Benha Veterinary Medical Journal 45 (2023) 136-140

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Staphylococcus aureus incidence in Egyptian meat outlets and butcheries, and their biofilm, antibiotic-resistance, and virulence capabilities

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ARTICLE INFO

Keywords

Staphylococcus Aureus

Equipment Surfaces.

Meat-Contact

Meca

Virulence and Biofilm Traits

Received 22/07/2023

Accepted 17/08/2023

Available On-Line 01/10/2023

ABSTRACT

The goal of this study was to identify the frequency of *Staphylococcus aureus*, as well as their virulence and biofilm-forming characteristics, in meat-contact and equipment surfaces at selling outlets, butcher shops, and supermarkets in Al-Menofia governorate, Egypt. A total of 100 swabs (50 from butcher shops and 50 from supermarkets) were collected to meet these objectives from ten butcher shops and ten supermarkets. Standard culture procedures, the VITEK2 compact system, and PCR techniques were all used to isolate and identify the targeted pathogens. The genetic elements that support virulence and biofilm development features were examined using PCR. *Staphylococcus aureus* was identified in 25% of the swabbed samples. Butchers had a higher detection rate of *S. aureus* than supermarkets (30% vs. 20%) ($P < 0.05$). All five *S. aureus* isolates had the *icaD* and pyrogenic exotoxin genes, whereas three shared the *icaA* and two of the three carried the *mecA* resistance gene. Strong virulence (pyrogenic exotoxin genes and *mecA* resistance gene) and a high incidence of biofilm-producing components in *S. aureus* isolated from meat-contact and equipment surfaces suggest poor hygiene of investigated selling outlets, and butcher shops, which can be attributed to either ineffective or absence of cleaning and disinfection program. This calls for more strict control from Egyptian food safety authorities because otherwise, such serious pathogens might pose concerns.

1. INTRODUCTION

Staphylococcus aureus is rated at the top of several priority pathogens by food safety agencies due to the frequency and severity of illness they cause (Bintsis, 2017). According to the European Union (EU) data from 2019, 9.6 % of samples tested were positive for *Staphylococcus* spp., and *S. aureus* accounted for 74 food-borne outbreaks, with 10.1 % (n=141) of human cases hospitalized (EFSA and ECDC, 2021).

The majority of health issues associated with this food-borne infection often affect the elderly, children, those with compromised immune systems, and healthy adults exposed to exceptionally high levels of a pathogen (CDC, 2020). The source and transmission of many foodborne illnesses, including *S. aureus*, is food animals (Heredia and García, 2018). *Staphylococcus* spp. are widely distributed in nature and can be encountered from infected hosts. *S. aureus* has also been recovered from animal products, including ground beef, pig sausage, ground turkey, salmon steaks, oysters, and shrimp (Bacon and Sofos, 2003; Bintsis, 2017).

To adapt to the harsh environment, the majority of bacteria in the natural environment survive by embedding themselves in biostructures known as biofilms (Zhao et al., 2017). Similarly, improper cleaning and disinfection of contaminated surfaces used in handling and processing meat allows these foodborne pathogens to survive and promote virulence potential (Capozzi et al., 2009; Ripolles-Avila et al., 2022). In a variety of food sectors, biofilms cause serious food safety issues (Srey et al., 2013). Poorly removed biofilm has the potential to cross-contaminate food products because it continuously sheds cells and spores from surfaces that come into contact with both food and non-food items (Kusumaningrum et al., 2003; González-Rivas et al., 2018). Thus, biofilm has been linked to many outbreaks (Dufrenne et al., 2001; Waak et al., 2002; Lapidot et al., 2006). Additionally, biofilms, especially mixed ones, are more durable and chemically resistant, leading to ineffective disinfection (Brooks and Flint, 2008). Also, mixed biofilms might play a crucial role in the horizontal transfer of antimicrobial resistance genes because the proximity of biofilm cells allows for the spread of resistance genes between them (Flemming et al., 2016). *S. aureus* have been found to persist for hours or days after initial contact on hands, clothes, utensils, and surfaces of food-processing facilities (Gajewska and Chajęcka-Wierzchowska, 2020).

The majority of the studies conducted in Egypt focused on estimating the prevalence of *S. aureus* in foods purchased from retail establishments, particularly supermarkets. However, the incidence of these pathogens on food-contact and/or processing surfaces, notably of butcheries, has only been evaluated in a relatively small number of investigations. Moreover, the genetic factors that contribute to the potential of *S. aureus* isolates from surfaces involved

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in food contact or processing to generate biofilms have not been extensively studied. Determining the prevalence and potential public health relevance of *S. aureus* isolated from equipment and meat-contact and -processing surfaces at retail outlets, notably butcher shops and supermarkets in Egypt's governorate of Al-Menofia, was the goal of the current study. The genetic factors influencing the isolated pathogens' capacity to form biofilms were also evaluated.

2. MATERIAL AND METHODS

Ethics approval

All protocols used in this work were approved by Benha University's Faculty of Veterinary Medicine's Institutional Animal Care and Use Committee Research Ethics number (BUFVTM 24-6-23).

2.1. Sample collection

In brief, one hundred environmental swabs were collected from meat-contact and -processing surfaces and equipment in Al-Menofia governorate from August to November 2021, Egypt, from selling outlets, butcher shops, and supermarkets. Surface samples of various surface areas were taken by rubbing with sterile cotton swabs wet with sterile physiological water (Bauwens et al., 2006, ISO, 2015a, b). Swabs were inserted into a screw-capped tube containing 10 mL of buffered peptone water.

2.2. Isolation and identification of Staphylococcus aureus

The ISO 6888-2 method was used to isolate *S. aureus* on Baird Parker agar plates (ISO, 1999). Five isolated colonies were chosen from the agar plates for *S. aureus* identification. The cultures were identified separately using the GPI card (Gram-positive identification) of the automated VITEK2 system (compact model, bioMérieux).

2.3. Molecular Characterization of Staphylococcus aureus isolates

A molecular analysis of five *S. aureus* isolates were conducted. These few isolates were chosen based on the distribution of isolates from various butcher shops because the prevalence of this disease in butcheries has only been evaluated in a very small number of studies due to the difficulties in accessing such stores. The cost and the availability of genes at the time of analysis were also significant factors in this selection. Using the QIAamp DNA Mini Kit (Cat. No. 51304, Qiagen, Hilden, Germany), the silica-membrane-based nucleic acid purification from diverse types of bacterial colonies was carried out following the manufacturer's instructions in 20 minutes. Table 1 contains a complete list of all primers and conditions for polymerase chain reaction (PCR) amplification of different *S. aureus* target genes, including 16S rRNA, icaA, zicaD, and mecA. A 25 µL reaction mixture containing 12.5 µL of Emerald Amp GT PCR Master Mix (Cat. No. RR310A, Takara Bio, Shiga, Japan), 1 µL (20 pmol / µL) of each primer (Midland Certified Reagent Company_ oilgos, USA), 5 µL target DNA, and the remaining volume needed to reach 25 µL was adjusted with deionized PCR grade water was prepared for PCR. The reaction was conducted using a thermal cycler T3 Biometra Trio (Biometra, Analytik Jena, Jena, Germany). Following completion of the amplification, PCR products (6 µL) were electrophoretically separated on a 1.5% agarose gel, stained with ethidium bromide, and examined under UV light in a gel documentation system (Alpha Innotech, Germany's Kasendorf).

2.4. Statistics

SPSS Statistics 20 (SPSS Inc., USA) was used for statistical analysis. The isolates obtained from butchers and supermarkets were compared using descriptive statistics like frequency, and the presence of variations in occurrences between retailers at $P < 0.05$ was identified using the T-test.

Table 1 PCR primers and conditions for *Staphylococcus aureus* gene amplification

Target Genes	Primer	Sequences (5' to 3')	Amplicon size (bp)	Annealing Temperature	Reference
16S rRNA	F	GTA GGT GGC AAG CGT TAT CC	228 bp	55˚C	(Monday and Bohach, 1999b)
	R	CGCACATCAGCGTCAG			
icaA	F	CCT AAC TAA CGA AAG GTA G	1315 bp	49˚C	(Ciftci et al., 2009)
	R	AAG ATA TAG CGA TAA GTG C			
icaD	F	ATGGTCAAGCCCAGACAGAG	198 bp	50˚C	(Ciftci et al., 2009)
	R	AGTATTTTCAATGTTTAAAGCAA			
*mec*A	*mec*A-1	GTAGAAATGACTGAACGTCCGATAA	310 bp	50˚C	(Stegger et al., 2012)
	*mec*A-2	CCAATTCCACATTGTTTCGGTCTAA			

3. RESULTS

The prevalence of *S. aureus* on swabbed surfaces and equipment from butchers and supermarkets is shown in Table 2 as both tentative and confirmed. *S. aureus* presumptive and VITEK2 compact system confirmed incidences were 60% and 25%, respectively. Butchers had a higher prevalence *S. aureus* than supermarkets ($P < 0.05$).

The virulence and biofilm genes of *S. aureus*(*n*=5) isolated from butcher shops and supermarkets are compared in Table 3 and illustrated in Supplementary Figure 1. All *S. aureus* isolates contained the 16s rRNA gene. The *mecA* gene was found in two of the five *S. aureus* isolates. All five *S. aureus* isolates tested positive for *icaD*, and three of them shared *icaA* (Figure 1a to 1d).

Table 2 Presumptive and confirmed prevalence of *Staphylococcus aureus* on food contact surfaces and equipment swabbed from Butchers and Supermarket (*n*=100).

Pathogen	Butchers Swab (*n*=50)				Supermarket Swabs (*n*=50)				Total confirmed (*n*=100)	
	Presumptive		Confirmed		Presumptive		Confirmed			
	No.	%	No.	%	No.	%	No.	%	No.	%
Staphylococcus aureus[1]	39	78	15	30	21	42	10	20	25	25

[1] All *S. aureus* strains were tentatively identified and confirmed using the VITEK2 compact system.

Table 3 Characterization of virulence and biofilm genes of *Staphylococcus aureus* isolated from food contact surfaces and equipment swabbed from Butchers and Supermarkets.

Pathogen (Targeted genes)	Serotypes (ID)	Origin	Genes
S. aureus (*n*=5) (Four genes were analyzed, including 16s rRNA, *icaA, icaD*, and *mecA*)	RBS1	Butcher	PTs,*icaA, icaD*
	RBS4	Butcher	PTs, *mecA, icaA, icaD*
	RBS7	Butcher	PTs, *mecA, icaA, icaD*
	RBS11	Butcher	PTs, *icaD*
	RBS15	Butcher	PTs, *icaD*

S. aureus, S. aureus; PTs, pyrogenic toxins estimated by 16s rRNA.

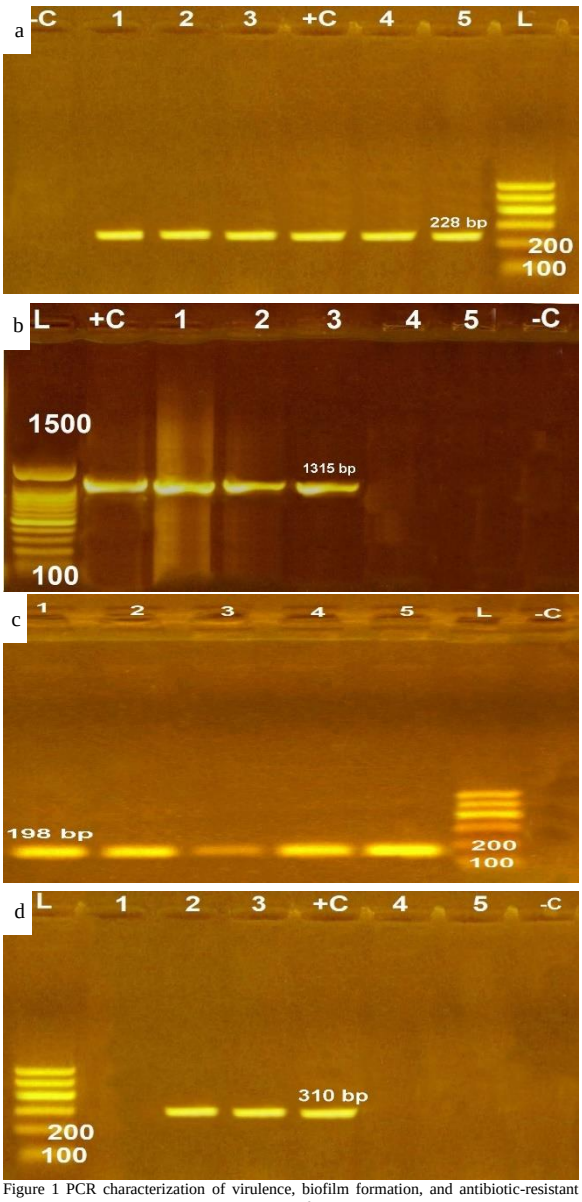

Figure 1 PCR characterization of virulence, biofilm formation, and antibiotic-resistant genes in *Staphylococcus aureus* (*n*=5) isolated from butcher shops and supermarkets with expected amplicon size. The amplified genes were a: 16S rRNA at 228 bp; b: *icaA* at 1315 bp; c: *icaD* at 198 bp and d: *mecA* at 310 bp. Lane M: 100 bp DNA ladder; C+: Positive control; C-: Negative control; Isolates of lanes from 1-8 or 1-2 or 1-5 in each gel were recorded for each targeted gene.

4. DISCUSSION

Ongoing screening of food and related processing, storage, and equipment for foodborne pathogens is required to determine the overall hygiene of food premises and the effectiveness of the cleaning and disinfection program implemented. It is also necessary to have a better understanding of the virulence factors of foodborne pathogens, which is a growing topic, to identify the most effective preventive and control measures within the security of food supplies.

S. aureus was confirmed on 25% of swabbed surfaces and equipment. From July 2011 to June 2016, 35.0% (647/1,850) of the retail meat and meat products in China tested positive for *S. aureus*(Wu et al., 2018). Additionally, retail beef livers, beef, and pork meats sold in Tulsa, Oklahoma, had isolation rates of *S. aureus* that were higher than those found in the current study: 80%, 50%, and 43.3%. *S. aureus* is one of the top five pathogens responsible for an estimated quarter million cases of acquired foodborne illnesses each year in the US (Abdalrahman et al., 2015). One of the strongest-resistant non-spore-forming pathogens, *S. aureus* can survive for extended periods in a dry state outside the body and has been isolated from air, dust, sewage, and water (Kozajda et al., 2019).

One of the most crucial strategies used by foodborne pathogens, such as *S. aureus*, to survive in host cells and harsh environments and to evade host immune responses is biofilm formation (Liu et al., 2023). The ability of bacteria to survive and colonize different environments is associated with significant metabolic, signaling, genetic, and transcriptional changes. In brief, genetic pathogen changes are manifested phenotypically in five stages: reversible attachment, irreversible adhesion, early development of biofilm structure, biofilm maturation, and cell separation, resulting in biofilm formation (Liu et al., 2023). Genetic changes and other multifactorial biofilm processes are specific to the bacteria involved, and Quorum sensing controls the majority of these indices (Funari and Shen, 2022). Quorum sensing (QS) is a communication mechanism between bacteria and control expression of several genes crucial for pathogenesis, such as biofilm formation, bacterial adhesion, host colonization, virulence factor, production of secondary metabolites, and stress adaptation mechanisms such as bacterial competition systems including secretion systems (SS) (Funari and Shen, 2022).

S. aureus isolates were characterized by the highly important *mecA* gene, which encodes high resistance to beta-lactam antibiotics. Two out of the five *S. aureus* isolates tested positive for the *mecA* gene. Isolates carrying such a gene are well-known as Methicillin-resistant *S. aureus* (MRSA), one of the most common causes of nosocomial infections worldwide (Wielders et al., 2002). The fact that this gene is a part of the mobile genetic staphylococcal chromosome cassette mec (SCCmec), which might also contain genetic elements encoding beta-lactam antibiotic resistance, exaggerates the severity (Ito and Hiramatsu, 1998). The *ica* operon in *S. aureus* indicates genetic potential for biofilm or slime production. Full biofilm synthesis in the ica operon necessitates the co-expression of *icaA* and *icaD* (Arciola et al., 2001, Cue et al., 2012). The five tested *S. aureus* isolates possess *icaD*, and three of them shared *icaA*. These results demonstrate the ability of the five isolates to form biofilms at various levels. Previously, these two genes were identified in 61% and 35.2% of clinical strains of *S. aureus* (Arciola et al., 2001, Satorres and Alcaráz, 2007). Other research on 146 *S. aureus* isolates revealed that 24 (16.4%) carried the *mecA* gene and that 75.0% of MRSA isolates carried the *icaA* gene, and that the *icaD* gene was not found in these strains (Omidi et al., 2020). These results are consistent with those of the present research, showing that a significant portion of MRSA strains possess strong biofilm-producing abilities. Twelve strains of *S.aureus* were isolated from food contact surfaces (FCS) of three hotels (Five stars hotels) kitchens located in Cairo, Sharm El-sheikh, and Hurghada governorates (30 samples of each), as well as one meat products processing plant located in Zahraa El-Maadi, Cairo governorate (30 samples), in an earlier study in Egypt. 100% (12/12) of the isolated strains showed a high capacity to generate biofilm, which was categorized as a strong type. In addition, the application of QACs, sodium hypochlorite, and iodine led to reductions in the production of biofilms of 76.77%, 71.38%, and 15.84%, respectively (Hamad et al., 2019). In Algeria, 39 (71.0%) and 23 (41.8%) of 55 *S.aureus* isolates from various sources generated slime and biofilm, respectively. All *S. aureus* strains isolated from food were capable of forming biofilms. The *fnbB* gene, which codes for

microbial surface components that recognize sticky matrix molecules, was detected in all biofilm-producing *S. aureus* isolates from food (Achek et al., 2020).

The ability of *S. aureus* isolates to produce immunomodulatory pyrogenic toxins (PTs), such as *staphylococcal* enterotoxins (SEs) and toxic shock syndrome toxin (TSST), was estimated using 16s rRNA. The 16s rRNA gene was found to be present in all isolates, demonstrating their capacity to produce pyrogenic toxins. The pyrogenic exotoxin (PT) family is one of several virulence factors that promote the staphylococcal ability to successfully persist within a range of hosts by evading host immunologic responses (Monday and Bohach, 1999a). PTs interact with antigen-presenting cells and T-lymphocytes to stimulate cellular proliferation and high-level cytokine expression, resulting in TSST (Monday and Bohach, 1999b). Furthermore, SEs have the unique ability to cause staphylococcal food gastroenteritis (Jablonski, 1997).

These biofilm-contaminated tools could potentially contaminate fresh meat products and carcasses (Vogeleer et al., 2014). Future trials utilizing novel antimicrobials and commercial disinfectants are intended to control isolated organisms.

5. CONCLUSION

In conclusion, *S. aureus* was confirmed in 25% of swabbed samples. Targeted pathogen detection rates were greater in butcheries than in supermarkets (30% vs. 20%, respectively) ($P < 0.05$). All five *S. aureus* isolates shared the *icaD* biofilm-forming gene and the pyrogenic exotoxin (PT) genes, and the three *S. aureus* isolates simultaneously shared the *icaA* and two of the three had the *mecA* resistance gene. Strong virulence (pyrogenic exotoxin genes and *mecA* resistance gene) and a high incidence of biofilm-producing components in *S. aureus* isolated from meat-contact and equipment surfaces indicate poor hygiene in investigated selling outlets, particularly butcher shops, which can be attributed to an ineffective or non-existent cleaning and disinfection program. This necessitates stricter control by Egyptian food safety officials, as this dangerous pathogen could cause severe problems.

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