IDENTIFICATION OF QUATERNARY AMMONIUM COMPOUND RESISTANT STAPHYLOCOCCI ISOLATED FROM THE FOOD INDUSTRY.

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SUMMARY

The occurrence of known QAC resistance genes among staphylococcal strains isolated from food processing plants has been studied by hybridization analysis. Of 191 isolates, 25 were resistant to benzalkonium chloride. Five of these strains gave no hybridization signals to probes for the known *qacA-C* genes. The QAC resistant staphylococci belonged to different species, some of which have been reported to produce enterotoxins. The finding of resistant staphylococci in different areas of the food processing industry indicates that QAC resistance is a potential problem in the food processing industry.

INTRODUCTION

Disinfectants based on quaternary ammonium compounds (QAC) are widely used in the hospital environment and the food industry in both manual processing lines and on surfaces not in contact with food because they are good penetrants, not corrosive or significantly toxic. Resistance to disinfectants based on QAC is widespread among clinical strains of staphylococci and has been reported and characterized in studies from many countries. Included amongst such isolates are strains of *Staphylococcus aureus*. Resistance to antiseptics and disinfectants in clinical strains of staphylococci is encoded by one of at least three separate multidrug resistant determinants, designated *qacA*, *qacB* and *qacC*. In contrast, very little is known about QAC resistance in the food industry.

Production of heat resistant enterotoxin by staphylococci is of concern in the food industry. For many years enterotoxin production was uniquely associated with *S. aureus*. More recently enterotoxin production has also been demonstrated in a number of other species (Valle *et al.* 1990, Bautista *et al.* 1988). In addition, coagulase negative staphylococci are regarded as possible genetic reservoirs of resistance, with the potential to spread resistance to enterotoxin producers.

Resistance to benzalkonium chloride has been reported in staphylococci isolated from the meat, poultry and bakery industries (Heir *et al.* 1995). Here we describe the occurrence and distribution of QAC resistance genes and the identification of the QAC resistant staphylococci strains.

MATERIALS AND METHODS

Isolation and identification of staphylococci. All staphylococci strains were cultured in Mueller-Hinton (MH) broth, Brain Heart Infusion (BHI) broth or on MH or BHI agar plates at 37 °C. Isolates were obtained from three separate meat and poultry processing plants during 1989-1990 by swabbing food cutting and other contact surfaces or equipment and from baguettes by inoculation into Baird-Parker agar. The strains were regarded as staphylococci if they were Gram-positive, catalase positive clusters of cocci resistant to bacitracin and sensitive to lysostaphin and furazolidone. Additional tests were performed for QAC resistant isolates not hybridizing to *qacA-C* specific probes: strains able to produce acid aerobically from glycerol in the presence of 0.4 mg l-1 erythromycin and strains fermenting glucose anaerobically verified the staphylococcus nature of these strains. API Staph analysis was performed according to the instructions of the producer.

Susceptibility tests. Minimum inhibitory concentrations (MICs) to benzalkonium chloride (BC) were tested in a microtiter assay. The lowest concentration of BC totally preventing growth was taken to be the MIC.

DNA techniques. Plasmid and chromosomal DNA from staphylococci was isolated. The 16S rRNA genes were amplified by PCR using the primers 27f (TAACACATGCAAGTCGAAGG) and 1492r (TACGGYTACCTTGTTACGACTT) or 1525r (AAGGAGGTGWTCCARCC). The amplified products were directly sequenced in both direction using exonuclease I, shrimp alkaline phosphatase and the Sequenase ver. 2.0 DNA sequencing kit together with the sequence primers 2768f (AGCGGCGGACGGGTGAGTAACACG) and 342r (CTGCTGCSYCCCGTAG).The *qacA /qacB* and *qacC* genes were identified by hybridization to a 22-mer oligo nucleotide (GCTGCATTTATGACAATGTTTG) and to a specific *qacC* PCR probe made from the *qacC* gene of plasmid pST827 (Heir *et al.* 1995), respectively. Computer analyses were performed employing the GCG program package.

RESULTS AND DISCUSSION

The 191 *Staphylococcus* strains investigated were screened for QAC resistance. Twenty five strains, including two which were coagulase positive, were resistant to BC having MICs between 4 and 11 mg l^{-1} compared to MICs between 0 and 2 mg l^{-1} for the sensitive strains. Plasmid and chromosomal DNA from the 25 isolates were hybridized with probes specific for *qacA/B* and *qacC* (not shown). The probes hybridized exclusively with plasmid DNA. Seven strains hybridized to the *qacA/B* probe only, 13 strains to the *qacC* probe only and none with both probes. No hybridization was evident for the remaining five strains though their MICs for BC were between seven and 11 mg l^{-1} and not diverging from the MICs

Table 1. Characterization of QAC resistant staphylococci from the food industry.

Isolate number	Resistance determinant	Isolated from *	MIC (mg l^{-1})	Sequence similarity[5]	Partial 16S rRNA analysis	API Staph
3	*qacA/B*	M	5	100.0	*S. hominis*	*S. hominis*[3], *S. epidermidis*[2] [6]
5	*qacA/B*	B	7	100.0	*S. arlettae*, *S. capitis*	*S. epidermidis*[2]
7	*qacA/B*	P	6	100.0	*S. arlettae*, *S. capitis*, *S. epidermidis*	*S. epidermidis*[2]
11	*qacC*	M	4	100.0	*S. epidermidis*	*S. epidermidis*[1]
13	*qacC*	P	7	85.2	*S. hominis*	*S. cohnii*[2]
19	*qacC*	P	11	100.0	*S. saprophyticus*	*S. saprophyticus*[3]
21	other	M	7	100.0	*S. warneri*	*S. aureus* and *S. hyicus*[4]
22	other	M	7	100.0	*S. warneri*	*S. hyicus*[2]
24	other	P	10	91.7	*S. caprae*	*S. aureus*, *S. simulans*[4]
25	other	P	11	100.0	*S. saprophyticus*	*S. saprophyticus*, and *S. simulans*[4] *S. aureus*[6], *S. simulans*[6], and *S. hominis*[4] [6]

** Isolated from poultry (P), red meat plants (M) and bread industry products (B), [5] sequence similarity relative to typestrain, [1] very good identification (% id ≥ 99.0), [2] good identification (% id ≥ 90.0), [3] acceptable identification (% id ≥ 80.0), [4] low discrimination (% id < 80.0), [6] results from second API Staph (if determination was uncertain).*

obtained for strains hosting *qacA/B* or *qacC*. The resistance may in these cases be a consequence of as yet uncharacterized determinants of the same type as *qacA/B* or *qacC* or of different mechanisms e.g. structural changes in the cell membrane influencing permeability to QAC. According to differences in plasmid profiles 16 of the 25 isolates were judged as different (not shown). Of these, 11 isolates were subjected to partial 16S rRNA analysis and API Staph analysis to determine the *Staphylococcus* species (Table 1).

The results show that the QAC resistant Staphylococci belong to different species. Some of these species have previously been reported to be enterotoxin producers (e.g. *S. epidermidis, S. saprophyticus, S. warneri, S. haemolyticus*) or cause other deceases in man (e.g. *S. epidermidis, S. saprophyticus*). In some cases there were discrepancies between the results from the two identification methods. Hybridization experiments showed that the QAC resistance determinants *qacA-C* were distributed among strains isolated from both the meat and the two poultry processing plants visited. *qacA/B* was also determined in staphylococci isolated from bakery industry products. The fact that the QAC resistant strains were isolated at different places, at different times and in addition in two completely different areas of food industry, indicates that genes encoding QAC resistance may be widespread among food associated staphylococci.
The general levels of resistance observed in pure cultures appear to be of little direct commercial significance. However, when tested against a commercial brand of QAC in the presence of other microbes and organic material, survival of staphylococcal strains has been observed (Sundheim *et al.* 1992). QAC resistance is thus a potential problem in the food processing industry.

CONCLUSIONS

QAC resistance is widespread in the food industry. The resistant strains belonged to different *Staphylococcus* species, some of which have been reported to produce enterotoxins. The results may be important when considering new formulations of disinfectants. Moreover, the results may be of importance when considering disinfecting procedures in the food industry.

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