Investigation of quaternary ammonium compound resistance in *Staphylococcus aureus* strains isolated from several foods and food production facilities

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ABSTRACT

Quaternary ammonium compounds (QACs) are the most significant disinfectants utilised to control the contamination of *Staphylococcus aureus* in food establishments. *S. aureus* is a significant pathogen that carries genes responsible for resistance to QACs, which pose a risk to public health and food safety. The objective of the study was to investigate the prevalence of QAC genes (*qacA/B*, *qacC*, *qacG*, *qacH*, *qacJ*, and *smr*) and benzalkonium minimal inhibitory concentration (MIC) values in *S. aureus* strains isolated from food products and food production facilities (n = 200). The analysis results indicated that the *qacC* gene was the most frequently detected, with a prevalence of 12%. The *qacA/B*, *qacG*, *qacH*, *qacJ*, and *smr* genes were identified at frequencies of 2%, 3%, 1%, 4.5%, and 5%, respectively. The highest MIC level was identified in the surface sample, which carried the *qacG* gene, at a concentration of 6.25 µg mL⁻¹. The study's results highlight the potential risks associated with disinfectant resistance in food establishments. To prevent the transfer of disinfectant resistance genes, which have become a global risk, it is imperative that the rules of disinfectant usage are observed rigorously and that scientific research in this field is diversified.

KEYWORDS

foods, food production facilities, quaternary ammonium disinfectant, qac genes, Staphylococcus aureus



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1. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a foodborne pathogen of global concern, classified by the World Health Organization as a high-level priority II pathogen (Gatadi et al., 2019). Since *S. aureus* is commonly found on the hands, nose, and mucosal membranes, personnel working in the food industry play an essential role as a source of contamination. It has been demonstrated that food handlers who carry *S. aureus* can contaminate food through hand contact or respiratory secretion (Bencardino and Vitali, 2019).

Quaternary ammonium compounds (QACs) are the most preferred disinfectants in the food industry, particularly in meat and dairy production facilities (Sidhu et al., 2001). The prolonged and high-level use of disinfectants in food production facilities has been observed to result in the development of resistance to these disinfectants amongst many microorganisms. Many studies have demonstrated that the inappropriate use of disinfectants can result in the emergence of cross-resistance to antibiotic compounds, which can subsequently facilitate the spread of antibiotic-resistance genes among microorganisms (Wu et al., 2023). The transfer of resistance genes *via* a unit transposon can lead to the spread of resistance development among microorganisms, which can present serious public health problems (Tong et al., 2021).

Gram-positive bacteria such as *S. aureus* are more susceptible to QACs than Gram-negative bacteria. This is because Gram-negative bacteria possess an outer membrane that precludes access of biocides to their cytoplasmic membrane, which is the target site (Boyce, 2023). *S. aureus* has been observed to exhibit acquired resistance, mainly against QACs. The *qac* and *smr* genes are responsible for resistance to QACs, one of the main disinfectants. A total of 6 different plasmids encoded as *qac* efflux pumps have been identified in *Staphylococcus* spp., belonging to two main protein families (Tong et al., 2021). The *qac*A and *qac*B belong to the Major Facilitator superfamily, while *qac*C, *qac*G, *qac*H, and *qac*J belong to the Small Multidrug Resistance (SMR) family (Heir et al., 1999).

This study aimed to investigate the prevalence of *qac* and *smr* genes in 120 *S. aureus* strains isolated from various food products and 80 *S. aureus* strains isolated from food processing facilities. Furthermore, the efficacy and minimum inhibitory concentration (MIC) values of benzalkonium chloride (BAC), frequently used as disinfectant in food establishments, are among the objectives of this study.

2. MATERIALS AND METHODS

2.1. Sample collection and bacterial isolation

A total of 120 *S. aureus* strains were isolated from foods (meat and meat products [n = 18], milk and milk products [n = 96], bakery products [n = 3], ready-to-eat foods [n = 3]) collected around Istanbul. In addition to the isolates above, samples were taken from the surfaces in direct contact with food in food processing facilities, personnel, instruments and equipment, and some food in the facilities, and transported to the laboratory in thermobox at +4 °C. The isolation of *S. aureus* from surface samples was achieved using a swab-rinse technique (Legnani et al., 2004). To achieve this objective, a 5×5 cm² area was sampled using a swab (Lp, Italy).

The isolation of *S. aureus* from food samples was conducted by the procedures outlined in the International Organization for Standardization (ISO) standard EN ISO 6881-1 (ISO 6888-



1:1999). The typical colonies on Baird Parker Agar (BPA, Oxoid, CM275, Basingstoke, UK) were subcultured and identified biochemically by Gram staining, catalase test, latex agglutination test, growth on mannitol (Mannitol Salt Agar; Oxoid CM0085B), and DNase activity (DNase agar; Oxoid, CM0321). The prescribed methodology conducted the DNA isolation procedure (Sudagidan and Aydin, 2009). This was achieved by screening for the presence of three genes (*nuc*, *coa*, and *spa*) by PCR for identification. The previously described PCR conditions and primer sequences were utilised (Hookey et al., 1998; Aires-De-Sousa et al., 2006; Sudagidan and Aydin, 2009).

2.2. Detection of disinfectant resistance genes in S. aureus

The presence of disinfectant resistance genes *qac*A/B-1, *qac*A/B-2 (Noguchi et al., 2006), *qac*A/B-3, *qac*A/B-4 (Opacic et al., 2010), *qac*C-1, qacC-2 (Sidhu et al., 2002), *qac*G-1, *qac*G-2, *qac*H-1, *qac*H-2, *qac*J-F, *qac*J-R (Smith et al., 2008), *smr*-1, *smr*-2 (Noguchi et al., 2006) and *smr*-3, *smr*-4 (Bjorland et al., 2001) was determined by PCR. The PCR products were electrophoresed in a horizontal electrophoresis system using 1xTris-acetate-EDTA (TAE) buffer containing 1.5% (w/v) agarose gel and 5% (v/v) fluorescent DNA dye (SafeView Classic, Applied Biological Materials Inc., Richmond, Canada). Visualisation of the gels was performed by the Infinity Gel Imaging System (Vilber Lourmat, Marne-la-Vallée, France). A total of two independent PCR experiments were performed for each isolate.

2.3. Determination of minimum inhibitory concentration (MIC) values of disinfectants

The efficacy and MIC values of BAC (Zefiran[®] Forte solution, 10g/100cc, Sandoz, Basel, Switzerland) QAC used as a disinfectant in the food industry, were determined by microdilution testing in 96-well microplates (Corning Costar, 3599, Corning, NY, USA).

Increasing concentrations of disinfectants were prepared as serial dilutions in Cation adjusted-Mueller Hinton Broth (CA-MHB, BD L007475) (0; 0.78; 1.56; 3.125; 6.25; 12.5; 50; 100; 200, and 400 μ g mL⁻¹). The overnight growth of *S. aureus* strains at 37 °C in Tryptone Soya Agar (TSA, Oxoid, CM0131) was adjusted to McFarland 0.5 in 0.9% NaCl using a densitometer (Den-1B, Biosan, Latvia). In duplicate, two hundred microlitres per well and twenty microlitres per well of the McFarland 0.5 bacterial suspension of each concentration were added to the wells. At a concentration of 0%, only bacteria and CA-MHB were utilised as a negative control. The 96-well microplates were incubated at 37 °C for 24 h, after which the absorbance values were measured at 600 nm using a microplate reader (EPOCH, Bio-Tek, USA). The calculations were performed by subtracting the negative control values from the obtained absorbance values (blank subtraction). *S. aureus* ATCC 25923 strains were used as a reference in this study.

3. RESULTS AND DISCUSSION

3.1. Detection of disinfectant resistance genes

S. *aureus* represents a significant pathogen for those engaged in the food industry. A total of 200 isolates identified from foods (n = 120) and food facilities (n = 80) as S. *aureus* within the study's scope were examined by PCR to determine the presence of genes responsible for disinfectant resistance. In addition to the findings of our study, it has been reported that S. *aureus* is prevalent in food and food-contact surface samples (including personnel and food



production facilities) (Marino et al., 2010; Bencardino and Vitali, 2019). In cases where hygiene and sanitation are inadequate, *S. aureus* contaminates food and significant public health problems occur because of the consumption of contaminated food. At the same time, the waste of contaminated food that cannot be consumed or the costs associated with its consumption cause significant economic problems.

The results demonstrated that the *qacJ* gene was the most frequently detected in *S. aureus* strains isolated from food samples (n = 9). Furthermore, the *smr* gene and all other studied genes were also positive in *S. aureus* strains (Table 1). Kroning et al. (2020) detected 9.6% and 3.2% *qacA* and *smr* genes in 31 *S. aureus* samples isolated from milk. In a study conducted with sheep milk, *smr/qacC* genes were detected at the highest rate in coagulase-negative staphylococci isolates, and the *qacA*/B gene was not detected (Turchi et al., 2020).

The studies in Türkiye were mainly carried out in milk samples and for the detection of qacA/B and qacC genes. Bayrakal and Aydin (2024) detected qacA/B (7.1%), qacC (7.1%), qacJ (17.8%), and smr (21.4%) genes, and Akin et al. (2020) detected qacA/B (18.8%) and qacC (2.2%) genes in *Staphylococcus* spp. isolates from milk samples.

The prevalence of disinfectant-resistant genes varies depending on the geographical location (Zaki et al., 2019). Studies for the detection of antimicrobial resistance genes in *S. aureus* strains have generally detected the *qac*A/B and *qac*C genes but not the *qac*G, *qac*H, and *qac*J genes (Zaki et al., 2019; Kroning et al., 2020). Similarly, in another study, the *qac*C gene was detected in 3 isolates out of 38 *S. aureus* samples isolated from dairy products. On the other hand, the *qac*A/*qac*B gene was not detected in any isolate (da Silva Abreu et al., 2021). In this study, genes were also detected that had not been detected in the above-mentioned studies, the number of *qac*A/B, *qac*C, *qac*G, *qac*H, and *qac*J positive samples were 4, 24, 6, 2, and 9, respectively. The observed differences in the rates of disinfectant resistance genes may be attributed to the location of the QAC resistance genes in different *S. aureus* isolates. These genes are typically carried on plasmids, facilitating rapid transmission, whereas in some *S. aureus* strains, different genes controlling QAC resistance are carried on the chromosome (Zaki et al., 2019). The differing prevalence of the genes identified in our study in comparison to those identified in other studies indicates that the development of resistance is an ongoing process, with geographical variations in the patterns of resistance.

A total of 21 (26.25%) S. aureus strains were found to contain the qacC gene, which originated from food facilities. Furthermore, the presence of smr genes was identified in 10%

Genes	Number of food samples with positive strains (n = 120)	Number of positive strains sampled from food production facilities $(n = 80)$	Total number ($n = 200$) of positive strains (%)
qacA/B-1/2	2	_	2 (1%)
qacA/B-3/4	2	_	2 (1%)
qacC	3	21	24 (12%)
qacG	5	1	6 (3%)
qacH	2	_	2 (1%)
qacJ	9	_	9 (4.5%)
smr-1/2	4	4	8 (4%)
smr-3/4	4	4	8 (4%)

Table 1. The distribution of disinfectant resistance genes in S. aureus strains (n = 200)



of the *S. aureus* strains isolated from contact surfaces. *qac*A/B, *qac*H, and *qac*J were not identified in any of the samples. Inadequate sanitation procedures for equipment in the food industry contribute to the transmission of microorganisms between personnel and equipment. The transmission of bacteria from personnel to food and from food to consumers represents a significant threat to public health. An investigation of studies conducted in the food industry revealed a high prevalence of contamination by *S. aureus* (Ye et al., 2012; da Silva Abreu et al., 2021). In a study conducted by Marino et al. (2010), *S. aureus* isolates were obtained from food processing surfaces (21.1 %) and gloves (46.3 %). Ye et al. (2012) observed that the prevalence rates of *qac*G, *qac*H, and *qac*J genes in human carriage isolates were low and that they were rarely transferred to food from humans. They reported that staphylococci colonising food animals play a more significant role in the food industry than human strains.

3.2. Determination of minimum inhibitory concentration values of disinfectants

The MIC levels of disinfectants were analysed in *S. aureus* strains with the detected disinfectant gene. The present study investigated the MIC levels of 15 foodborne and 3 *S. aureus* strains isolated from food production facilities (Table 2). The benzalkonium MIC level was determined as $3.125 \,\mu\text{g mL}^{-1}$ in 75% (9/12) of the foodborne *S. aureus* isolates.

The highest benzalkonium MIC level was identified in one surface sample as $6.25 \,\mu g \,m L^{-1}$, in which the *qacG* gene was also detected. In addition, a high level ($3.125 \,\mu g \,m L^{-1}$) of benzal-konium was identified in two additional surface samples, in which the *smr* gene was also present.

The efficacy of BAC on *S. aureus* was evaluated by a disinfectant tolerance test, and MIC values were determined. As there is no standard for MIC values, the data were interpreted by comparison with other studies or by comparison of isolates. Turchi et al. (2020) found that 12/20 (60%) of the isolates had a MIC value $\geq 2 \,\mu g \, m L^{-1}$, and the highest MIC value for BAC

	S. aureus codes	Isolated food types/surfaces	Genes	Benzalkonium chloride MIC (µg mL ⁻¹)
Type of food	PY-5	Local cheese	qacG	3.125
	PY-100C	White cheese	qacA/B, qacG	1.56
	PY-128D	Local cheese	qacJ	3.125
	PY-358	Curd cheese	smr, qacC	3.125
	PY-368A	Sheep-goat cheese	qacA/B	1.56
	PY-417A	Curd cheese	qacH, qacJ	3.125
	S-176A	Raw milk	smr	3.125
	S-267	Raw milk	qacH	1.56
	SE-21D	Beef meat	qacJ	3.125
	TE-2	Chicken skin	qacG	3.125
	Y-5	Cake	qacC	3.125
	Y-41	Semolina halva	smr, qacC	3.125
Surfaces	Y-6	Hand swab	qacG	6.25
	Y-22	Hand swab	smr, qacC	3.125
	Y-70	Surface swab	smr	3.125

Table 2. The distribution of QAC resistance genes and MIC values of benzalkonium chloride among *S. aureus* strains



was in the *qac* gene negative isolate. Another study identified a 30% reduction in susceptibility among isolates exhibiting MIC higher than 8 mg L⁻¹ for BAC and documented a strong positive correlation between the presence of *qac* genes (*qacA/B*, *qacJ*, and *smr*) and elevated BAC MIC levels (Zaki et al., 2019). Our results were similar to the aforementioned studies and showed high MIC sensitivity in samples carrying the disinfectant gene. The data obtained shows the risk in the facilities, and the highest value obtained from the hand swab shows the importance of personnel in contamination in food establishments. As a result of not using appropriate hand sanitisers, not paying attention to their duration of effect, and not cleaning hands properly, sensitivity to sanitisers decreases and resistance increases.

4. CONCLUSIONS

The most significant future risk is horizontal gene transfer, which demonstrates that resistance genes can be transmitted between bacteria and humans, animals, and food. The findings of our study indicate that the detection of disinfectant-resistant genes in *S. aureus* strains isolated from food businesses and foods suggests a potential risk associated with these foods. To prevent the formation and transfer of resistance, it would be prudent to consider the expansion of disinfectants that are sensitive to the environment and living organisms as an alternative to the current disinfectants that are widely used. It is recommended that disinfectants with varying effects be used in combination. It is imperative to prevent the formation of biofilms. Furthermore, it is essential to ensure the appropriate selection of a disinfectant, along with the correct duration and concentration of the chosen agent. It is imperative to conduct comprehensive molecular investigations into gene transfer and resistance mechanisms to avert the advent of more substantial risks in the future.

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