللكشف على الجينات المكونة للسموم البكتيرية فى ميكروب المكور العنقودي الذهبى. تم تجميع عدد ٢٠٠ عينة منها ١٠٠ عينة من الدواجن الطازجة والمجمدة من الصدور والأوراك و ١٠٠ عينة من منتجات اللحوم الحمراء بواقع ٢٥ عينة من كل من اللحوم المفرومة واللانشون والبرجر والسجق لإجراء هذا البحث. تم إجراء الفحص البكتريولوجى و البيوكيميائى والجينى باستخدام تفاعل إنزيم البلمرة المتسلسل. دلت النتائج على أن معدل إنتشار ميكروب المكور العنقودي الذهبى كان ٧٢% و ٦٧% و ٥٢% و ٤٠% فى صدور وأوراك الدواجن الطازجة والمجمدة على التوالى و ٢٠% و ٢٤% فى اللحوم المفرومة واللانشون بينما كانت عينات البرجر والسجق خالية من هذا الميكروب. معدل تواجد الجين أ كان ٥١% فى الدواجن الطازجة و ٣٥% فى الدواجن المجمدة و ١٠٠% فى اللحوم المفرومة واللانشون ومعدل تواجد الجين ج كان ٣٧% فى الدواجن الطازجة و ١٧% فى الدواجن المجمدة و ١٠٠% فى اللحوم الحمراء المفرومة واللانشون بينما خلقت جميع العينات من الجين ب. خلصنا من هذا البحث أن قدرة ميكروب المكور العنقودي الذهبى على إحداث التسمم الغذائى للإنسان تعتمد أساسا على مدى إحتوائه على الجينات المكونة للسموم البكتيرية وأن الجين أ هو الجين الأشهر والرئيسى لإحداث هذا التسمم الغذائى فى الإنسان من خلال إستهلاك اللحوم والدواجن.