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Distribution of antiseptic resistance genes in *Staphylococcus* spp. from bovine mastitis

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ABSTRACT: The purpose of this study was the determination of antiseptic resistance genes (*qacA*/*B* and *qacC*) from staphylococcal mastitis in cattle in various regions of Turkey. In total, 283 isolates (Burdur: 36, Hatay: 47 and Van: 200) were studied, and the antiseptic resistance genes were detected using simplex PCR. The distribution of the *qacA*/*B* and *qacC* genes, mediating resistance against quaternary ammonium compounds, was found to vary among the different isolates. The *qacA*/*B* genes were found in three of the Burdur isolates, six of the Hatay isolates and seven of the Van isolates. The *qacC* gene was found in two of the Burdur isolates, none of the Hatay isolates and two of the Van isolates. The presence of these genes and transmission among *Staphylococcus* spp. strains may pose risks in the control of mastitis, as well as to public health.

Keywords: antiseptics; isolates; DNA; *qacA*/*B* genes

Antiseptics and disinfectants, especially quaternary ammonium compounds (QACs), are products commonly used in teat dipping applications during the milking process, to maintain udder health and prevent the transmission of mastitis agents in dairy cattle (NMC 1999). In addition, they are usually used to prevent the colonisation of microorganisms in milking machines, milk tanks and the equipment used in the manufacturing of milk products (Saran 1995; Ucuncu 2015).

Genetic resistance against antiseptics, particularly QACs, is a longstanding problem (Gillespie et al. 1986; Lyon and Skurray 1987; Russell 2004). Resistance against QACs is primarily encoded in the *qac* genes, and their roles and effects have been well described. The presence of these genes, with their different mechanisms of antiseptic resistance, can affect the use of antiseptics (Jaglic and Cervinkova 2012; Cervinkova et al. 2013). It has been suggested that resistance develops as a result of the selective pressure of antiseptic applications, or that it is closely related to the acquisition of antibiotic resistance genes (Leelaporn et al. 1994; Thomas et al. 2000; Sidhu et al. 2002).

In several studies, different antiseptic and disinfectant resistances, mainly against QACs, have been determined in *Staphylococcus* spp. (Heir et al. 1999; Mayer et al. 2001; Zhang et al. 2011; Mc Gann et al. 2013). These studies were usually carried out in the food industry or in hospital isolates, particularly in relation to methicillin-resistant *Staphylococcus aureus*. However, studies aimed at determining the occurrence of antiseptic resistance genes in *Staphylococcus* spp. from mastitis have been limited (Bjorland et al. 2001; Bjorland et al. 2005). As is the case for antibiotic resistance genes, the existence and spread of antiseptic resistance genes are important factors in public health (Levy 2000).

The goal of this study was to determine the frequency of the *qacA*/*B* and *qacC* genes responsible

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Table 1. The isolates used and their sources from Burdur (36 isolates from 10 farms), Hatay (47 isolates from 10 farms) and Van (200 isolates from 40 farms); *S.* = *Staphylococcus*

Strains	Strains according to origin		
	Burdur	Hatay	Van
S. aureus	13 (36.11%)	2 (4.26%)	111 (55.5%)
Staphylococci other than *S. aureus*	23 (63.89%)	45 (95.75%)	89 (44.5%)

for QAC resistance in staphylococci isolated from bovine mastitis in three different regions (Burdur, Hatay and Van) of Turkey.

MATERIAL AND METHODS

Bacterial strains. In this study, a total of 283 staphylococcal isolates from bovine mastitis were used. They were obtained from the culture collection of the veterinary faculties in the cities of Burdur (36 strains isolated in 2010–2012 from 10 herds), Hatay (47 strains isolated in 2011–2012 from 10 herds) and Van (200 strains isolated in 2008–2014 from 40 herds). Teat dipping applications with QACs were performed on farms in Hatay and Burdur regularly, but on farms in Van, only rarely. The identification of the strains was confirmed by PCR (Cantekin et al. 2014), and the properties and origins of the isolates are shown in Table 1.

Isolation of DNA. For total bacterial DNA extraction, each of the isolates was treated with lysozyme (20 mg/ml) and lysostaphin (40 mg/ml) at 37 °C for 30–60 min, and DNA was then isolated using the phenol/chloroform extraction method. The extracted DNA was stored at –20 °C until the PCR analyses (Sambrook and Russel 2001).

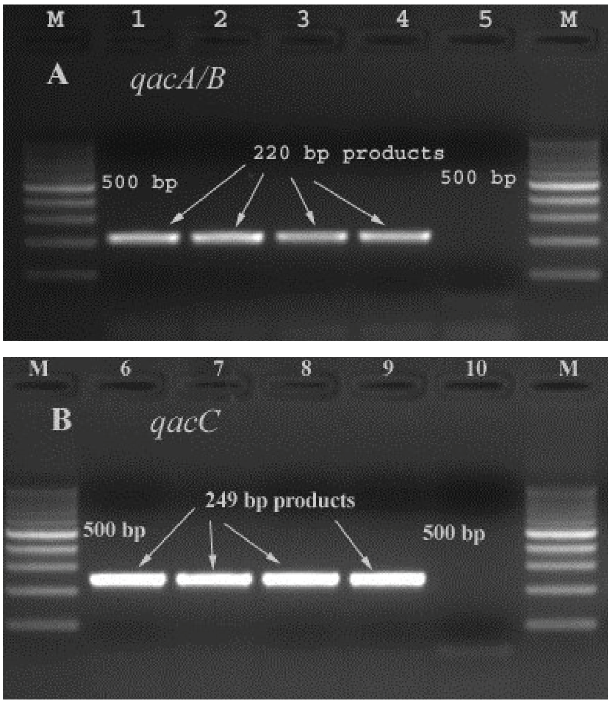

Figure 1. Results of the PCR analyses. 1–4 = 220 bp qacA/B-specific bands; 5, 10 = negative controls without template DNA; 6–9 = 249 qacC-specific bands; M = VC 100 bp DNA ladder

PCR amplification. Two simplex PCRs were conducted for the detection of the *qacA*, *qacB* and *qacC* genes using the primers recommended by Zmantar et al. (2011). The properties of the primers are shown in Table 2. Each PCR was performed in a 25 µl reaction volume containing 2 µl of extracted DNA, 2.5 µl of the Taq buffer (10X, Vivantis, Malaysia), 200µM of each of the deoxynucleoside triphosphates, 20µM of each of the forward and reverse primers, and 1 U of the Taq DNA polymerase (Vivantis, Malaysia). The reaction mixtures were heated to 94 °C for 5 min, then subjected to 35 cycles at 94 °C for 60 s, annealing at 56 °C for 30 s and extension at 72 °C for 60 s, ending with a final extension at 72 °C for 10 min.

RESULTS

In the results of the PCR analyses, a 220-bp specific amplification product for *qacA/B,* and a 249-bp specific amplification product for *qacC* were observed (Figure 1).

The *qacA/B* genes were detected in three (8.33%) of 36 isolates from Burdur, six (12.76%) of 47 from

Table 2. Properties of primers used in this study

Target genes	Primer names	Primer sequences	Length of amplification products
qacA/qacB	qacA/B f qacA/B r	5'-TCCTTTTAATGCTGGCTTATACC-3' 5'-AGCCKTACCTGCTCCAACTA-3'	220 bp
qacC	qacC f qacC r	5'-GGCTTTTCAAAATTTATACCATCCT-3' 5'-ATGCGATGTTCCGAAAATGT-3'	249 bp

Hatay and seven (3.5%) of 200 from Van. The *qacC* gene was detected in two (5.55%) of 36 isolates from Burdur, two (1%) of 200 from Van, and in none of the Hatay isolates. Overall, the *qacA/B* and *qacC* genes were found together in just one isolate from Burdur. The distribution of these resistance genes according to the province is shown in Table 3.

Overall, the *qac* genes were detected in four (13.88%) isolates from Burdur, six (12.76%) from Hatay and nine (4.5%) from Van. In total, 19 (6.71%) of the 283 isolates were positive for *qac* genes. Calculation of farm-level positivity revealed three positive farms (30%) from 10 in Burdur, four farms (40%) from 10 in Hatay and six (15%) from 40 in Van. The results are shown in Table 4.

DISCUSSION

In this study, the distributions of the *qacA/B* and *qacC* genes in *Staphylococcus* spp. from bovine mastitis were determined using simplex PCR analysis. This research was conducted on 283 isolates from three different provinces in Turkey (Burdur: 36, Hatay: 47 and Van: 200), and *qac* genes were detected in four isolates (13.88%) from Burdur, six (12.76%) from Hatay and nine (4.5%) from Van. In total, 19 (6.71%) of the 283 isolates were found to be positive for *qac* genes.

Table 3. Distribution of resistance genes for each positive isolate according to the province; *S.* = *Staphylococcus*

	Isolates	*S.* spp.	*S. aureus*	*qacAB* genes	*qacC* genes
Burdur (36 isolates)					
1	B14	+	–	+	–
2	B15	+	–	+	+
3	B28	+	–	+	–
4	B33	+	–	–	+
				3 (8.33%)	2 (5.55%)
Hatay (47 isolates)					
1	H33	+	–	+	–
2	H38	+	–	+	–
3	H41	+	–	+	–
4	H42	+	–	+	–
5	H44	+	–	+	–
6	H45	+	–	+	–
				6 (12.76%)	
Van (200 isolates)					
1	V44	+	–	–	+
2	V45	+	–	–	+
3	V131	+	+	+	–
4	V199	+	+	+	–
5	V208	+	–	+	–
6	V290	+	+	+	–
7	V339	+	–	+	–
8	V340	+	+	+	–
9	V341	+	–	+	–
				7 (3.5%)	2 (1%)

Little is known about QAC resistance genes in *Staphylococcus* spp. from mastitis. In one study, Bjorland et al. (2001) studied the distribution of the *smr* gene, responsible for QAC resistance in *Staphylococcus* spp., in four dairy herds. In one herd, they reported a widespread distribution of the *smr* gene among the staphylococcal species. In addition, they described how the use of teat cream containing QACs over several years for the control of mastitis can cause the development of QAC resistance. Their study indicated that the occurrence and spread of QAC resistance may be a problem for public health, which requires further investigation. Bjorland et al. (2005) also studied resistance to QACs in *Staphylococcus* spp. from 127 dairy cow herds and 70 dairy goat herds. They identified QAC resistance genes (*qacA/B*, *smr*, *qacG* and *qacJ*) in 21% of the cow herds and 10% of the goat herds, and concluded that there was widespread distribution of QAC resistance genes in the *Staphylococcus* spp. in both dairy cow and goat herds. In this study, 19 (6.71%) of the 283 isolates were found to be positive for *qac* genes. With respect to geographical distribution, *qac* genes were detected at rates of 13.88% in Burdur, 12.76% in Hatay and 4.5% in Van. Farm-level positivity was found to differ among the three regions. It was found that three (30%) from 10 farms in Burdur, four (40%) from 10 in Hatay and six farms (15%) from 40 farms in Van were positive. These differences among the regions may result from the frequency of the implementation of teat dipping disinfectants.

In this report, the presence and distribution of the *qacA/B* and *qacC* genes in staphylococci from bovine mastitis were determined in Turkey. The use of QACs may not be effective against resistant strains of *Staphylococcus* spp. These resistant strains may be

Table 4. Distribution of antiseptic resistance genes in Burdur (36 isolates from 10 farms), Hatay (47 isolates from 10 farms) and Van (200 isolates from 40 farms)

Target gene	Result of PCR		
	Burdur	Hatay	Van
qacA/qacB	3 (8.33%)	6 (12.76%)	7 (3.5%)
qacC	2 (5.55%)	–	2 (1%)
Total isolates	4 (13.88%)	6 (12.76%)	9 (4.5%)
Farm level positive	3 (30%)	4 (40%)	6 (15%)

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transmitted between udders, from cow to cow and from cow to human, and the transmission of these genes among isolates may create potential risks for food security and public health. In addition to detection of antibiotic resistance, studies aimed at estimating antiseptic resistance may be useful for the control of bovine mastitis. Further studies for detection of *qacA/B* and *qacC* genes in strains from other animals or milk products would be a useful contribution to our knowledge of the distribution of QAC resistance.

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