Staphylococcus aureus Isolated from Raw Meat Products and Food handlers: Prevalence, Antimicrobial Susceptibility and Molecular characterization

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Abstract: The current work aimed to estimate the occurrence of *Staphylococcus aureus* (*S. aureus*) in raw meat products and their food handlers, beside studying the antimicrobial susceptibility of the obtained isolates of *S. aureus* as well as molecular characterization of *clf*A gene specific for coagulase positive *S. aureus* and enterotoxin associated genes were attempted. A total of 75 meat products including minced beef, sausage and beef burger (25 / each) beside 50 human hand and nasal swabs (25 / each) were collected randomly from different local supermarkets and butcher shops in Behera Governorate, Egypt between January and June, 2017. It was found that the prevalence of *S. aureus* was 48, 56, 36, 76 and 64 % in minced beef, sausage, beef burger, hand and nasal swabs, respectively. Moreover, it was recorded that the prevalence of coagulase positive *S. aureus* in the examined samples was 20, 20, 12, 32 and 28% in the examined samples, respectively while the prevalence of coagulase negative *S. aureus* was 28, 36, 24, 44 and 36% of the examined samples respectively. As regard to antimicrobial susceptibility profile, it was noticed that coagulase positive *S. aureus* strains scored the highest sensitivity to Amikacin then moderate sensitivity to Doxycycline and Erythromycin while they were found to be weakly sensitive to Ciprofloxacin, Gentamycin and Flumequine. On contrary, they were non-sensitive to Enrofloxacin and Oxytetracycline. On the other side, it was noticed that Coagulase negative *S. aureus* isolates were highly sensitive to Amikacin and Erythromycin and quite sensitive to Ciprofloxacin and Oxytetracycline while they were resistant to Doxycycline and Flumequine.
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1. Introduction:

Staphylococcus aureus is representing the most frequent zoonotic food- borne pathogen isolated from food of animal origin that requires understanding its molecular ecology in food, especially raw meat harboring isolates containing multiple toxin genes **(Song et al., 2015).** Also, it remains one of the most intensively investigated bacterial species in human and animals. It is an adaptable, opportunistic pathogen with abilities to persist and multiply in a variety of environments and causes a wide scale of diseases **(Cucarella et al., 2004).** In man, *S. aureus* is a common bacterium found on the skin and nasal passages of healthy people. Approximately 25- 40% of the population is colonized with *S. aureus*. Moreover, it is a common cause of skin and soft tissue infections **(Francois et al., 2005)** and sometimes causes severe disease such as pneumonia, bacteremia, meningitis, sepsis, and pericarditis and food poisoning **(Gao and Stewart, 2004)**.

It is generally agreed that the internal tissues of healthy slaughter animals are free of bacteria at the time of slaughter. However, under the current practices of meat processing, it is impossible to guarantee sterility of the final products **(Schaumburg et al., 2014).** Meat products are often get contaminated by *S. aureus* from humans during handling as a result to bad hygienic habits of handlers such as coughing, sneezing and contaminated hands during handling products and equipment as nasal pathways (anterior nares, nasopharnx) and skin of these persons are colonized with *S. aureus* **(Zouharova and Rysanek, 2008)**.

S. aureus can produce several virulence factors including enterotoxins which are heat stable, retain their biological activity even after thermal processing of food and also resistant to gastrointestinal proteases such as pepsin **(Cremonesi et al., 2007)**. To date, 19 types of Staphylococcal enterotoxins (SEs), including the classical types (SEA to SEE) and the newly discovered types (SEG to SEU) have been reported (**Pelisser et al., 2009**). Classical enterotoxins types A, B, C, D and E were responsible for 95% of these outbreaks (**Bergdoll and Adlam, 1983**), the majority have been attributed to SEA and the remaining 5% were associated with newly identified SEs **(Rosec and**

Gigaud, 2002) therefore, the presence of *S. aureus* in food can be considered a potential health risk.

Most food borne illness outbreaks are resulted from ingestion of food containing from 20ng to >1 µg of SE that is sufficient to cause intoxication in human. These symptoms appear within few hours (1-6 hours) after consuming food characterized by nausea, vomiting, abdominal cramps and diarrhea. Luckily, these symptoms resolve within 24-48 hours without treatment and deaths rarely occur specifically in the very young or elderly (**Normanno et al., 2005**). Sanitary food handling, proper cooking and refrigerating could prevent such food borne illness **(FSIS, 2003).**

Antimicrobial resistance is a main public health worry worldwide. The expansion of resistance both in humans' and animals' bacterial pathogens has been allied with the widespread remedial use of antimicrobials or their administration to food producing animals as growth promoters **(Barber et al., 2003).** *S. aureus* has become resistant to various antimicrobial agents including the commonly used penicillin-related antibiotics as oxacillin, methicillin and other beta lactams **(Boyce et al., 2005).**

In the last few years, the use of molecular methods for bacterial typing has proven a helpful method in human and veterinary epidemiological investigations to identify bacterial strains, virulence factors and targeting antibacterial drugs for more effective disease control **(Middleton et al., 2002).** Moreover, phenotypic identification of bacterial contamination of meat products is considered as time consuming and often problematic in many aspects. Polymerase chain reaction (PCR) has been reported to be very successful and reliable technique for detection of the genes that are responsible for production of enterotoxins in *S. aureus* **(Johnson et al., 1991).**

Considering the aforementioned points, our investigation was conducted to study the prevalence, antimicrobial susceptibility and molecular characterization of *S. aureus* isolated from some raw meat products including minced beef, sausage and beef burger retailed for sale in Alexandria province, Egypt and the role of food handlers in contamination of these products.

2. Materials and Methods:

2.1. Samples:

Meat products:

A grand total of 75 raw meat products samples represented by minced meat, sausage and beef burger (25 of each) collected randomly from different supermarkets and butcher shops at Alexandria Province during the period extended from January to June 2017. Samples were kept in a separate plastic bag and transferred with the minimum delay to the laboratory under possible aseptic conditions to be examined for detection of *S. aureus*.

Swabs of food handlers:

A special consent was obtained from food handlers at each supermarket and butcher shop for collection of hand and nasal swabs. A total of 25 hand swabs were collected by rolling a moistened sterile swab over the palm of hands, area between fingers, finger tips and nails and then inserted into tubes containing buffered peptone water (BPW) for pre-enrichment **(Cobeljic et al., 1996)**. Hand swab samples were obtained during work time. In addition, a total of 25 nasal swabs were collected by rubbing a moistened sterile swab into one naris, rotated it against the anterior nasal mucosa and repeated with the same swab in the 2nd naris **(VandenBergh et al., 1999).**

2.2. Processing of samples:

Meat products:

It was performed according to the procedures describe by **APHA, (2001**). 25 g of each sample were aseptically transferred into sterile blender flask containing 225 ml of sterile BPW 1% and homogenized using stomacher (Lab. Blender 400, Seward Lab, London) and incubated at 37 °C for 24 hours.

Swabs of food handlers:

Each swab was inoculated into a sterile tube containing BPW and incubated at 37 °C for 24 hours.

2.3. Isolation and identification of *S. aureus*

Isolation and identification of *S. aureus* were carried out according to per Bergey's manual of determinative bacteriology **(Holt et al., 1994).** From each of previously prepared dilution, 0.1 ml was evenly spread over a dry surface of Baird parker agar plate medium with egg yolk Tellurite with a sterile bent glass rod using surface plating Technique. The inoculated plates were incubated at 37° C for 24 hours in an inverted position. The black shiny colonies with narrow white margins surrounded by a clear zone were *S. aureus*. Screening for pathogenic *S. aureus* was done by performing various biochemical assays, including Coagulase test, DNase test **(Baird, 1996),** and Thermostable nuclease test (TNase) **(Lachica et al., 1971).**

2.4. Antimicrobial susceptibility

The antimicrobial susceptibility test was performed for isolated Coagulase positive and Coagulase negative *S. aureus.* Standard agar disk diffusion method was employed according to the recommendations of the Clinical and Laboratory Standards Institute **(CLSI), (2012)** using commercial antibiotic disks (Oxoid). Suspension of each of the test organisms was made by collecting a loopful of colony from each plate and inoculating in a nutrient broth. The tubes of the subcultured organisms were

incubated at 37°C for 24 hours. Using different sterile swab sticks, 24 hour old culture of each of the test organisms was collected. The swab sticks containing the different bacterial cultures were swirled into different test tubes containing 10 ml of sterile water. The content of each of the tubes was properly homogenized before the inoculation. Another set of sterile swab sticks were dipped into each of the bacterial solution and were used to inoculate the solidified Nutrient agar plates ensuring that the plates were completely covered for uniform growth.

Antibiogram discs:

The antimicrobial discs were obtained from (**Oxoid, England**). The graduated rule to 0.5 mm was used for reading the diameter of the zones of inhibition twice at right angles. The used antibiotic discs were Ciprofloxacin (CIP) (5 mg), Gentamycin (CN) (10 mg), Flumequine (30 mg) (UB), Enrofloxacin (5 mg) (ENR), Amikacin (AK) (30 mg) Doxycycline (30 mg) (DO), Oxytetracycline (30 mg) (OT) and Erythromycin (15 mg) (E).

2.5. Molecular identification of *clf*A specific for coagulase positive *S. aureus* and enterotoxins associated genes:

The technique was carried out according to **Reinoso et al., (2004).** Genomic DNA extraction was carried out using GeneJET Genomic DNA Purification Kit (Fermentas) following the instruction procedures. The collected DNA was then kept at - 20 °C until used. 20 ng of chromosomal DNA was used per reaction. Amplifications were performed in 25 µl of buffer solution containing 3 µM of oligonucleotides, 200µM of each deoxy nucleoside triphosphate, 3.5 mM $MgCL_2$ and 2.5U of DNA Taq polymerase. The mixtures were then overlaid with mineral oil and amplification was performed in PCR thermal cycler.

Table (1): Oligonucleotides sequences of coagulase positive *S. aureus* specific genes (*clf*A) and enterotoxins associated genes.

Gene	Primer sequence (5'-3')	Length of amplified products	Reference
*clf*A	GCAAAATCCAGCACAACAGGAAACGA	638 bp	**Mason et al., (2001)**
	CTTGATCTCCAGCCATAATTGGTGG		
Sea	GGTTATCAATGTGCGGGTGG	102 bp	**Mehrotra et al., (2000)**
	CGGCACTTTTTTCTCTTCGG		
Seb	GTATGGTGGTGTAACTGAGC	478 bp	
	CCAAATAGTGACGAGTTAGG		
Sec	AGATGAAGTAGTTGATGTGTATG	257 bp	
	CACACTTTTAGAATCAACCG		
Sed	CCAATAATAGGAGAAAATAAAAG	317 bp	
	ATTGGTATTTTTTTTCGTTC		

The amplification consisted of a cycle of predenaturation at 94° C for 5 minutes followed by 40 cycles of 1 minute at 93° C, 1.5 minute at 55° C and 1 minute at 72° C and final extension at 72° C for 8 minutes The amplified products were analyzed on agarose gel (consisted of 2% agarose and 5 µL of ethidium bromide in 1 x Tris – Acetate EDTA (TAE) buffer. Samples were then electrophoreses at 100 volts for one hour, the products were visualized under ultra violet transiluminator and photographed.

3. Results:

Table (1): Prevalence of *S. aureus* in meat products and human samples

Samples (n = 25 / each)	Coagulase positive *S. aureus*		Coagulase negative *S. aureus*		Total	
	No.	%	No.	%	No.	%
Minced meat	5	20.0	7	28.0	12	48.0
Sausage	5	20.0	9	36.0	14	56.0
Beef burger	3	12.0	6	24.4	9	36.0
Hand swabs	8	32.0	11	44.0	19	76.0
Nasal swabs	7	28.0	9	36.0	16	64.0
Chi^2	10.55**				9.44**	

** = Significant at (P < 0.01).

Table (2): Antimicrobial susceptibility profile of *S. aureus* recovered from raw meat products and human samples

Isolates / Antimicrobial disc	Coagulase positive *S. aureus*		Coagulase negative *S. aureus*	
	Zone around discs	Indication of Sensitivity	Zone around discs	Indication of sensitivity
Ciprofloxacin (CIP)	3 mm	+	6 mm	++
Gentamycin (CN)	2 mm	+	8 mm	+++
Flumequine (UB)	2 mm	+	0 mm	-
Enrofloxacin (ENR)	0 mm	-	6 mm	++
Amikacin (AK)	16 mm	++++	15 mm	++++
Doxycycline (DO)	4 mm	++	0 mm	-
Oxytetracycline (OT)	0 mm	-	7 mm	+++
Erythromycin (E)	4 mm	++	10 mm	++++

-: Resistance **+: Weakly sensitive** **++: Moderately sensitive**
+++: Quite sensitive **++++: Highly sensitive**

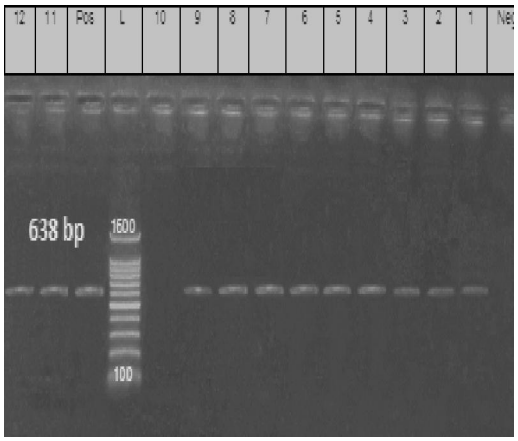

Photo (1): PCR products of *S. aureus* isolates of *clf*A coding gene

Lane L: 100 bp ladder
Lane Neg Negative control
Lane Pos Positive control
638 bp representing coagulase positive *S. aureus* specific genes (*clfA*)

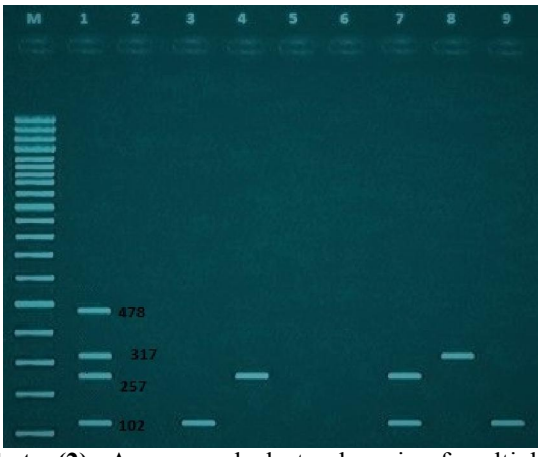

Photo (2): Agarose gel electrophoresis of multiplex PCR of *sea* (102 bp), *seb* (478 bp), *sec* (257 bp) and *sed* (317 bp) enterotoxin genes for characterization of *S. aureus*

Lane M: 100 bp ladder.
Lane 1: Control positive for *sea, seb, sec* and *sed* genes.
Lane 2: Control negative.
Lanes 3, 9: Positive *S. aureus* strains for *sea* gene.
Lane 4: Positive *S. aureus* strains for *sec* gene.
Lanes 5, 6: Negative *S. aureus* strains for enterotoxins.
Lane 7: Positive *S. aureus* strain for *sea* and *sec* genes.
Lane 8: Positive *S. aureus* strain for *sed* gene.

4. Discussion:

Staphylococci are normal Inhabitants of the skin and mucous membranes of animals and humans. Pathogenic strains are usually coagulase- positive **(Braddy, 2002)** and have been found to cause diseases in their hosts throughout the world **(Collins et al., 2010)**. Staphylococcal food poisoning (SFP) is

one of the most common foodborne illnesses resulting from ingestion of staphylococcal enterotoxins produced in food by enterotoxigenic strains of *S. aureus* (**Asao et al., 2003**). Based on the previous facts, it was of utmost importance to focus on the occurrence of *S. aureus* in raw meat products as a mean of tracing back their sources of contamination (nasal and hand swabs of food handlers). Also, studying the antimicrobial susceptibility of the obtained isolates of *S. aureus* as well as molecular characterization of *clf*A gene specific for coagulase positive *S. aureus* and enterotoxin associated genes were attempted.

The recorded data in **Table (1)** showed the incidences of *S. aureus* among examined raw meat products and food handlers. It was observed that there was a significant difference ($P < 0.01$) of the incidence of coagulase positive *S. aureus* and coagulase negative *S. aureus* among different examined samples of raw meat products and human samples. The results clarified that the highest incidence of coagulase positive *S. aureus* was observed in hand swabs samples 8 (32 %) followed by nasal swabs 7 (28 %) then minced meat and sausage 5 (20% of each), while the lowest incidence was observed in beef burger 3 (12 %). Meanwhile, the highest incidence of coagulase negative *S. aureus* was also observed in hand swabs 6 (44 %) followed by nasal swabs and sausage 9 (36 % of each) while the lowest incidence occurred in minced meat 7 (28 %). Moreover, statistical analysis cleared that, the total incidence of *S. aureus* differed significantly ($P < 0.01$) among examined raw meat products and food handlers confirming the role of food handlers in contaminating raw meat products with *S. aureus*. The highest incidence of *S. aureus* was recorded in hand swabs 19 (76 %) followed by nasal swabs 16 (64 %), sausage 14 (56 %), minced meat 12 (48 %) and lastly beef burger 9 (36 %). Detection of pathogenic *S. aureus* in the examined meat products was a matter of concern making these products non-complying with the Egyptian Standards noted that raw meat products should be free from pathogenic *S. aureus* **(EOS, 2009)**.

The recorded result was nearly similar to that recorded by **Tarabees et al., (2015)** who examined a total of 120 samples of beef burger, minced meat and fresh sausage (40/each) and found that *S. aureus* was isolated at the percentage of 27.5, 70 and 45%, respectively by using traditional methods. Also, the presented data in the current study was lower than that obtained by **Al-Kour, (2001) and Ouf, (2001).** On contrary, a higher incidence was obtained by **Abou-Hussien, (2004)** and **Hassanin, (2007).**

Man was the main reservoir of *S. aureus* with 30 to 50% of human population carried bacteria on their skin and nares **(Jay, 1986)**. Moreover, Staphylococci present as normal flora in the throat, nasal area and under the fingernails **(Brooks et al., 2012)**. The role of food handlers in contaminating raw meat products with could not be denied that was supported by **Colombari, (2007)** who stated that contaminated hands from nasal carriers were a major source of cross-contamination.

It was noticed that the prevalence of *S. aureus* was 76 and 64 % in hand and nasal swabs of food handlers, respectively. This result was supported by the findings of **Abdel All et al., (2010)** who recorded that 82% of hand swabs examined were positive for *S. aureus,* **Gwida and EL-Gohary, (2013)** who isolated *S. aureus* from 60% of hand swabs examined. On the other hand, lower prevalence was documented by **El-Gedawy et al., (2014)** (10%). Concerning the prevalence of *S. aureus* in nasal swabs of food handlers in our study (64%), it was higher than that recorded by **Rinsky et al., (2013)** in North Carolina (40%), **Jordá et al., (2015)** in Argentina (32.5%) and **Torky and Abu Tabeikh (2016)** in Egypt found that 30% of examined human pharyngeal swabs were positive for *S. aureus*. On the other side, it was lower than that obtained by **Sarkar et al. (2014)** (72%).

Wide spread use of antibiotics has evolved the emergence of multidrug resistant strains and it makes eradication more difficult and incidence to increase. Multi-resistant *S. aureus* is rather common in hospital settings and farms **(Sakoulas and Moellering, 2008)**. The presented data in **Table (2)** clarified the antimicrobial susceptibility profile of coagulase positive *S. aureus* strains. It was noticed that Amikacin scored the highest sensitivity then doxycycline and erythromycin scored moderate sensitivity while ciprofloxacin, gentamycin and flumequine were found to be weakly sensitive. On contrary, enrofloxacin and oxytetracycline were found non-sensitive against the isolated strains. On the other side, it was noticed that Coagulase negative *S. aureus* isolates were highly sensitive to amikacin and erythromycin and quite sensitive to ciprofloxacin and oxytetracycline while they were resistant to Doxycycline and Flumequine. Nearly similar results were obtained by **Sciezyńska et al., (2012), Saleh et al., (2016)** who found that *S. aureus* isolates were highly susceptible to penicillin, rifompion, ampicillin and novobiocin. In contrast, the isolates showed high resistance to oxacillin, sulphatrimethoprim, vancomycin and Cefotoxin, **Torky and Abu Tabeikh (2016)** who observed that coagulase negative *S. aureus* isolates were highly sensitive to ciprofloxacin and amikacin while intermediate sensitive for ampicillin and gentamycin while they were resistant to amoxicillin and trimethoprime+ sulphamethaxol and **El-Mahrouk and Hanaa (2017)** who found that

amikacin was the drug of choice to *S. aureus* followed by gantamycin and amoxyicillin while they were resistant to methicillin, penicillin, ampicillin, ciprofloxacin and sulpha-methoxazola.

Staphylococcal food poisoning (SFP) is one of the most common foodborne illnesses resulting from ingestion of staphylococcal enterotoxins produced in food by enterotoxigenic strains of *S. aureus.* These enterotoxins are heat-stable and resistant to the action of digestive enzymes (**Brooks, et al. 2001)**. The most common types of these enterotoxins are *sea* to *see*. Isolates carrying toxin genes *sea* to *see* are responsible for 95% of staphylococcal food poisoning outbreaks **(Bergdoll, et al. 1983)**. Therefore, the presence of *S. aureus* in food can be considered a potential health risk. Various typing methods have been used to characterize *S. aureus* isolates. PCR has been used as a simple technique for detecting enterotoxigenic strains **(Asperger and Zangerl, 2003)**. Although the PCR-based approach is specific, highly sensitive and rapid, it can only detect the presence of enterotoxigenic genes, not the production of the SE proteins **(Boerema et al., 2006)**.

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