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- RESEARCH ARTICLE-

Investigation of antiseptic resistance genes in *Staphylococcus* spp. isolates

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Abstract

Antiseptic and disinfectants are used very frequently in all health institutions, including hospital and veterinary application areas, in the home environment and food production industry, to prevent infections and contaminations. At present, the quaternary ammonium compounds, benzalkonium chloride and chlorhexidine digluconate is one of the divalent cations most commonly used chemicals such as antiseptics and disinfectants. However, the widespread use of biocides has brought about the emergence of bacteria resistant to antiseptics/disinfectants. It is known that bacteria develop resistance mechanisms against antibiotics as well as disinfectants. Epidemiological data on antiseptic susceptibility and distribution of resistance genes are very important for nosocomial infections. Some species, including the species belong to genus *Staphylococcus*, cause foodborne poisoning and various clinical infections such as skin and soft tissue and surgical site infections, endocarditis, mastitis, pneumonia and bacteremia in humans and animals. *Staphylococcus* strains can contain plasmid-derived *qacA/B* and *qacC* genes that provide resistance to quaternary ammonium compounds (QAC). In this study, the presence of antiseptic resistance genes (*qacA/B* and *qacC*) in 90 *Staphylococcus* spp. strains isolated from chicken carcass, bovine tank milk, various cheeses and bovine clinical mastitis samples were determined by simplex polymerase chain reaction. *QacA/B* was found in %18.8 and *qacC* in %2.2 of the studied isolates. Of antiseptic resistance genes, *qacA/B* was detected in cheese and bovine clinical mastitis samples, and *qacC* in chicken carcass.

Keywords:

Antiseptic resistance genes, Bovine clinical mastitis, Chicken carcass, Cheese, Bovine tank milk.

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Introduction

Staphylococcus spp. strains are important pathogens that cause infections such as skin and soft tissue infections, endocarditis, bacteremia, mastitis, synovitis, endometritis, furuncle, suppurative dermatitis, pneumonia, and septicemia in humans, other mammals, and birds (Schleifer & Bell, 2009).

Antiseptics are widely used to reduce the temporary microbial flora in the hands of clinical field workers, prevent the transmission of microbes from person to person, prepare the skin before invasive procedures, and provide hand antisepsis prior to surgical procedures. The most commonly used antiseptics are alcohols, chlorhexidine, iodine and iodophores, chloroxylenol, quaternary ammonium compounds (QAC) and triclosan (Boyce & Pittet, 2002). QACs are membrane-active cationic agents. Cationic agents react with phospholipid components in the cytoplasmic membrane and perform membrane destruction with osmotic effects. In microorganisms exposed to cationic agents, respectively; adsorption and penetration of the agent into the cell wall, reaction with cytoplasmic membrane (lipid or protein) and subsequent degradation of membrane integrity, infiltration of intracellular low molecular weight components, destruction of proteins and nucleic acids, wall lysis due to otolytic enzymes takes place (McDonnel & Russell, 1999). Quaternary ammonium compounds are substances that consist of a nitrogen atom directly attached to four alkyl groups, which may differ in structure and complexity. Alkyl benzalkonium chlorides are the most commonly used antiseptics among this wide family of compounds (Weber et al., 2007).

Although *qac* genes (*qacA/B* and *qacC*) that provide resistance to quaternary ammonium compounds and derivative biocidal agents were first identified in human-induced *Staphylococcus aureus* and coagulase negative staphylococci, *qacA/B* and *qacC* resistance genes have also been reported in samples isolated from food and food production sites (Lyon & Skurray, 1987; Bjorland et al., 2003; Bischoff et al., 2012; Wendlant et al., 2013; Ebner et al., 2013; Monecke et al., 2013; Wassenaar et al., 2015; Damavandi et al., 2017; Ignak et al., 2017; Do Vale et al., 2019; Cantekin et al., 2019). These genes have often been reported as plasmid-borne genes. These plasmid-borne genes encode proteins that allow the removal of hydrophobic compounds containing intercalation dyes and other cationic biocides, including QACs. *QAC* genes are located on mobile genetic elements, and their location allows them to interact between different species of *Staphylococcus* (Hegstad et al., 2010).

qacA and *qacB*, qac resistance genes, are encoded on large plasmids, but other qac resistance genes such as *smr*, *qacG*, *qacH*, and *qacJ* are encoded on plasmids smaller than 3 kb. Resistance genes on small plasmids are usually available as gene cassettes and encode the protein portion of the small multi-drug resistance (SMR) family (Bjorland et al., 2005). The *qacA* and *qacB* genes encode the protein portion of the primary facilitating superfamily (MFS). All proteins encoded by resistance genes are embedded in the cell membrane (Bragg et al., 2013). QacA is a 514 amino acid transmembrane protein located on the pSK1 plasmid (Gillespie et al., 1989). The *qacB* gene, which is very similar to *qacA*, is carried in plasmid pSK23, only six amino acids differ between the two genes. Expression of both genes is regulated by transcriptional repressor qacR (Wassenaar et al., 2015). QacC proteins were first identified in pSK89, a small plasmid of *Staphylococcus aureus*. (Lyon & Skurray, 1987). The QacC protein is 107 amino acids long, and in other types of bacteria, it can be 108 or 109 in length. QacC proteins contain four transmembrane domains that form dimers in the bacterial membrane. There is no need for a transcriptional regulator for the expression of the

qacC gene (Wassenaar et al., 2015). This gene, which is responsible for staphylococcal multidrug resistance, has been named smr in different studies (Grinius et al., 1992; Grinius et al., 1994).

Biocide and disinfectant resistance can significantly prevent hygiene strategies and disinfection measures taken to reduce nosocomial infections. Studies on the qac genes in Staphylococcus populations found in other settings with the clinic have gained importance. In this study, the presence of qac antiseptic resistance genes (*qacA/B* and *qacC*) in 90 *Staphylococcus* spp. isolated from bovine clinical mastitis, cheese, bovine tank milk and chicken carcass samples were determined by PCR.

Material and methods

Bacterial strains

A total of 90 isolates of *S. aureus* isolates were included in this study. All the strains were kindly provided from the laboratory culture collection of Dr. Zafer Cantekin (Mustafa Kemal University Veterinary Faculty). *S. aureus* isolates were grown on Trypticase soy agar. DNA extraction from the isolates was done according to the phenol-chloroform method of Sambrook and Russell (2001).

PCR Detection of Genes

The primers used for PCR amplification of qacA or qacB (qacA/B) and qacC were the same as used previously (Zmantar et al., 2011). The nucleotide sequences of the primers used for this detection were: 5'-TCCTTTTAATGCTGGCTTATACC-3'and 5'-AGCCKTACCTGCTCCAACTA-3' for qacA/B (product size 220 bp); and 5'-GGCTTTTCAAAATTTATACCATCCT-3' and 5'-ATGCGATGTTCCGAAAATGT-3' for qacC (product size 249 bp). Each PCR mixture contained 2µl of DNA extract, 2 µM (each) primer, 200 µM (each) deoxynucleoside triphosphate, 2.5 µM MgCl2 (1× reaction buffer), and 1 U of *Taq* DNA polymerase in a total volume of 25 µl. PCR amplification was run with an initial cycle of denaturation (3 min at 94°C) followed by 35 cycles. The conditions for each cycle were denaturation for 45 sec at 94°C, annealing for 45 sec at 56°C. Finally, reaction mixtures were incubated at 72°C for 10 min. Each PCR was performed in duplicate. The PCR products were separated by electrophoresis in a 1.5 % agarose gel, stained with Safe Red, and visualized under UV light. The amplification products were photographed, and their sizes were determined using a 100-1000 bp molecular size marker (Vivantis 100 bp plus).

Results and Discussion

In 6 of the 19 cheese-derived *Staphylococcus* spp. isolates, the *qacA/B* gene was identified and the *qacC* gene was not detected. No study was found to determine antiseptic resistance genes of cheese-derived *Staphylococcus* isolates. In *Enterococcus faecalis* strains isolated from milk and dairy products, it was reported that qac genes (*qacA, qacB, qacC, smr* [*qacC + qacD*], *qacEΔ1, qacG, qacH, qacJ*) were screened by PCR and 4 *Enterococcus faecalis* strains were positive in terms of qac genes. Of these strains, it was stated that smr genes (*qacC + qacD*) were detected in only one of the isolates originating from cheese ("Camembert" cheese) (Bischoff et al., 2012).

QacA/B genes were identified in 11 of 15 *Staphylococcus* spp. isolates from clinical mastitis (Figure 1), but no qacc genes were detected in these isolates. By PCR analysis, 3.3% of 30 *Staphylococcus* spp. strains isolated from subclinical mastitis goats from Hatay province in 2019

were reported to contain the *qacA/B* gene (Cantekin et al., 2019). A new plasmid containing the smr gene was found in 3 *Staphylococcus aureus* isolates resistant to penicillin, tetracycline, and QAC resistant, which was isolated from cows that used breast cream containing cetyltriamonium bromide, a type of QAC, for 10 years (Bjorland et al., 2001). In a study with *Enterococcus faecalis* isolates from bovine blood, *qacA/B* was detected in one isolate (Bischoff et al., 2012).

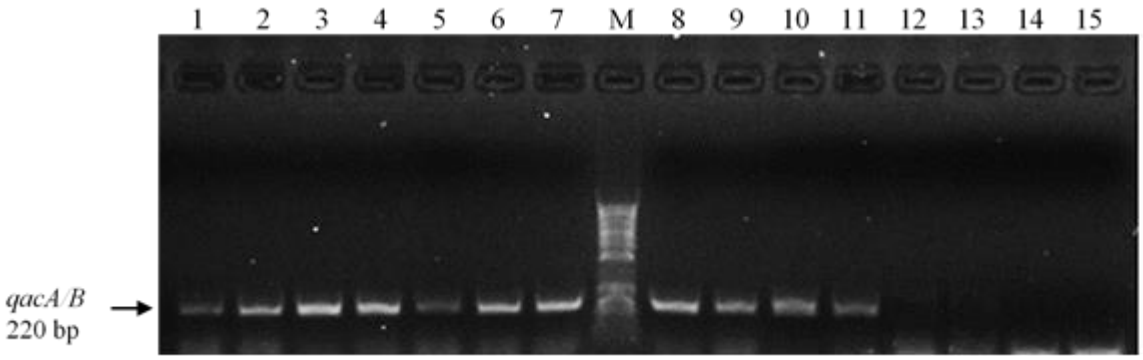

Figure 1. Simplex amplification agarose gel electropherogram of *qacA/B* genes from bovine clinical mastitis derived from *Staphylococcus* spp. isolates (Marker 100-1000bp).

QacC gene was detected in 2 of 32 *Staphylococcus* spp. isolates isolated from chicken carcasses and *qacA/B* genes were not found. Ebner et al. (2013), found that 65% of 34 *Staphylococcus aureus* isolates isolated from two different slaughterhouse-derived chicken carcasses were positive for *qacC* gene by DNA microarray analysis. 15 of 131 MRSA strains isolated from turkeys and chickens by microarray hybridization method were detected with the *qacC* gene (Monecke et al., 2013). In addition, as a result of microarray analysis screening in living chickens, the quaternary ammonium resistance gene, *qacC*, was found (Wendlandt et al., 2013). In a similar study with pig carcass, the presence of PCR and antiseptic resistance genes of 100 MRSA isolates was determined and 45 strains contained *smr* (*qacC*+*qacD*) and 8 of the *smr*-positives contained *qacG*. Strains were reported not to contain *qacA/B*, *qacH* or *qacJ* genes (Wong et al., 2013).

QacA/B and *qacC* genes were not detected in 24 *Staphylococcus* spp. strains isolated from bovine tank milk samples. Similarly, Ammar et al. (2016), stated that 55% of MRSA strains isolated from milk and meat products (Egypt) are resistant to benzalkonium chloride, but these isolates do not contain the *qacA/B* and *smr* genes. Turchi et al (2020) reported that 12.3% of 120 coagulase-negative *Staphylococcus* strains isolated from bovine tank milk collected in Lazio, Tuscany (Italy) contained *qac* genes, and also *smr*-*qacC* genes were commonly found in these strains.

As a result, *qacA/B* in 18.8% and *qacC* gene in 2.2% of the total isolates included in the study were found. The *qacA* and qacB genes (Paulsen et al., 1996; Wassenaar et al., 2015) encoded on the plasmid, which are very similar in terms of amino acid sequence and provide resistance to various dyes and antiseptics, including quaternary ammonium compounds, were found only in cheese and bovine clinical mastitis samples. QacC (Lyon & Skurray, 1987), which is generally responsible for antiseptic resistance limited to quaternary ammonium compounds, was detected

only from chicken carcass samples. In the literature where the susceptibility to quaternary ammonium compounds is determined in *Staphylococcus* spps isolated from various sources, it is seen that the percentages of qac genes vary greatly according to the geographical region studied (Bjorland et al., 2003; Bischoff et al., 2012; Wendlant et al., 2013; Ebner et al., 2013; Monecke et al., 2013; Wassenaar et al., 2015; Damavandi et al., 2017; Ignak et al., 2017; Dovale et al., 2019; Cantekin et al., 2019). Different studies have reported that *qacA/B* positive *Staphylococcus* spp strains are more common and this gene pair is common in MRSA isolates from Europe and Asia (Noguchi et al., 2006; Wang et al., 2008; Schlett et al., 2014). It has been reported that there is a genetic link between the genes responsible for qac and certain antibiotic (erythromycin trimethoprim and aminoglycoside) resistance carried on the same staphylococcal plasmids (Noguchi et al., 2005; Conceição et al, 2016), and hence the spread of antibiotic resistance genes can occur with selective repression due to frequent use of chlorhexidine (Anthonisen et al., 2002; Noguchi et al., 2006; Sheng et al., 2009). Similarly, frequent antibiotic use is thought to create a selective pressure in the direction of resistance to quaternary ammonium compounds.

Conclusion

The increase of antiseptic resistant bacteria is a serious threat to public health. Therefore, understanding the distribution and selection of resistance genes is important for establishing long-term strategies in clinical and economic terms. In studies conducted with *Staphylococcus* spp. in our country, disinfectant resistances of staphylococcal strains isolated from various sources were determined at the genetic level with the study presented. It is thought that the data obtained within the scope of the study can contribute to the infection control programs that will prevent the development of new resistance mechanisms by providing useful data to studies on antiseptic resistance in our country. As a result, it is recommended to study antiseptic resistance, which has social and economic importance, in more detail in different isolates and species in order to contribute significantly to public health.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical decleration: The manuscript in part or in full has not been submitted or published anywhere

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