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Frequency of Disinfectant Resistance Genes and Genetic Linkage with β-Lactamase Transposon Tn*552* among Clinical Staphylococci

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A total of 61 strains of *Staphylococcus aureus* and 177 coagulase-negative staphylococcal strains were isolated from the blood of patients with bloodstream infections and from the skin of both children under cancer treatment and human immunodeficiency virus-positive patients. The MIC analyses revealed that 118 isolates (50%) were resistant to quaternary ammonium compound-based disinfectant benzalkonium chloride (BC). The frequencies of resistance to a range of antibiotics were significantly higher among BC-resistant staphylococci than among BC-sensitive staphylococci. Of 78 BC-resistant staphylococcal isolates, plasmid DNA from 65 (83%), 2 (3%), 43 (55%), and 15 (19%) isolates hybridized to *qacA* or *-B* (*qacA*/*B*), *qacC*, *blaZ*, and *tetK* probes, respectively. The *qacA*/*B* and *blaZ* probes hybridized to the same plasmid in 19 (24%) staphylococcal strains. The plasmids harboring both *qacA*/*B* and *blaZ* genes varied from approximately 20 to 40 kb. The *Staphylococcus epidermidis* Fol62 isolate, harboring multiresistance plasmid pMS62, contained *qacA*/*B* and *blaZ* together with *tetK*. Molecular and genetic studies indicated different structural arrangements of *blaZ* and *qacA*/*B*, including variable intergenic distances and transcriptional directions of the two genes on the same plasmid within the strains. The different organizations may be due to the presence of various genetic elements involved in cointegration, recombination, and rearrangements. These results indicate that *qac* resistance genes are common and that linkage between resistance to disinfectants and penicillin resistance occurs frequently in clinical isolates in Norway. Moreover, the higher frequency of antibiotic resistance among BC-resistant strains indicates that the presence of either resistance determinant selects for the other during antimicrobial therapy and disinfection in hospitals.

Staphylococci, including *Staphylococcus aureus* and the coagulase-negative staphylococci (CNS), and enterococci account for approximately one-third of all bloodstream infections and up to 50% of nosocomial bloodstream infections. In the past, CNS were considered to be very rarely involved in disease, but since the 1980s there has been increasing evidence that CNS can act as opportunistic pathogens and can be a frequent and important cause of disease. In recent years there has been a dramatic increase in the prevalence of multiple-drug-resistant strains. Worldwide emergence of antimicrobial resistance in staphylococci is probably a serious and increasing problem, especially in hospitals (16).

Disinfectants based on quaternary ammonium compounds (QACs), e.g., benzalkonium chloride (BC), cetylpyridinium chloride, cetrimide, proceine, and detizor, are frequently used in hospitals to disinfect and to prevent the spread of pathogens. It has been suggested that the widespread use of QACs may impose a selective pressure and contribute to the emergence of disinfectant-resistant microorganisms in these environments (22, 26). Known *qac* resistance genes reported in clinical staphylococci, *qacA*, *qacB*, and *qacC*/*smr*, are generally plasmid borne and are widely distributed in the environment (12, 14, 15, 18).

Large consumption of antibiotics by both humans and animals has resulted in the development and spread of a large number of antibiotic resistance determinants among bacterial populations, thus creating critical public health problems. Concerns have arisen regarding the potential emergence of cross-resistance and coresistance between widely used disinfectants and antibiotics (20, 22, 26). Resistance genes on transferable genetic elements such as plasmids and transposons may lead to the epidemic spread of resistance between species. On some of the published plasmids (pST6, pSK4, and pSK41) and transposons (Tn*552* and Tn*4002*), *qac* resistance determinants are located together with antibiotic resistance genes encoding resistance to gentamicin (GEN), trimethoprim (TMP), penicillin, kanamycin (KAN), and tobramycin (3, 12, 14, 27).

Staphylococcal β-lactamase structural gene *blaZ* and two closely linked genes, *blaI* and *blaR*, that control its expression have been identified on several transposons, e.g., Tn*552* (24, 25), and large plasmids, e.g., pST6 (27), as well as on the chromosome (23). Staphylococcal β-lactamase transposon Tn*552* is closely related to staphylococcal transposons Tn*4002*, Tn*3852*, and Tn*4201* and to Tn*4201*-like elements. Staphylococcal transposons contains terminal inverted repeats (TIR) at their ends that serve as recognition sequences for transposase in their role of fusing the ends of the transposon with the recipient DNA. The three genes (*blaI*, *blaR*, and *blaZ*) involved in β-lactamase production constitute the right half of transposon Tn*552* (Tn*552*-like *bla* gene module). The left half of Tn*552* consists of genes p*271*, encoding a potential ATP-binding protein, p*480*, encoding a transposase, *binL*, encoding a resolvase, and a resolution site, *resL*. Partial and complete genetic structural arrangements of Tn*552* in staphylococci from different geographic locations have been described (3, 4, 21, 27). Some staphylococci harbor only the right or left half of

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TABLE 1. Screening for phenotypic susceptibility to BC and PEN among clinical staphylococci

Source	Bacterium	N[a]	No. of strains with indicated susceptibility[b] to:			
			BC		PEN	
			R	S	R	S
Skin isolate[c]	CNS	61	30	31	22	39
Skin isolate[d]	CNS	56	17	39	30	26
Bloodstream infection	*S. aureus*	61	37	24	41	20
	CNS	60	34	26	50	10
Total		238	118	120	143	95

[a] N, number of strains studied.
[b] Susceptibility was tested by the microdilution method. R, resistant; S, sensitive.
[c] Isolates from children with cancer.
[d] Isolates from HIV-positive outpatients.

Tn*552*. Staphylococcal insertion sequence IS*257*, associated with Tn*552* and IS*256*, has been shown to be an active mobile genetic element (5, 6, 13, 17, 27).

There is limited knowledge concerning the frequency of QAC resistance and the genetic linkage between *qac*-mediated resistance and antibiotic resistance genes. In this study, we investigated the presence and genetic linkage of known *qac* resistance determinants and antibiotic resistance genes, along with genetic mobile elements, in staphylococci isolated from clinical environments.

MATERIALS AND METHODS

Bacterial strains, culture media, and growth conditions. A total of 61 isolates of *S. aureus* and 177 CNS isolates were included in this study (Table 1). The staphylococci were isolated from patients in four different hospitals. *S. aureus* ($n = 61$) and CNS ($n = 60$) were isolated from patients with bloodstream infections at two hospitals (Molde County Hospital and Norwegian Radium Hospital) during 1991 to 1992 and 1995 to 1996 (10). The CNS isolates ($n = 56$) from the skin of human immunodeficiency virus (HIV)-positive patients were from Ullevål Hospital, while the remaining 61 CNS isolates were obtained at two hospitals (Ullevål Hospital and Rikshospitalet University Hospital) from the skin of children under cancer treatment receiving cytostatica and undergoing multiple-antibiotic cures. In all cases, repeat isolates from the same patient were excluded. This strain collection represents staphylococci from different types of hospitals and patients subject to various degrees of antimicrobial treatment. Reference strains were *Staphylococcus haemolyticus* NVH97A (resistant to BC and penicillin) and *S. aureus* RN4220, *S. haemolyticus* DSM20623, and *S. aureus* ATCC 25923 (sensitive to BC and penicillin). All staphylococcus strains were cultured in Mueller-Hinton (MH) broth or on MH agar plates (Difco Laboratories, Detroit, Mich.) at 37°C. Isolates were preserved in MH broth with 15% glycerol at −80°C.

Antimicrobial agents. BC was from the Norwegian Medical Depot (Oslo, Norway). Ampicillin (AMP), penicillin G (PEN), methicillin (MET), tetracycline (TET), erythromycin (ERY), KAN, GEN, chloramphenicol, TMP, and ethidium bromide (EBR) were purchased from Sigma Chemical Co. (St. Louis, Mo.), and chlorhexidine (CHX) was from Nycomed (Oslo, Norway). Amphoteric disinfectant Tego 103G was purchased from Otto Olsen AS (Lillestrøm, Norway). Neo-Sensitabs disks (Rosco, Medkjemi A/S, Taastrup, Denmark) containing antimicrobial agents were used for susceptibility testing.

Susceptibility tests. MICs of antimicrobial agents and dyes were determined by the microdilution method (28) and by E test (10) using MH medium. The lowest concentration of an antimicrobial agent and dye that totally inhibited bacterial growth after 24 h of incubation at 37°C was considered the MIC. Antibiotic susceptibility patterns were also examined by the disk diffusion method (Neo-Sensitabs user's guide, Rosco). The diameters of the inhibition zones around antimicrobial disks were measured in millimeters after 2 days of incubation at 37°C and compared to standard MIC breakpoints recommended by "Susceptibility testing standardization groups" (Neo-Sensitabs user's guide, Rosco). The results were interpreted as recommended by the Norwegian AFA Group (Neo-Sensitabs user's guide). Susceptibility tests for the isolates resistant to BC and PEN were repeated two times on different days.

MIC testing with disinfectants BC and Tego 103G was carried out by using 1-μg/ml intervals from 0 to 10 μg/ml. For CHX the interval was 0.5 μg/ml from 0 to 4 μg/ml. MIC tests with PEN, AMP, and EBR were performed by using twofold dilutions starting from 320 μg/ml.

DNA isolation and analysis. Cells were lysed with lysostaphin (Sigma-Aldrich, St. Louis, Mo.) at a concentration of 40 μg/ml and incubated at 37°C for 30 min. Total DNA and plasmid DNA were isolated by Easy-DNA (Invitrogen, Carlsbad, Calif.) and QIAprep spin (Qiagen GmbH, Hilden, Germany) kits, respectively. After agarose gel electrophoresis, the DNA was stained with EBR and visualized under UV light.

Plasmid curing. Strains were grown in increasing sublethal concentrations of novobiocin in MH broth as described by Heir et al. (7).

Southern blotting and hybridization. Plasmid DNA and total DNA were transferred from agarose gel to a Hybond-N^+ nylon membrane (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) along with positive and negative control DNA by vacuum blotting, according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). PCR products (see below) specific for genes *qacA* and *qacB* (common probe), *qacC*, *qacG*, *qacH*, *blaZ*, *blaR*, *blaI*, *ermC*, *cat, tetK*, *aacA-aphD*, and *dfrA* and insertion sequences IS*257* and IS*256* were used as probes. The PCR products were purified with a Qiagen PCR product purification kit and sequenced on an ABI PRISM 377 (Applied Biosystems, Perkin-Elmer Cetus Corp., Norwalk, Conn.) before being used as probes. The labeling of probes and hybridization were done with an AlkPhos direct gene image kit in accordance with the manufacturer's instructions (Amersham Pharmacia Biotech). Membrane stripping was done as recommended by the manufacturer.

PCR amplification. The primers used for PCR amplifications were designed from previously known sequences (Table 2). All the designed primers were first tested for their specificity on known positive and negative control strains. To obtain probes for DNA hybridization, standard PCR was carried out for *qacA* or *-B* (*qacA/B*), *qacC*, *qacG*, *qacH*, *blaZ*, *blaR*, *blaI*, *ermC*, *cat*, *tetK*, *aacA-aphD*, *dfrA*, IS*257*, and IS*256*. Each PCR mixture contained 50 ng of plasmid DNA, 2 μM (each) primer, 200 μM (each) deoxynucleoside triphosphate, 2.5 μM $MgCl_2$, 1× reaction buffer (Promega Corp., Madison, Wis.), and 2.5 U of *Taq* DNA polymerase (Promega Corp.) in a total volume of 50 μl. Reaction mixtures were subjected to 30 cycles of amplification. The conditions for each cycle were denaturation for 1 min at 95°C, annealing for 1 min at between 50 and 55°C depending on the primer set, and primer extension for 2 min at 72°C. Finally, reaction mixtures were incubated at 72°C for 10 min. The PCR products were separated by electrophoresis in a 1% agarose gel, stained with EBR, and visualized under UV light.

XL-PCR. The primers used for extralong PCR (XL-PCR) amplification are included in Table 2. The XL-PCR was carried out with a GeneAmp XL-PCR kit (Applied Biosystems) in accordance with the manufacturer's instructions with slight modifications. The GeneAmp XL-PCR kit (Perkin-Elmer Cetus Corp.) was used for amplification of the DNA regions between the β-lactamase genes and disinfectant resistance gene *qacA/B*. The primer combinations used were qac IV and blaI R2 and qac IV and blaZ 2F (see Fig. 2; Table 2). The DNA region between *qacA/B* and staphylococcal insertion element IS*257* was amplified by using primers qac IV and IS*257* F (see Fig. 2). Each PCR mixture contained 10 ng of plasmid DNA, 2 μM (each) PCR primer, 200 μM (each) deoxynucleoside triphosphate, 1 mM $MgCl_2$, and 1.5 U of *rTh* DNA polymerase-XL in a total volume of 50 μl. The cycle conditions were preliminary denaturation for 2 min at 93°C, followed by 25 cycles of denaturation at 93°C for 1 min, annealing for 45 s at between 47 and 50°C depending on the primer set, and primer extension for 7 min at 68°C. Finally, reaction mixtures were incubated at 68°C for 5 to 12 min depending on the primer set. The PCR products were examined by electrophoresis in a 0.7 or 1.0% agarose gel, stained with EBR, and visualized under UV light.

DNA sequencing. The nucleotide sequences of PCR products and XL-PCR products were determined by using an ABI PRISM BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems) with synthetic oligonucleotide primers on an ABI PRISM 377 automatic sequencer (Applied Biosystems) as recommended by the manufacturer. Before application on the sequencer, the extension products were purified as described by the manufacturer.

Unknown flanking DNA fragments were isolated by a two-step PCR method (A. B. Sørensen, M. Duch, and F. S. Pedersen, Dynalogue customer report **3:**2-3, 1999). The unknown DNA flanking regions were amplified with primers coupled to biotin at the 5′ end and degenerate flanking primers (Table 2) in first-step PCR. The PCR products were bound to Dynabead-streptavidin beads and purified with a Dynabeads kilobaseBINDER kit (Dynal, Oslo, Norway). The puri-

TABLE 2. Primers used in PCR and XL-PCR amplification

Primer	Sequence (5′–3′)[a]	Annealing position[b] or source	Accession no. or reference
qacA/B R	TGGCCCTTTCTTTAGGGTTT	1278–1259	X56628
qacA/B F	ATTCCATTGAGTGCCTTTGC	1061–108	X56628
qacA/B	GCTGCATTTATGACAATGTTTG	1693–1713	X56628
qac II	AATCCCACCTACTAAAGCAG	2321–2302	X56628
qac IIR	CTGCTTTAGTAGGTGGGATT	2302–2321	X56628
qac IV	TTTAAATGGCGAATGGTGT	249–267	X56628
qacC F	GGCTTTTCAAAATTTATACCATCCT	621–645	Z37964
qacC R	ATGCGATGTTCCGAAAATGT	870–850	Z37964
qacG F	TAACTTACGCAACATGGGCA	170–190	Y16944
qacG R	TCAATGGCTTTCTCCAAATAC	325–303	Y16944
qacH F	CAAGTTGGGCAGGTTTAGGA	180–200	Y16945
qacH R	TGTGATGATCCGAATGTGTTT	321–300	Y16945
blaI F	ATGTCTCGCAATTCTTCAA	3190–3208	X52734
blaI R	CTATGGCTGAATGGGAT	3520–3503	X52734
blaI R2	CAAAGAAATTGAAGAATTGCGA	3195–3216	X52734
blaR F	CATCTGATAAATGTGTAGC	3612–3630	X52734
blaR R	GGTATCTAACTCTTCTTGC	5177–5159	X52734
blaZ 1F	TACAACTGTAATATCGGAGGG	5372–5392	X52734
blaZ 2F	GAGGCTTCAATGACATATAGTG	5741–5762	X52734
blaZ R	CAATAGGTTCAGATTGGCCC	6149–6129	X52734
blaZ 3F	CACCTGCTGCTTTCGGTAAGAC	5916–5937	X52734
blaZ 4F	GTTGATAAGTGAAACCG	6201–6218	X52734
tetK F	CTACTCCTGGAATTACAA	138–154	S67449
tetK R	TACTATACACTCCAGAAG	1291–1273	S67449
dfrA F	CACTTGTAATGGCACGGAAA	119–139	AF045472
dfrA R	CTGGTCAATCATTGCTTCGT	335–315	AF045472
ermC F	ATCTTTGAAATCGGCTCAGG	2639–2620	J01755
ermC R	CAAACCCGTATTCCACGATT	2345–2364	J01755
cat F	ATGGTTCGGGGAAATTGTTT	1627–1647	J01754
cat R	TCCTGCATGATAACCATCACA	1853–1832	J01754
aacA-aphD F	CAGAGCCTTGGGAAGATGAA	150–170	M18086
aacA-aphD R	TTGCCTTAACATTTGTGGCA	564–544	M18086
mecA F	GTGGAATTGGCCAATACAGG	478–497	X52594
mecA R	TGAGTTCTGCAGTACCGGAT	1797–1816	X52594
IS*256* F	CAGAACAGCTGGATCCTATGG	523–543	M18086
IS*256* R	GTCGACTTTTAGCCTCACGCG	970–990	M18086
IS*257* F	TTGGGTTCAAGAATATGCCC	202–222	U40386
IS*257* R	CTTCGTTGAAGGTGCCTGAT	473–453	U40386
Bio-qacA/B[c]	Biotin-CTGCATTTATGACAATGTTTG	1692–1713	X56628
Bio-blaZ 2F[c]	Biotin-GAGGCTTCAATGACATATAGTG	5740–5762	X52734
Bio-FP[c]	Biotin-CAGTTCAAGCTTGTCCAGGAATTC	This study	CR[e]
Degen-FP1[d]	CAGTTCAAGCTTGTCCAGGAATTCNNNNNNNGGCCT	This study	CR
Degen-FP2[d]	CAGTTCAAGCTTGTCCAGGAATTCNNNNNNNGCGCT	This study	CR
Degen-FP3[d]	CAGTTCAAGCTTGTCCAGGAATTCNNNNNNNGCCCT	This study	CR
Degen-FP4[d]	CAGTTCAAGCTTGTCCAGGAATTCNNNNNNNGCGGT	This study	CR

[a] Underlined sequences indicate differences among degenerate primers.
[b] Annealing position in published sequence.
[c] Biotinylated primer.
[d] Degenerate primer.
[e] CR, Sørensen et al., Dynalogue customer report **3:**2–3, 1999.

fied PCR products from the first-step PCR were then used as templates for nested PCR followed by DNA sequencing. Nucleotide sequences were analyzed by using the BLAST website program (National Center for Biotechnology Information, National Institutes of Health), the Sequencher, version 3.0, software package (Gene Codes Corporation, Ann Arbor, Mich.), and the GCG sequence analysis software package, version 8 (Genetics Computer Group, Madison, Wis.).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this study have been assigned accession no. AF426833, AF426834, and AF426835 in the GenBank database.

RESULTS

Susceptibility testing. The 238 staphylococcus isolates were screened for QAC (BC) and PEN resistance (Table 1). The strains were categorized as BC resistant or sensitive according to the BC MICs. One hundred eighteen (50%) isolates were considered BC resistant (BC MICs between 3 and 8 μg/ml), and 120 isolates were sensitive to BC (BC MICs ≤ 2 μg/ml). Of the 238 staphylococcus isolates, 143 were found resistant to PEN. One hundred twenty-one CNS isolates from patients with bloodstream infections ($n = 60$) and from the skin of children under cancer treatment ($n = 61$) were analyzed for correlation between BC and antibiotic resistance (Fig. 1). This analysis showed that the frequency of antibiotic resistance was higher among the BC-resistant strains. As an example, a large number of BC-resistant strains also showed PEN resistance. On the basis of Fisher's exact test (1), these distributions appear not to be random ($P \leq 0.01$ for PEN, oxacillin, cephalothin, cefuroxime, imipenem, TMP, GEN, doxycycline, and fusidic acid). None of the isolates were resistant to vancomycin,

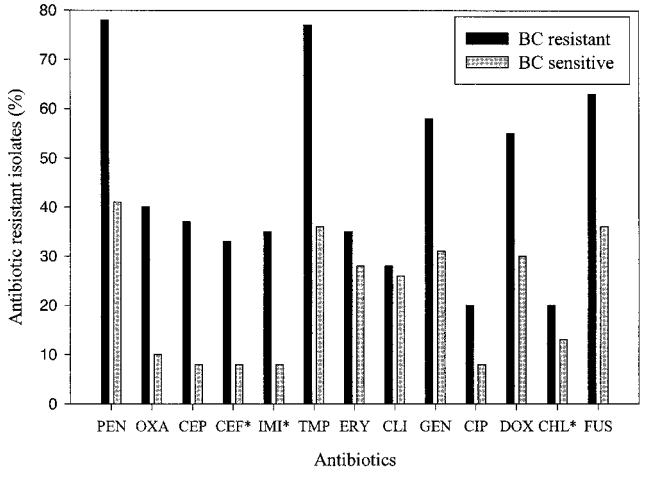

FIG. 1. Percentages of antibiotic-resistant CNS isolates from patients with bloodstream infections ($n = 60$) and from the skin of children under cancer treatment ($n = 61$) among BC-resistant and BC-sensitive isolates. Antibiotics used are PEN, oxacillin (OXA), cephalothin (CEP), cefuroxime (CEF), imipenem (IMI), cotrimoxazole (COT), ERY, clindamycin (CLI), GEN, ciprofloxacin (CIP), doxycycline (DOX), chloramphenicol (CHL), and fusidic acid (FUS). Susceptibility to CEF, IMI, and CHL was tested only among CNS isolates from patients with bloodstream infections (asterisks).

and less than 3% of the staphylococcus isolates were resistant to teicoplanin and rifampin (results not shown). The CNS from HIV-positive outpatients and *S. aureus* from patients with bloodstream infections were excluded from the analyses of the correlation between BC and antibiotic resistance, because the frequencies of antibiotic resistance among these isolates were relatively low (data not shown).

The 118 BC-resistant isolates were screened for susceptibility to a second disinfectant, CHX (Table 3). Twenty-eight of the BC-resistant isolates showed low resistance to CHX (MIC between 1.5 and 3.0 μg/ml), whereas 90 isolates were sensitive (MIC ≤ 1.0 μg/ml). Likewise, of 42 BC-resistant staphylococcus isolates randomly selected among strains for which the BC MIC was ≥4 μg/ml, 11 and 37 isolates were resistant to disinfectant Tego 103G and dye EBR, respectively (Table 3). Systematic cross-resistance between BC and EBR and no systematic cross-resistance between BC and CHX or Tego 103G were found in staphylococci.

The MIC tests and disk diffusion test revealed that an isolate from a bloodstream infection, *Staphylococcus epidermidis* Fol62, was resistant to BC (MIC, 5 μg/ml), EBR (MIC, 160 μg/ml), Tego 103G (MIC, 10 μg/ml), PEN (MIC, 160 μg/ml), AMP (MIC, 160 μg/ml), MET (MIC, 50 μg/ml), TET (MIC, 70 μg/ml), KAN (MIC, 7 μg/ml), ERY (MIC, 80 μg/ml) and TMP (MIC, 8 μg/ml).

TABLE 3. BC-resistant isolates were screened for susceptibility to disinfectants and dye by a microdilution method

Bacterium	No. of isolates[a] with indicated susceptibility[b] to:					
	CHX (118)		Tego 103G (42)		EBR (42)	
	R	S	R	S	R	S
S. aureus	8	3	3	13	14	2
CNS	20	77	8	18	23	3
Total	28	90	11	31	37	5

[a] Total numbers of BC-resistant strains tested are in parentheses.
[b] R, resistant; S, sensitive.

DNA hybridizations. Of the 118 BC-resistant isolates, 78 isolates for which the BC MICs were high (MICs between 4 and 8 μg/ml) were selected for DNA hybridization studies. Plasmid DNA from these isolates was screened for the presence of known gram-positive bacterium *qac* genes mediating resistance (*qacA*/*B*, *qacC*, *qacG*, and *qacH*), β-lactamase genes (*blaZ*, *blaI*, and *blaR*), other antibiotic resistance genes (*ermC*, *cat*, *dfrA*, and *tetK)*, and staphylococcal insertion sequences IS*257* and IS*256* by Southern blotting and hybridization (Table 4). Sixty-seven of the BC-resistant isolates harbored either *qacA*/*B* or *qacC* on plasmid DNA, and the *qacA*/*B* gene was detected by PCR (total DNA was the template) in the remaining 11 isolates. Neither the *qacG* nor the *qacH* gene was detected among the isolates. Plasmids from 43 strains also hybridized with β-lactamase probes. In 19 of these strains, *qac* and *blaZ*, *blaI*, and *blaR* resided on the same plasmid. Only *S. epidermidis* Fol62 plasmid pMS62 (32 kb) hybridized with other antibiotic resistance gene probes tested (*tetK*, IS*257*, *ermC*, *aacA-aphD*, and *dfrA*) in addition to *qacA*/*B*, *blaZ*, *blaI*, and *blaR*. *tetK* and a copy of IS*257* were localized adjacent to *blaZ*. However, no XL-PCR products were obtained when DNA stretches between *tetK* or *qacA*/*B* and antibiotic resistance gene *ermC*, *aacA-aphD*, or *dfrA* were amplified. The determinant for MET resistance (*mecA*) in *S. epidermidis* Fol62 was chromosomally encoded.

Genetic organizations of the *qac* and β-lactamase genes. From the 19 staphylococcus isolates harboring disinfectant *qac* and β-lactamase genes on the same plasmids, we selected six isolates for a study of gene organization, isolates Fol24 (pMS24, 30 kb), Fol33 (pMS33, 35 kb), Fol62 (pMS62, 32 kb), Fol89 (pMS89, 40 kb), Fol90 (pMS90, 35 kb), and Fol100 (pMS100, 40 kb) (plasmid designations and approximate sizes are in parentheses). These isolates were all identified as *S. epidermidis*. Isolates Fol24, Fol33, and Fol62 were from patients having bloodstream infections, and isolates Fol89, Fol90, and Fol100 were from the skin of children treated for cancer. Results obtained by XL-PCR and DNA sequencing indicated different structural arrangements of the β-lactamase genes and the disinfectant resistance *qacA*/*B* genes, including variable intergenic distances and different transcriptional orientations of genes located on the same plasmids (Fig. 2).

Staphylococcal plasmids pMS24, pMS33, and pMS89 harbor a complete copy of transposon Tn*552*, comprising p*271*, p*480*, and *binL*, encoding a potential ATP-binding protein, a transposase, and a resolvase, respectively, in addition to the β-lactamase structural gene (*blaZ*) and regulatory genes (*blaR* and *blaI*). Staphylococcal insertion sequence IS*257* was found downstream of the *blaZ* gene in these plasmids. There were sequences homologous to the right TIR (TIR_R) of Tn*552* downstream of *blaZ*, a *resL* site between *blaI* and *binL*, and a sequence homologous to TIR_L of Tn*552* downstream of p*271*. Isolates Fol24, Fol33, and Fol89 harbored the *qacA*/*B* gene together with their regulatory gene, *qacR*, downstream of the p*271* gene. However, the region between p*271* and *qacR* was different for different isolates. Fol24 and Fol89 had very similar

TABLE 4. Screening for the presence of disinfectant and various antibiotic resistance genes on plasmid DNA among BC-resistant staphylococci

Isolate source	N[a]	Bacterium	No. of isolates with hybridization[b] to:									
			qacA/B	*qacC*	*blaZ*[c]	*ermC*	*tetK*	*cat*	*dfrA*	IS*257*	IS*256*	*qacA/B* + *blaZ*[d]
Skin[e]	25	CNS	23	1	11	8	4	5	6	13	6	6
Bloodstream cultures	26	*S. aureus*	17	0	14	4	2	5	0	8	2	5
	27	CNS	25	1	18	12	9	10	9	15	9	8
Total	78		65	2	43	24	15	20	15	36	17	19

[a] N, number of strains studied.
[b] The *qacABC, blaZ, ermC, tetK, cat*, and *dfrA* genes encode QAC, PEN, ERY, TET, chloramphenicol, and TMP resistance, respectively, from plasmids.
[c] Hybridized with *blaZ*, *blaI*, and *blaR* probes.
[d] *qacA/B* and *blaZ* on the same plasmid.
[e] Isolates from children treated for cancer.

regions and contained the *sin* gene, encoding a putative recombinase, while the *sin* gene was absent in Fol33. Plasmid pMS62 harbored the incomplete right half of the transposon Tn*552*-like *bla* gene module upstream of the *binR* and *sin* genes. Also in pMS62, IS*257* was found downstream and adjacent to *blaZ*. The *tetK* gene, encoding TET resistance, was located approximately 2.4 kb further downstream.

In Fol90 and Fol100 the orientation of β-lactamase gene clusters was inverted relative to the *qacA/B* gene cluster, so that *qacR* was situated downstream of *blaZ*. In pMS90 the intergenic sequence between *blaZ* and *qacR* contained only the inverted repeat of Tn*552*, while in pMS100 this region contained a *sin* and an additional noncoding 743 bp of DNA. DNA hybridization and PCR confirmed the presence of staphylococcal insertion sequence IS*257* on pMS90 and pMS100.

When the DNA stretches between the β-lactamase genes and disinfectant resistance gene *qacA/B* were amplified by using primer combinations qac IV and blaI R2 and qac IV and blaZ 2F, both primer sets gave rise to PCR products in strains Fol33 and Fol89 (Fig. 2). DNA sequencing of the PCR products of qac IV and blaZ 2F revealed that the *blaZ* and the *qacR* genes were located very close to each other, with the TIR of Tn*552* between the genes. These strains apparently harbored the gene clusters in two orientations.

The nucleotide and deduced amino acid sequences derived from complete and incomplete (Tn*552*-like *bla* gene module) Tn*552* transposons of plasmids pMS24, pMS33, pMS89, and pMS62 were identical to those derived from staphylococcal β-lactamase transposon Tn*552* (25). The deduced amino acid sequences derived from *qacR* of plasmids pMS24, pMS33, pMS62, pMS89, pMS90, and pMS100 and *qacA/B* of pMS33 were similar to those derived from pST6, pSK156, and pSK23. Similarly, the deduced amino acid sequences derived from the resolvase gene (*binR*) of pMS62 was 100% identical to that derived from pST6 (AY028779) and 99% similar to that derived from pNVH96 (AJ302698). The recombinase genes (*sin*) of pMS62 and pMS89 were 100% identical and 99% similar to those of pST6 and pNVH97A (AJ400722), respectively. The *sin* gene of Fol100 is 100% identical to that of *S. epidermidis* strain SR1 and 90% similar to the *sin* genes of Fol62 and Fol89.

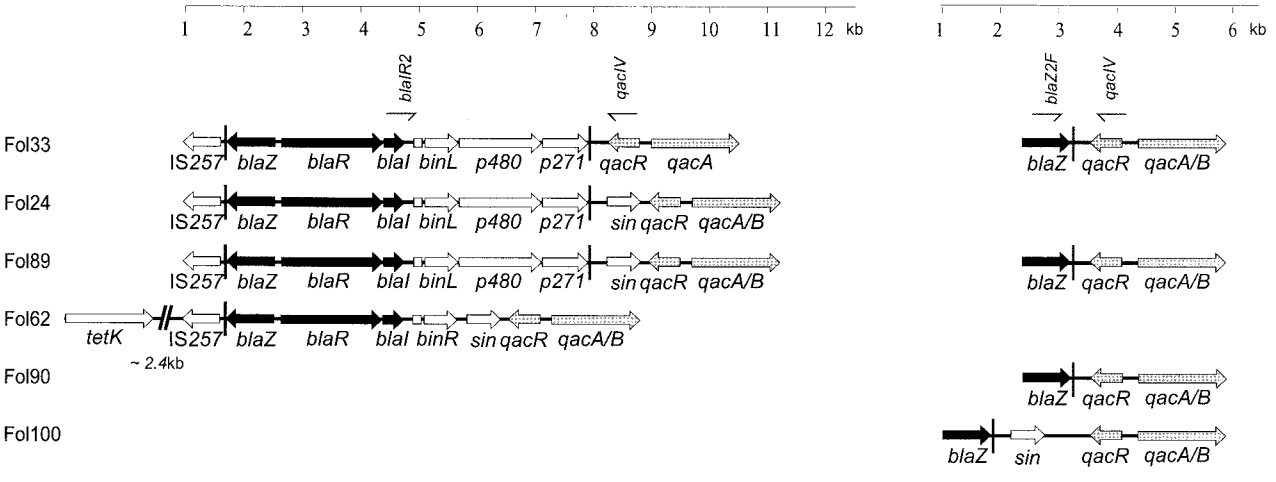

FIG. 2. Genetic organization of *qac* and β-lactam resistance genes in staphylococcal isolates Fol24, Fol33, Fol62, Fol89, Fol90, and Fol100. Genes involved in *qac* and β-lactam resistance are gray and black, respectively. *qacA/B* encodes QAC resistance; *qacR* encodes a putative repressor of *qacA/B*; *sin* is a putative staphylococcal recombinase gene; p*271* encodes a potential ATP-binding protein; p*480* encodes a transposase; *binR* and *binL* are resolvase-encoding genes; *blaZ* is a β-lactamase structural gene; *blaR* and *blaI* are β-lactamase regulatory genes; IS*257* is a staphylococcal insertion sequence. Primers for detection of specific PCR products are indicated for Fol33. Vertical bars and boxes downstream of *blaI*, TIR of transposon Tn*552* and a resolution site (*res*), respectively.

DISCUSSION

Little is known about the occurrence and possible genetic linkage of *qac* and antibiotic resistance in staphylococci. Of the 238 human clinical isolates from Norway investigated in this study, 50% were phenotypically resistant to BC. Plasmid-borne *qacA/B* and *qacC* genes were detected in 83 and 3% of the BC-resistant staphylococcus isolates, respectively. The *qacA/B* genes were also detected in the remaining BC-resistant isolates, indicating that the genes were either chromosomally located or present on large plasmids not obtained by the plasmid isolation procedure. Previous investigators have reported a similar distribution of these three *qac* resistance genes in clinical *S. aureus* and CNS (2, 11, 15) although additional staphylococcal disinfectant resistance genes also have been indicated (18). A lower prevalence of QAC resistance (13%) was reported for staphylococci from food and the food-processing industry (7, 8, 9). Here, both *qacA/B* and *qacC* as well as *qacG* and *qacH* were reported, with *qacC* as the most prevalent determinant. Overall, it seems likely that the presence, maintenance, and widespread dissemination of multidrug efflux *qac* genes in staphylococci are a consequence of the selective pressure exerted by the use of antiseptics and disinfectants (11, 18, 20, 26).

Many of the staphylococci included in this study were resistant to different antibiotics, but in general the antibiotic resistance was considered low compared to findings in other studies (10). Interestingly, we observed that staphylococci resistant to BC were generally more often resistant to antibiotics than BC-sensitive isolates (Fig. 1). The results are compatible with selective advantages of isolates carrying both disinfectant and antibiotic resistance genes, and the data indicate that the presence of *qac* genes in staphylococci results in selection of antibiotic-resistant bacteria (20). Russell also speculated that disinfectant resistance might contribute to antibiotic resistance by coresistance or cross-resistance mechanisms or coselection (26).

This study detected a systematic genetic linkage between resistance to BC and PEN although less than one-half of the plasmid-encoded β-lactamase resistance was linked to disinfectant resistance genes (19 of 43 isolates). Previous investigators have also reported genetic linkage between disinfectant (*qac*) and antibiotic resistance genes (*blaZ*, *aacA-aphD*, *dfrA*, and *ble*) on the same staphylococcal plasmids from clinics (3, 14, 21), food environments (27), and an animal clinic (I.-L. Anthonisen et al., unpublished data). To our knowledge, this paper is the first report of closely linked *qac*, β-lactamase, and *tetK* genes on a multiresistance plasmid (pMS62). Plasmid pMS62 also harbored *ermC*, *dfrA*, and *aacA-aphD*, encoding resistance to ERY, TMP, and GEN-KAN-tobramycin, respectively. No genetic linkage between *qac* and these or other antibiotic resistance genes tested was detected. The observed cross-resistance between BC and EBR is compatible with the ability of all known *qac* resistance determinants to cause the efflux of structurally similar, monovalent cationic agents. A low-level CHX resistance phenotype probably reflects isolates expressing *qacA*, which also encodes resistance to divalent cations (19), while resistance to the amphoteric disinfectant Tego 103G is probably due to other mechanisms.

The genetic organizations between *qac* and β-lactamase genes in six plasmids (pMS24, pMS33, pMS62, pMS89, pMS90, and pMS100) were studied (Fig. 2). The plasmids originated from isolates from different sources and distant geographic areas, and their genetic organizations showed similarities with those of other known staphylococcal plasmids, e.g., pSK1, pSK4, pSK23, pSK57, pSK108, pSK156, pNVH97A, pNVH96, pI258, and pI9789 (3, 4, 12, 14, 20). Our results generally confirmed previous findings that β-lactamase-related transposons Tn*552* and Tn*4002* preferentially insert within TIR_L located upstream of the *sin* gene (4, 21). Insertion of Tn*552*-like transposons adjacent to *qac* genes also occurs frequently, as observed in isolates Fol133, Fol89, Fol90, and Fol100 (this study). Overall, this suggests that genetic organizations containing IS*257*, Tn*552*, *binR*, and *sin* and/or *qacR* and *qacA/B* are widely distributed among staphylococci from clinical as well as food environments. Various rearrangements and DNA inversions generating two alternative staphylococcal β-lactamase transposons have been reported (4). We also found the presence of additional organizations of Tn*552* and *qacA/B* within the genomes of strains Fol33 and Fol89 (Fig. 2).

On pMS62, the resolvase- and recombinase-encoding genes (*binR* and *sin*, respectively) were present between *qac* and the incomplete Tn*552*-like *bla* gene module as in *S. aureus* plasmid pS1 (21). The truncated Tn*552* observed in pMS62 has probably lost its ability to transpose, as the transposase gene (p*480*) was not detected.

Staphylococcal insertion sequence IS*257* plays a central role in cointegration events (3, 6, 17). Large, conjugative plasmid pSK41 has integrated several small plasmids, where all the cointegrated plasmids are all flanked by copies of IS*257* (3). IS*257* has been found closely associated with Tn*552* (27), *qacB* (pSK156) (22), *qacA* (AJ400722), and *qacC* (11, 12) as well as antibiotic resistance determinants, e.g., *dfrA*, *tetK*, and *aacA-aphD* (3, 6, 12, 13, 14, 27). The presence of IS*257* downstream of *blaZ* on pMS33, pMS24, pMS62, and pMS89, and also detected on pMS90 and pMS100, indicates that IS*257* integration events were involved in the formation of the structures of these plasmids. The presence of insertion sequences IS*257* and IS*256* on some of the plasmids harboring either *qacA/B* or β-lactamase genes suggests that these plasmids may act as a source for formation of new multiresistance plasmids (5, 13, 17).

Taken together the above observations indicate that a number of different recombination events have occurred to yield the various genetic organizations. To investigate the stability of the plasmids harboring disinfectant and antibiotic resistance genes, we tried to cure the isolates for plasmids. None of the strains were cured for the resistance plasmids. This indicated that plasmids are stably maintained in these strains.

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