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Conjugative Transfer of Staphylococcal Antibiotic Resistance Markers in the Absence of Detectable Plasmid DNA

NÉVINE EL SOLH,* JEANINE ALLIGNET, ROLAND BISMUTH, BERNADETTE BURET, AND JEANNE-MARIE FOUACE

Laboratoire des Staphylocoques et des Streptocoques (Unité des Antigènes Bactériens), Institut Pasteur, 75724 Paris Cedex 15, France

Received 12 November 1985/Accepted 17 April 1986

Eleven *Staphylococcus aureus* clinical isolates were tested for transfer of resistance markers by transduction and filter mating. The resistance markers of six of the strains could be transferred only by transduction; however, the five remaining strains transferred their resistance both by transduction and filter mating. The resistance markers that were cotransferred in filter matings (transfer of resistance to penicillin and streptogramin A was accompanied, in each case, by the transfer of one or more markers, i.e., resistance to aminoglycosides, cadmium, or tetracycline, depending on the donor) were not cotransduced. The filter mating transfers were *recA* independent and were observed with both *Staphylococcus aureus* and *Staphylococcus epidermidis* recipients. Experiments to elucidate the mechanism of transfer by filter mating suggested that conjugation requiring cell-to-cell contact may have been involved. These transfers occurred in the absence of detectable plasmid DNA.

Most of the drug resistance genes of multiply resistant *Staphylococcus aureus* strains isolated in Europe are reported to be located on the chromosome (6, 12, 27, 30, 55; N. El Solh, J. Allignet, R. Bismuth, B. Buret, and J. M. Fouace, *in* J. Jeljaszewicz, ed., *Staphylococci and Staphylococcal Diseases*, in press; N. El Solh, N. Moreau, B. Michel, and D. Ehrlich, *in* J. Jeljaszewicz, ed., *Staphylococci and Staphylococcal Diseases*, in press). Evidence for the chromosomal location of these genes includes their low frequency of transduction ($<1 \times 10^{-9}$ transductants per PFU) and the absence of extrachromosomal DNA in the transductants. Further evidence was obtained from hybridization experiments with probes consisting of aminoglycoside resistance genes. These probes did not hybridize with the plasmids isolated from the wild-type strains; hybridization with cellular DNA from both the wild-type strains and the plasmid-free transductants supported the hypothesis of the chromosomal location of these genes (13a).

Although the transfer by conjugation of the antibiotic resistance genes in the absence of detectable plasmids occurs in various bacterial genera (4, 7, 18, 20, 21, 23, 24, 34, 36, 44, 49, 50, 56), reports of such conjugative transfer in staphylococci have so far been limited to plasmid-borne determinants (2, 3, 15, 19, 33, 37, 53, 54).

We report in this study the transfer by conjugation of apparently chromosome-borne resistance markers from wild-type *S. aureus* strains into staphylococcal recipients belonging to different species.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. The wild-type *S. aureus* strains were isolated in French hospitals from independently obtained clinical specimens.

Media. Brain heart infusion broth (BHI; Difco Laboratories, Detroit, Mich.) and BHI agar (Difco) were used for bacterial growth, mating experiments, and the preparation of all of the selective media except those containing trimethoprim. Nutrient broth no. 2 (Oxoid Ltd., London, England) and nutrient broth agar (Oxoid) were used for bacteriophage propagation and transduction experiments. Mueller-Hinton agar (Institut Pasteur Production, Paris, France) was used for disk susceptibility tests and preparation of selective media containing trimethoprim.

Mating procedures on agar media. A 0.3-ml portion of a mixture containing 1×10^7 CFU of donors and 10-fold more recipients, both obtained from late-logarithmic-phase broth cultures, was spread either on nitrocellulose filters (type HAEP; pore size, 0.45 μm; Millipore Corp., Bedford, Mass.), as described by Horodniceanu et al. (20), or directly on agar plates. After 18 h of incubation at 37°C, the cells on each filter were suspended in 1 ml of BHI, and the suspension was spread on BHI agar or Mueller-Hinton agar containing the appropriate selective drug. Controls, consisting of donor or recipient cells alone, were treated similarly. For two crosses, the donor and recipient cultures were separated during incubation (18 h at 37°C) by a nitrocellulose filter: a filter was placed on the agar and spread with the donor culture, a second filter was placed on the donors, and the recipient culture was spread on the top. The same experiment was also done with the positions of the donor and recipient reversed. The drugs used for selection of the donor markers were cadmium acetate (0.15 μmol ml^{-1}), erythromycin (5 μg ml^{-1}), kanamycin (100 μg ml^{-1}), streptogramin A (20 μg ml^{-1}), tetracycline (5 μg ml^{-1}), and trimethoprim (10 μg ml^{-1}). The drugs used for counterselection of bacterial donors were either fusidic acid (25 μg ml^{-1}) and rifampin (100 μg ml^{-1}) used together or novobiocin (25 μg ml^{-1}), depending on the recipient used. The clones which acquired drug resistance, designated transcipients, were scored after 48 h of incubation at 37°C. The frequency of transfer was expressed as the number of resistant recipients per donor CFU at the end of the mating. The transcipients were purified on media containing the drugs used for their selection, picked onto drug-free agar, and analyzed for unselected markers both by replica plating on appropriate selective

* Corresponding author.

TABLE 1. Bacterial strains

Strain[a]	Relevant markers[b]	Plasmid size (kb) and markers[b]	Reference or source
Wild types			
BM3121[c]	Ak Gm Km Lv Nm Nt Pm Sm Ss Tm Sp/As Cd/Mc Pc/MLS/Mn Tc/Su	22; Pc/Cd/As 3.4[d] 2.3[d]	11, 13a
BM3152[c]	Ak Gm Km Lv Nm Nt Pm Sm Ss Tm Sp/As Cd/Mc Pc/MLS/Mn Tc/Su	22; Pc/Cd/As	11, 13a
BM3247	Ak Gm Km Lv Nm Nt Pm Sm Ss Tm Sp/As Cd Hg/L SgA[e]/Mc Pc/Mn Tc/Su	None	10
BM3248	Ak Gm Km Nt Ss Tm/As Cd Hg/Pc/L SgA[e]	None	10
BM3249	Ak Gm Km Lv Nt Pm Sm Ss Tm/As Cd/L SgA[e]/Mc Pc/Mn Tc/Su	None	10
BM3250	Ak Gm Kn Nt Sm Ss Tm/As Cd Hg/L SgA[e]/Cm/Mc Pc/Su	2.8; not studied	10
BM3251	Ak Gm Km Nt Sm Ss Tm/As Cd Hg/L SgA[e]/Mc Pc	2.3; not studied	10
BM3252	Ak Gm Km Lv Nm Nt Pm Sm Ss Tm/As Cd Hg/L SgA[e]/Mc Pc//Mn Tc/Su	None	10
BM3318	Ak Gm Km Lv Nm Nt Pm Sm Ss Tm Sp/As Cd Hg/Mc Pc/MLS/SgA[e] SgB[e]/Mn Tc/Su	22; Pc/Cd/As	10
BM3377	Ak Gm Km Lv Nt Pm Sm ss Tm/As Cd/L SgA[e] SgB[e]/Mn Tc/Pc	3.5; not studied	This study
BM3385	As Cd/L SgA[e]/Mc Pc/Rf/Su/Tp	None	This study
Recipients			
BM224	Fa/Rf	None	1[b]
ISP1127	Nv	None	41
BM231	Fa/Pc/Rf/Sm, restrictionless	None	22
RN980	*RecA1*	None	27
BM3449	Fa/Rf, *recA1*	None	Mutant of RN980
BM3319	Fa/Rf	None	16
BM3302	Rf/Fa/As/Cd	35; not studied	16
Plasmid-containing controls			
BM3002	Ak Km Lv Nm Tm/Cd/Fa/Nv/Rf/SgA[e]SgB[e]	35; Ak Km Lv Nm Tm/Cd/SgA[e], SgB[e]	13
TC41	Cd/Pc, Eti+ii, Ba	42; Cd, Etii, Ba	40
BM224(pAM899-1)	Ak Km Nt Ss Tm/Fa/Rf	42; Ak Gm Km Nt Ss Tm	15

[a] All are *S. aureus* strains except BM3302, which is *S. epidermidis*.

[b] Abbreviations (resistance): Ak, amikacin; As, sodium arsenate; Cd, cadmium acetate; Cm, chloramphenicol; Fa, fusidic acid; Gm, gentamicin; Hg, mercuric nitrate; Km, kanamycin; L, lincosamides; Lv, lividomycin; Mc, methicillin; MLS, macrolides-lincosamides-streptogramin B; Mn, minocycline; Nm, neomycin; Nt, netilmicin; Nv, novobiocin; Pc, penicillinase production; Pm, paromomycin; Rf, rifampin; SgA, streptogramin A; SgB, streptogramin B; Ss, sisomycin; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim. Antibiotics belonging to the same family are grouped and separated from other families by shills. Other abbreviations (production): Eti+ii, epidermolytic toxins belonging to serotypes i and ii (also called A and B); Ba, bacteriocins.

[c] Strain carries both the *aphA* gene, encoding the aminoglycoside phosphotransferase APH(3')III, and the *aacA* gene, encoding the bifunctional protein AAC(6')-APH(2"), which has both aminoglycoside acetyltransferase and phosphotransferase activities (13a).

[d] Plasmid was not transferred with the following transduced resistance markers: Sodium arsenate, cadmium acetate, kanamycin, minocycline, macrolides-lincosamides-streptogramin B, spectinomycin, or tetracycline (13a).

[e] An enzyme inactivating this antibiotic was detected by a Gots test performed as described in Materials and Methods.

media and by the disk diffusion method. The media used for purification and replica plating contained either the same concentrations of antibiotics as those used for selection or higher concentrations in the case of cadmium acetate (0.25 μmol ml^{-1}), streptogramin A (30 μg ml^{-1}), and trimethoprim (20 μg ml^{-1}). Unselected markers were tested on media containing gentamicin (20 μg ml^{-1}), neomycin (40 μg ml^{-1}), or sodium arsenate (8 μmol ml^{-1}). The disk diffusion method was performed with both commercial antibiotic disks (Institut Pasteur Production) and disks loaded with cadmium acetate (2×10^{-1} μmol), mercuric nitrate (2×10^{-1} μmol), sodium arsenate (2 μmol), spectinomycin (100 μg), streptogramin A (20 μg), or streptogramin B (40 μg). Penicillinase production was detected by using the chromogenic cephalosporin substrate nitrocefin (Glaxo Pharmaceuticals, France) according to the instructions of the supplier.

Broth mating procedures. Ten milliliters of BHI was inoculated with 0.3 ml of a mixture containing the donor and recipient strains, prepared as described above for matings on agar media. After 18 h of incubation at 37°C, the bacterial cells were pelleted and spread on the appropriate selective media as described above.

Transduction procedures. The technique used for transduction was described previously (17). Transductions were performed either with the *S. aureus* transducing phage ϕ11, 1503, 1305, or 80α, propagated on the donor strains, or with the phage(s) harbored by the donors and induced by UV irradiation (38). The multiplicity of infection was 1 PFU/10 CFU. The media used for the selection of transductants contained 5 μg of both fusidic acid and rifampin ml^{-1} and one of the following drugs: cadmium acetate (0.15 μmol ml^{-1}), erythromycin (5 μg ml^{-1}), tetracycline (3 μg ml^{-1}), or trimethoprim (10 μg ml^{-1}). The drug-resistant recipients (transductants) were purified and analyzed for unselected markers as described above for transcipients.

Detection of antibiotic-inactivating enzymes. For the detec-

tion of antibiotic-inactivating enzymes, a modified Gots test was performed as described previously (10).

Curing procedures. Portions (0.2 ml) of 18-h BHI cultures were inoculated into 5-ml portions of BHI containing increasing concentrations of ethidium bromide (1 to 400 μg ml^{-1}) or novobiocin (0.4 to 200 μg ml^{-1}) and incubated at 45°C for 18 h. Isolated colonies obtained from the cultures grown at the highest subinhibitory concentration of the curing agent were tested by replica plating on media supplemented with the appropriate drugs.

DNase I, calcium, and antiphage serum assays. The sensitivity of filter mating transfer to DNase I (Sigma Chemical Co., St. Louis, Mo.) was tested as described by Jacob and Hobbs (25). DNase I was added at a final concentration of 100 μg ml^{-1} to both the broth media used for donor and recipient cultures and to the media used for matings. Calcium chloride was added at a final concentration of 0.01 M to the broth and agar media mentioned above. For one of the crosses (BM3248 × BM224), a rabbit antiphage serum was added to the donor and recipient cultures before mating. This antiserum, prepared with phage 1339/789, as described by Rountree (46), contained antibodies specific for *S. aureus* phages belonging to serogroup F and was shown to inhibit the lytic and transducing activities of the phages(s) carried by the donor strain, BM3248.

Plasmid DNA isolation. Six techniques, including both rapid and large-scale procedures, were used to screen for plasmid DNA in the staphylococcal strains. The rapid procedures were those described by Goering and Ruff (19), O'Reilly et al. (41), and Portnoy et al. (43). The latter technique was adapted to staphylococci as follows: the bacterial pellet obtained from 2 ml of an overnight BHI culture was suspended in 40 μl of buffer, lysostaphin (Sigma) was added to give a final concentration of 50 μg ml^{-1}, and the suspension was incubated for 30 min at 37°C. Otherwise, the procedures were as originally described (43).

Three large-scale plasmid isolation techniques were used. The bacterial cells obtained from 200 ml of an overnight BHI culture were treated with lysostaphin and sarcosyl, as described by Novick et al. (40). The crude lysate was enriched in plasmid DNA either by centrifuging the lysate at 49,000 × *g* (40) or by shearing and pelleting it on a glycerol cushion, as described by Labigne-Roussel et al. (30). In the third technique, the bacterial cells obtained from 100 ml of an overnight BHI culture were harvested and washed once with 0.01 M Tris (pH 7). The washed cells were suspended in 5 ml of the same buffer containing 50 μg of lysostaphin ml^{-1}. After 30 min of incubation at 37°C, the suspension was treated with sodium dodecyl sulfate (pH 12.4) and neutralized. The single-stranded DNA was precipitated as described by Portnoy et al. (43). Plasmid DNA was further purified in a cesium chloride-ethidium bromide density gradient, as described by Novick et al. (40). Digestions with restriction enzymes (Boehringer GmbH, Mannheim, Federal Republic of Germany) were performed according to the instructions of the supplier. Gel electrophoresis was performed as described previously (12).

RESULTS

Background mutation frequencies. In each transfer experiment both the donor and recipient strains were tested for background mutation to resistance to the antibiotics used in the selective media. No resistant mutants appeared when donors were plated on media containing the drugs used for counterselection (fusidic acid and rifampin or novobiocin) or when the recipients were plated on tetracycline. Mutations of recipient strains to erythromycin resistance occurred at a very low frequency ($\simeq 2 \times 10^{-10}$); the mutant clones appeared as dwarf colonies on the erythromycin-containing media. Mutant recipient strains resistant to kanamycin, streptogramin A, or trimethoprim appeared at higher frequencies (2×10^{-9} to 3×10^{-10}). The kanamycin-resistant mutants were also resistant to all of the aminoglycosides listed in Table 1; most of these mutants appeared as dwarf colonies on the kanamycin-containing media. The streptogramin A-resistant mutants appeared either as dwarf or normal-sized colonies; these mutants did not produce an enzyme inactivating streptogramin A, as did the donor strains and the corresponding transcipients or transductants (see below). The dwarf mutant colonies selected on each of the three antibiotics were translucent and smaller than the antibiotic-resistant transcipients or transductants (diameters of <0.5 and 1 to 1.8 mm, respectively, after 72 h of incubation at 37°C).

Filter mating transfers and analysis of transcipients. Eleven wild-type strains (Table 1) were crossed on membrane filters with the staphylococcal recipient BM224 or ISP1127 (Table 1). Media containing kanamycin, streptogramin A, or trimethoprim and, in some cases, cadmium acetate, erythromycin, or tetracycline were used to select the transcipients. For each donor and each selective drug, at least three independent filter mating experiments were performed. The resistance profiles of at least 100 transcipients, recovered from each of the three independent filter mating experiments, were analyzed. When BM3248 was used as the donor, more than 500 kanamycin-resistant transcipient clones, recovered from at least 10 independent mating experiments, were studied. Five of the wild-type donors transferred some of their resistance markers, whereas for the six other wild-type donors no detectable transfer was observed. The transfer frequencies and results of the analysis of the transcipients for 18-h matings are shown in Table 2. When transcipients were selected on kanamycin, streptogramin A, or trimethoprim, transfer occurred at frequencies varying from 1.8×10^{-6} to 2.8×10^{-8}. For the cross BM3318 × BM224, the transfer frequencies of kanamycin resistance were determined for 2-, 4-, 6-, and 18-h filter matings. Transfer occurred at a frequency of 3.5×10^{-8} after 2 h, reached a maximum after 4 h (1.4×10^{-7}), and then remained constant (1.3×10^{-7} to 1.7×10^{-7}). All of the transcipients obtained from a given donor acquired the same set of resistance markers. No transcipients were obtained by selection on cadmium acetate, erythromycin, or tetracycline.

Two of the wild-type strains, BM3248 and BM3318, were also crossed with the staphylococcal recipient strains BM231, BM3449, BM3319, and BM3302 (Table 1), and transcipients were selected on kanamycin. For each of these donors, the transfer frequencies varied with the recipient used (Table 2). The transfer frequencies obtained with recipients BM3319 and BM3302 were 10- to 100-fold lower than those obtained with the recipient BM224. *S. aureus* BM3319 was characterized, like the *Staphylococcus epidermidis* recipient BM3302, by teichoic acid different from that of most *S. aureus* strains (26). The transfer frequencies with the *recA S. aureus* recipient strain, BM3449, were similar to those obtained with BM224. Depending on the donor used, the transfer frequencies with the restrictionless recipient, BM231, were either 10-fold higher than or similar to those obtained with BM224. Analysis of transcipients for unselected markers revealed that the same resistance markers

TABLE 2. Filter mating experiments

Donor (no. [CFU ml^{-1}])	Recipient	Selective donor marker(s)[a]	Transfer frequency (transcipients donor cell^{-1}	Resistance markers transferred[a]
BM3247 (6×10^9)	BM224	Km	5.3×10^{-8}	Gm Km Tm/Pc/SgA[b]
		SgA	4.3×10^{-8}	Gm Km Tm/Pc/SgA
		Tc	$<1.6 \times 10^{-10}$[c]	
BM3248 (3.5×10^{10})	BM224	Km	4.3×10^{-7}	Gm Km Tm/Pc/SgA[b]
		SgA	2.8×10^{-7}	Gm Km Tm/Pc/SgA
	BM231	Km	6.6×10^{-6}	Gm Km Tm/Pc/SgA
	BM3449	Km	2.2×10^{-7}	Gm Km Tm/Pc/SgA
	BM3319	Km	5.1×10^{-8}	Gm Km Tm/Pc/SgA
	BM3302	Km	1.7×10^{-8}	Gm Km Tm/Pc/SgA
BM3318 (3×10^{10})	BM224	Km	1.3×10^{-7}	Cd/Gm Km Nm Tm/Pc/SgA[d]
		SgA	6×10^{-8}	Cd/Gm Km Nm Tm/Pc/SgA
		Cd, Em, or Tc	$<3.3 \times 10^{-11}$[c]	
	BM231	Km	1.5×10^{-7}	Cd/Gm Km Nm Tm/Pc/SgA/Tc[d]
	BM3449	Km	3.3×10^{-7}	Cd/Gm Km Nm Tm/Pc/SgA/Tc
	BM3319	Km	1.7×10^{-8}	Cd/Gm Km Nm Tm/Pc/SgA/Tc
	BM3302	Km	3×10^{-9}	Cd/Gm Km Nm Tm/Pc/SgA/Tc
BM3377 (2×10^{10})	BM224	Km	3.5×10^{-8}	Cd/Gm Km Nm Tm/Pc/SgA/Tc
		SgA	2.8×10^{-8}	Cd/Gm Km Nm Tm/Pc/SgA/Tc
BM3385 (1.2×10^{10})	ISP1127	SgA	1.8×10^{-6}	Pc/SgA/Tp
		Tp	4.1×10^{-7}	Pc/SgA/Tp
		Cd	$<8.3 \times 10^{-11}$[c]	

[a] Abbreviations and shill are defined in Table 1, footnote *b*.
[b] Amikacin, netilmicin, and sisomycin resistance was also transferred. The aminoglycoside resistance profile suggested the transfer of the *aacA* gene.
[c] No transcipients were detected after all bacteria obtained on each filter were spread on selective media.
[d] Amikacin, lividomycin, netilmicin, and sisomycin resistance was also transferred. The aminoglycoside resistance profile suggested the transfer of both the *aacA* and *aphA* genes.

were consistently transferred from a given donor, independently of the recipient used.

For each of the three donors BM3247, BM3377, and BM3385, three transcipients, obtained from separate experiments (BM224 or ISP1127 as recipients), were examined for plasmid content by using the large-scale plasmid isolation technique derived from Portnoy et al. (43). For BM3248 and BM3318, 10 transcipients obtained from filter mating experiments with BM224, BM231, or BM3319 as the recipient were analyzed by the six plasmid isolation techniques described in Materials and Methods. No plasmids could be detected in any of these transcipients. As a control, the 35- and 42-kilobase (kb) plasmids from strains BM3002, TC41, and BM224(pAM899-1) (Table 1) were visualized by the same techniques. The 22-kb penicillinase plasmid harbored by the donor BM3318 (Table 1) was not detected in the corresponding penicillinase-producing transcipients. This suggests that BM3318 carries two distinct sets of penicillinase genes, one of them located on a nonconjugative 22-kb plasmid and the other on a conjugative element apparently integrated into the chromosome.

Transduction experiments and analysis of transductants. Transduction experiments were performed with the 11 wild-type strains (Table 1). The phages used were either those harbored by these strains and induced by UV irradiation of the bacterial cells (38) or *S. aureus* transducing phages propagated on the wild-type strains. The recipients for the transduction experiments were derivatives of strain BM224, obtained by lysogenizing BM224 with each of the phages used in the experiments. The transduction frequencies obtained were similar for the 11 donors. The data obtained with the five donors that transferred resistance markers by filter mating (Table 2) are shown in Table 3. When the transductants were selected on media containing erythromycin, kanamycin, streptogramin A, tetracycline, or trimethoprim, the transduction frequencies were very low (2×10^{-9} to 1.5×10^{-10} transductants per PFU). Transfer frequencies were higher when the transductants were selected on cadmium acetate (1.2×10^{-7} to 1×10^{-8}). With strain BM3318 as the donor, the transduction frequency for kanamycin resistance was about 100-fold higher, whereas the transduction frequency of cadmium acetate resistance decreased 10-fold when the phage lysate was subjected to UV irradiation. This treatment lowered the PFU by a factor of 10.

When kanamycin was used for selection (Table 3), two types of kanamycin-resistant transductants were obtained: those which acquired resistance to the aminoglycosides inactivated by AAC(6′)-APH(2″) (amikacin, gentamicin, kanamycin, netilmicin, sisomycin, and tobramycin) and those which acquired resistance to the aminoglycosides inactivated by APH(3′)III (amikacin, kanamycin, lividomycin, neomycin, and paromomycin). The latter transductants also acquired low-level resistance to streptomycin (MIC, 32 to 128 μg ml^{-1}). Both types of kanamycin-resistant transductants were also obtained from four (BM3121, BM3152, BM3249, and BM3252) of the six clinical isolates that did not transfer resistance markers by filter matings (data not shown). The enzymes AAC(6′)-APH(2″) and APH(3′)III have recently been characterized in the transductants from strains BM3121 and BM3152 (13a).

As described previously for strains BM3121 and BM3152 (13a), all of the tetracycline-resistant transductants were also resistant to minocycline, and all of the erythromycin-resistant transductants were also resistant to spectinomycin.

Transductants resistant to streptogramin A were obtained only when strain BM3385 was used as the donor (Table 3).

TABLE 3. Frequencies of transfer by transduction

Donor strain	Selective donor marker[a]	Phage[b]	Transfer frequency (transductants PFU^{-1})	Resistance markers transferred[a]
BM3247	Km	Donor lysate	4×10^{-10}	Gm Km Tm[c]
BM3248	Km	Donor lysate	8×10^{-10}	Gm Km Tm[c]
		ϕ11	2×10^{-10}	Gm Km Tm[c]
	SgA	Donor lysate	$<4 \times 10^{-10}$	
BM3318	Km	Donor lysate	2×10^{-9}	Km Nm Sm[d]
		1305	1.2×10^{-9}	Km Nm Sm[d]
			2×10^{-10}	Gm Km Tm[c]
		1305 + UV[e]	8.4×10^{-8}	Km Nm Sm[d]
	Tc	1305	6.4×10^{-9}	Mn Tc
	Em	1305	4×10^{-9}	Em/Sp
	Cd	1305	1.2×10^{-7}	As Cd/Pc
		1305 + UV[e]	1×10^{-8}	As Cd/Pc
	SgA	1305	$<2 \times 10^{-10}$	
BM3377	Km	1503	2×10^{-10}	Gm Km Tm[c]
	Tc	1503	$<1 \times 10^{-10}$	
	SgA	1503	$<1 \times 10^{-10}$	
BM3385	Tp	Donor lysate	$<2 \times 10^{-10}$	
		1503	1.5×10^{-10}	Pc/Tp
		1305	5×10^{-10}	Pc/Tp
	SgA	1305	6.2×10^{-10}	SgA
		Donor lysate	$<2 \times 10^{-10}$	
		1503	$<5 \times 10^{-11}$	

[a] Abbreviations and shill are defined in Table 1, footnote *b*.
[b] The phages used for transduction were either those harbored by the donors and induced by UV irradiation (37) or those propagated on the donor strains.
[c] Amikacin, netilmicin, and sisomycin resistance was also transferred. The aminoglycoside resistance profile of the transductants suggested the transfer of the *aacA* gene.
[d] Amikacin, lividomycin, and paromomycin resistance was also transferred. The aminoglycoside resistance profile of the transductants suggested the transfer of the *aphA* and *stm* genes.
[e] UV irradiation of the phage lysate decreased the PFU ml^{-1} from 1×10^9 to 9.5×10^7. Transduction experiments were performed with 4.7×10^8 PFU of irradiated phage instead of 5×10^9 PFU of nonirradiated phage.

These transductants could be distinguished from the mutant clones because, like the donor strain, they produced an enzyme which inactivated streptogramin A, as revealed by the modified Gots test.

Trimethoprim-resistant transductants were selected when strain BM3385 was used as a donor. Two types of transductants were selected, those which were resistant to trimethoprim and penicillin by penicillinase production (Table 3) and those which were resistant to trimethoprim alone. The latter could not be distinguished from the trimethoprim-resistant mutants.

For strain BM3318 (Table 3), as well as for strains BM3121 and BM3152 (12, 13a), the transductants selected for cadmium resistance were also resistant to sodium arsenate and produced penicillinase.

For each donor, three transductants obtained on each selective drug used were examined for plasmid DNA content by two large-scale plasmid isolation techniques (30, 43). Plasmids encoding penicillinase production, as well as resistance to cadmium acetate and sodium arsenate, were isolated from all of the cadmium-resistant transductants obtained from strains BM3121, BM3152, and BM3318. These plasmids had the same size (22 kb) and the same restriction pattern whether they were digested by *Eco*RI, *Bgl*II, or *Hin*dIII (data not shown). The donor strains also harbored plasmids with the same restriction patterns (10, 12) as those isolated from the transductants (data not shown). No plasmids could be detected in any of the other analyzed transductants selected on erythromycin, kanamycin, streptogramin A, tetracycline, or trimethoprim.

Filter mating retransfers. For the five wild-type strains that transferred resistance markers by filter matings (Table 2), at least three transcipients and three transcipients selected on kanamycin or trimethoprim were used as donors in a second round of transfer with the recipient strain, ISP1127 or BM224. The transcipients used as donors retransferred their resistance markers by filter matings, with frequencies similar to those obtained in the first round of transfer with the wild-type donors. Retransfer occurred in the absence of detectable prophages in the transcipients. Whereas all of the transcipients studied retransferred all of the resistance markers acquired from the wild-type strains, none of the transductants retransferred any of its resistance markers by filter mating.

Effect of various conditions on transfers. Various mating conditions were studied to elucidate the mechanism involved in filter mating experiments. Three wild-type strains were mated with the recipient strain BM224, with kanamycin used for selection. Based on the number of kanamycin-resistant clones, either all or at least 100 clones were tested for their resistance pattern (Table 4).

When the crosses were done directly (without the membrane filter) on an agar medium, the transfer frequency for kanamycin resistance was 100-fold less than that obtained by filter mating (mating 1). Analysis of the kanamycin-resistant clones showed that 5% had the same resistance profile as

TABLE 4. Effects of various mating consitions on transfer frequencies

Donor	Recipient (mating no.)	Mating condition	Transfer frequency (Km^r transcipients $donor^{-1}$)	Resistance markers transferred[a]
BM3248	BM224 (1)	Filter	2.7×10^{-7}	Gm Km Tm/Pc/SgA
		No filter[b]	4×10^{-9}	Gm Km Tm/Pc/SgA or Gm Km Tm
		Filter + calcium	3.2×10^{-7}	Gm Km Tm/Pc/SgA
		No filter + calcium	5.6×10^{-10}[c]	Gm Km Tm
		Filter + antiphage serum	3.8×10^{-7}	Gm Km Tm/Pc/SgA
		No filter + calcium P + antiphage serum	$<3.5 \times 10^{-11}$[c]	
		Filter + DNase I	5.7×10^{-7}	Gm Km Tm/Pc/SgA
		Donor and recipient separated by filter	$<1 \times 10^{-10}$[c]	
		Broth	$<1 \times 10^{-10}$	
BM3318	BM224 (2)	Filter	1×10^{-7}	Cd/Gm Km Nm Tm/Pc/SgA
		No filter + calcium	6×10^{-10}	Gm Km Tm or Km Nm Sm
		Filter + DNase I	2.3×10^{-7}	Cd/Gm Km Nm Tm/Pc/SgA
		Donor and recipient separated by filter	$<1 \times 10^{-10}$[c]	
		Broth	$<1.5 \times 10^{-10}$	
BM3152	BM224 (3)	Filter	$<1.5 \times 10^{-11}$[c]	
		No filter + calcium	1.3×10^{-10}[c]	Gm Km Tm

[a] Abbreviations and shill are defined in Table 1, footnote *b*.
[b] Matings done directly on agar media, without membrane filters.
[c] Ten filters were used, and all the bacteria obtained were spread on selective media.

that of the kanamycin-resistant transductants (Table 3), whereas the other 95% resembled the transcipients obtained by filter mating (Table 2).

When the matings were carried out on a filter, the addition of calcium (0.01 M), which is known to enhance phage adsorption (47), had no effect either on the kanamycin resistance transfer frequencies or on the phenotype of the kanamycin-resistant clones (mating 1). In contrast, in the absence of a filter, the addition of calcium lowered the transfer frequencies 1,000-fold and all of the kanamycin-resistant clones had the same resistance patterns as those of the transductants (matings 1 and 2). In mating 3, although no transfer could be detected by filter matings, a few kanamycin-resistant clones with a resistance profile similar to that of transductants (13a) could be detected when matings were performed on agar medium supplemented with calcium and without a filter.

For the donor BM3248, the addition to the agar media of an antiphage serum, which inhibited the lytic effect of the phage harbored by this strain, had no effect on the frequency of kanamycin resistance transfer by filter mating. No transfer could be detected in the presence of this serum when the same mating (mating 1) was carried out on agar media supplemented with calcium and without a filter.

The presence of DNase I in the media had no effect on the transfer frequencies in filter matings (matings 1 and 2).

No detectable transcipients were obtained when donor BM3248 or BM3318 was mated with strain BM224 on agar media with an interposing filter and in broth media (matings 1 and 2) or when viable donor cells were replaced either by the sterile filtrates of the donor cultures or by the UV-induced phages harbored by the wild-type donors (matings 1 and 2; data not shown in Table 4).

Curing experiments. The spontaneous loss of resistance to aminoglycosides (AG^r) by strain BM3248 or to aminoglycosides, streptogramin A, and penicillin by one of its transcipients, BM3368, was sometimes observed when the cultures were conserved at 4 or −80°C. AG^r was spontaneously lost from strain BM3248 at a frequency of 13%; if ethidium bromide was added to the culture, the efficiency of curing was 100%. Strain BM3368 spontaneously lost all of the resistance markers it had acquired during filter mating (aminoglycosides, streptogramin A, and penicillinase production) with a frequency of 9%; treatment with ethidium bromide did not significantly modify the frequency (12%). In contrast, the resistance markers transferred by filter matings of the wild-type strains BM3318 and BM3385, as well as those of their transcipients, BM3481 and BM3417, respectively, were stably maintained even when the cultures were treated with ethidium bromide or novobiocin. The same treatment did not provoke the cure of the 22-kb penicillinase plasmid harbored by strains BM3318, BM3121, and BM3152.

DISCUSSION

In this study, we showed that for 5 of the 11 *S. aureus* clinical isolates analyzed, some of the resistance markers cotransferred by filter matings, whereas they were not cotransduced. The transfers occurred into staphylococcal recipients, belonging to different species, in the absence of detectable plasmids.

Several experiments were performed to elucidate the mechanism of transfer by filter mating. Because no transfer occurred when the donor cultures in broth media were replaced by the sterile filtrates or when matings were performed in broth media or on agar media with an interposing filter between the donor and the recipient, close contact between a large number of viable donor and recipient cells is required for this type of transfer. Transformation does not seem to be involved, because DNase I had no effect on transfer frequencies.

Transduction with the phages detected in four of the five wild-type donors studied (BM3247, BM3248, BM3318, and BM3385) is not likely to have occurred, because (i) no transfer occurred by filter matings when the phage(s) harbored by the wild-type donors were used instead of the viable bacterial cells or when the donor and recipient cultures were coincubated separated by an interposing filter; (ii) for each of these wild-type donors, the transcipients obtained by filter mating had phenotypes different from those

of the transductants selected for the same donor marker, suggesting the involvement of different transfer mechanisms; (iii) the filter mating conditions used in this study were shown to inhibit transduction; (iv) the addition of an anti-phage serum which inhibited transduction from one of the wild-type donors, BM3248, did not affect the frequency of transfer in filter matings; and (v) transfers occurred into recipients (BM3319 and BM3302) with teichoic acids which differ from those of most *S. aureus* strains. Strain BM3319, a mutant of *S. aureus* 187 (29), and *S. epidermidis* BM3302 were not susceptible to the lytic effect of the phages harbored by the wild-type *S. aureus* strain analyzed. Although phages that lyse both *S. aureus* and *S. epidermidis* have been characterized (45), no *S. aureus* phages that transduce DNA into *S. epidermidis* have been described. Moreover, *S. aureus* 187 was shown to carry a teichoic acid different from that of most *S. aureus* strains (26) and to be susceptible to phage 187, but not to the other *S. aureus* phages (29).

Phages were not detected in the transcipients used as donors in a second round of transfer or in the wild-type strain BM3377; nevertheless, these strains transferred resistance markers by filter matings. This also suggests that the mechanism of transfer was not transduction. However, as Dyer et al. demonstrated (9), the absence of plaque-forming bacteriophages does not exclude the possibility that a cryptic transducing particle is present in the donor; these investigators also reported that RN450 (39), the parental strain of the derivative mutants BM224 and ISP1127, might be lysogenized with defective phages. Even if defective phages were involved in the genetic transfer by filter matings, our results suggest that they do not transduce the same DNA fragments as those transduced by known *S. aureus* transducing phages.

All of the results reported here suggest that conjugation, requiring close cell-to-cell contact, may be the mechanism involved in the transfer of antibiotic resistance markers by filter matings. Until now, transfer of staphylococcal resistance markers by filter matings has been attributed to conjugative plasmids (2, 3, 15, 19, 33, 37, 53, 54). In this study, conjugative plasmids were not detected in either the donors or the transcipients. Since plasmids larger than 60 kb (33) have not been isolated from staphylococci, the available techniques may not be sensitive enough for the detection of larger plasmids. Possibly, large conjugative plasmids were present in the *S. aureus* clinical isolates studied, but were not detectable even with techniques adapted for the isolation of large plasmids (30, 43).

The same resistance markers that transferred by filter matings were transduced at low frequencies ($<10^{-9}$/PFU). The transduction frequencies increased when the PFU of the phage lysate was decreased by UV irradiation in an Arber experiment (1). Although these results suggest a chromosomal location of the genes, a plasmid location cannot be excluded, because the same response in Arber experiments has recently been described for plasmid transduction (9).

In curing experiments, the aminoglycoside resistance markers carried by the wild-type strain BM3248 and all of the markers acquired by filter mating of one of the transcipients were lost spontaneously at low frequencies (13 and 9%, respectively). The frequency of loss of the markers of the transcipient was not affected by treatment with ethidium bromide. However, the resistance markers that transferred by filter matings were stably maintained in the two other wild-type strains studied (BM3318 and BM3385), as well as in the corresponding transcipients. The use of curing as a criterion to establish the plasmid location of genes remains open to question because all attempts to cure the 22-kb penicillinase plasmid harbored by some donors failed and spontaneous loss of chromosomal antibiotic resistance markers has been reported for a group A streptococcal strain (32).

If conjugative plasmids do not exist as autonomous replicons in the staphylococcal strains described here, they, nevertheless, may be present but integrated into the chromosome, as previously suggested for streptococcal strains (20, 23). The chromosomal integration of an entire plasmid, pI258, has been reported for staphylococci (47). This integration occurred after cointegration with prophage ϕ11; the resulting recombinant plasmid, pI258-ϕ11de, was recently shown to be capable of autogenous transduction in the absence of both a helper bacteriophage and detectable plaque-forming bacteriophage (8). This plasmid was found to be integrated in the chromosome of the transductants (8). The presence in the strains studied here of a chromosomal element similar to pI258-ϕ11de is unlikely, because transduction did not appear to be involved in the filter mating transfers.

The transfers by filter matings observed here could also be mediated by chromosomally integrated conjugative transposons similar to Tn*916* (28), Tn*918* (5), and Tn*919* (14) or to other types of translocatable elements, such as those described by Le Bouguenec et al. (32). All attempts to translocate the staphylococcal genes encoding resistance to kanamycin or streptogramin A (genes that were transferable in the filter matings) onto the penicillinase plasmid pIP1066 introduced into some of the clinical isolates studied were unsuccessful (data not shown). Further analysis is needed to properly identify the elements responsible for the conjugative transfer of staphylococcal resistance markers carried by about one-half of the *S. aureus* clinical isolates studied.

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LITERATURE CITED

1. **Arber, W.** 1960. Transduction of chromosomal genes and episomes in *Escherichia coli*. Virology **11**:273–288.
2. **Archer, G. L., and J. L. Johnston.** 1983. Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. Antimicrob. Agents Chemother. **24**:70–77.
3. **Asch, D. K., R. V. Goering, and E. A. Ruff.** 1984. Isolation and preliminary characterization of a plasmid mutant derepressed for conjugal transfer in *Staphylococcus aureus*. Plasmid **12**:197–202.
4. **Buu-Hoï, A., and T. Horodniceanu.** 1980. Conjugative transfer of multiple antibiotic resistance markers in *Streptococcus pneumoniae*. J. Bacteriol. **143**:313–320.
5. **Clewell, D. B., Y. A. Florence, B. A. White, and C. Gawron-Burke.** 1985. *Streptococcus faecalis* sex pheromone (cAM373) also produced by *Staphylococcus aureus* and identification of a conjugative transposon (Tn*918*). J. Bacteriol. **162**:1212–1220.
6. **Dixson, S., W. Brumfitt, and M. T. Hamilton-Miller.** 1984. Stability of aminoglycoside resistance *in vitro* in gentamicin-resistant *Staphylococcus aureus*. J. Hyg. **93**:43–49.
7. **Docherty, A., G. Grandi, R. Grandi, T. J. Gryczan, A. G. Shivakumar, and D. Dubnau.** 1981. Naturally occurring macrolide-lincosamide-streptogramin B resistance in *Bacillus*

licheniformis. J. Bacteriol. **145**:129–137.
8. **Dyer, D. W., M. I. Rock, and J. J. Iandolo.** 1984. Autogenous transduction of o11de in *Staphylococcus aureus*: transfer and genetic properties. J. Bacteriol. **158**:689–695.
9. **Dyer, D. W., M. I. Rock, C. Y. Lee, and J. J. Iandolo.** 1985. Generation of transducing particles in *Staphylococcus aureus*. J. Bacteriol. **161**:91–95.
10. **El Solh, N., R. Bismuth, J. Allignet, and J. M. Fouace.** 1984. Résistance à la pristinamycine (ou virginiamycine) des souches de *Staphylococcus aureus*. Pathol. Biol. **32**:362–368.
11. **El Solh, N., and J. M. Fouace.** 1984. Caractéristiques des souches résistantes aux bêta-lactamines isolées en France, p. 27–49. *In* F. Vachon and B. Régnier (ed.), Problèmes actuels de pathologie infectieuse et de réanimation. Les infections à Staphylocoques meticilline résistants. Librairie Arnette, Paris.
12. **El Solh, N., J. M. Fouace, J. Pillet, and Y. A. Chabbert.** 1981. Plasmid DNA content of multiresistant *Staphylococcus aureus* strains. Ann. Microbiol. (Paris) **132B**:131–156.
13. **El Solh, N., J. M. Fouace, Z. Shalita, D. B. Bouanchaud, R. P. Novick, and Y. A. Chabbert.** 1980. Epidemiological and structural studies of *S. aureus* R plasmids mediating resistance to tobramycin and streptogramins. Plasmid **4**:117–120.
13a. **El Sohl, N., N. Moreau, and S. Ehrlich.** 1986. Molecular cloning and analysis of *Staphylococcus aureus* chromosomal aminoglycoside resistance genes. Plasmid **15**:104–118.
14. **Fitzgerald, G. F., and D. B. Clewell.** 1985. A conjugative transposon (Tn*919*) in *Streptococcus sanguis*. Infect. Immun. **47**:415–420.
15. **Forbes, B. A., and D. R. Schaberg.** 1983. Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugative exchange of resistance. J. Bacteriol. **153**:627–634.
16. **Fouace, J.** 1981. Mixed cultures of *Staphylococcus aureus*: some observations concerning transfer of antibiotic resistance. Ann. Microbiol. (Paris) **132B**:375–386.
17. **Fouace, J., and V. Ferré.** 1973. Transduction chez le staphylocoque: modification des lysotypes observées chez des souches du groupe III après acquisition de certains caractères de résistance aux antibiotiques. Ann. Microbiol. (Paris) **124A**:381–392.
18. **Franke, A. E., and D. B. Clewell.** 1981. Evidence for a chromosome-borne resistance transposon (Tn*916*) in *Streptococcus faecalis* that is capable of "conjugal" transfer in the absence of a conjugative plasmid. J. Bacteriol. **145**:494–502.
19. **Goering, R. V., and E. A. Ruff.** 1983. Comparative analysis of conjugative plasmids mediating gentamicin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **24**:450–452.
20. **Horodniceanu, T., L. Bougueleret, and G. Bieth.** 1981. Conjugative transfer of multiple-antibiotic resistance markers in beta-hemolytic group A, B, F and G streptococci in the absence of extrachromosomal deoxyribonucleic acid. Plasmid **5**:127–137.
21. **Horodniceanu, T., A. Buu-Hoï, F. Delbos, and G. Bieth.** 1982. High-level aminoglycoside resistance in group A, B, G, D (*Streptococcus bovis*), and viridans streptococci. Antimicrob. Agents Chemother. **21**:176–179.
22. **Horodniceanu, T., A. Buu-Hoï, C. Le Bouguenec, and G. Bieth.** 1982. Narrow host range of some streptococcal R plasmids. Plasmid **8**:199–206.
23. **Inamine, J., and V. Burdett.** 1984. Structural organization of a 67-kilobase streptococcal conjugative element mediating multiple antibiotic resistance. J. Bacteriol. **161**:620–626.
24. **Ingram, L., R. B. Sykes, J. Grinsted, J. R. Saunders, and M. H. Richmond.** 1972. A transmissible resistance element from a strain of *Pseudomonas aeruginosa* containing no detectable extrachromosomal DNA. J. Gen. Microbiol. **72**:269–279.
25. **Jacob, A. E., and S. J. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. **117**:360–372.
26. **Karakawa, W. W., and J. A. Kane.** Immunochemical analysis of a galactosamine-rich teichoic acid of *Staphylococcus aureus*, phage type 187. J. Immunol. **106**:900–906.
27. **Kayser, F. H., F. Homberger, and M. Devaud.** 1981. Aminocyclitol-modifying enzymes specified by chromosomal genes in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **19**:766–772.
28. **Krolewski, J. J., E. Murphy, R. P. Novick, and M. G. Rush.** 1981. Site-specificity of the chromosomal insertion of *Staphylococcus aureus* transposon Tn*554*. J. Mol. Biol. **152**:19–33.
29. **Krynski, S., J. Galinski, and E. Becla.** 1976. Phage type 187 as a separate taxonomic subunit within the *Staphylococcus aureus* species, p. 165–167. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustave Fischer Verlag KG, Stuttgart, Federal Republic of Germany.
30. **Labigne-Roussel, A., G. Gerbaud, and P. Courvalin.** 1981. Translocation of sequences encoding antibiotic resistance from the chromosome to a receptor plasmid in *Salmonella ordonez*. Mol. Gen. Genet. **182**:390–408.
31. **Lacey, R. W., and V. L. Lord.** 1980. Transfer of gentamicin resistance between cultures of *Staphylococcus aureus* in nutrient broth, serum and urine. J. Med. Microbiol. **13**:411–421.
32. **Le Bouguenec, C., T. Horaud, G. Bieth, R. Colimon, and C. Dauguet.** 1984. Translocation of antibiotic resistance markers of a plasmid-free *Streptococcus pyogenes* (group A) strain into different streptococcal hemolysin plasmids. Mol. Gen. Genet. **194**:377–387.
33. **Lyon, B. R., J. W. May, and R. A. Skurray.** 1984. Tn*4001*: a gentamicin and kanamycin resistance transposon in *Staphylococcus aureus*. Mol. Gen. Genet. **193**:554–556.
34. **Magot, M.** 1983. Transfer of antibiotic resistances from *Clostridium innocuum* to *Clostridium perfringens* in the absence of detectable plasmid DNA. FEMS Microbiol. Lett. **18**:149–151.
35. **Malamy, M. H., and F. P. Tally.** 1981. Mechanism of drug-resistance transfer in *Bacteroides fragilis*. J. Antimicrob. Chemother. **8**:(Suppl. D):59–75.
36. **Mays, T. D., C. J. Smith, R. A. Welch, C. Delfini, and F. L. Macrina.** 1982. Novel antibiotic resistance transfer in *Bacteroides*. Antimicrob. Agents Chemother. **21**:110–118.
37. **McDonnell, R. W., H. M. Sweeney, and S. Cohen.** 1983. Conjugational transfer of gentamicin resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **23**:151–160.
38. **Morse, M. L.** 1959. Transduction by staphylococcal bacteriophage. Proc. Natl. Acad. Sci. USA **45**:722–727.
39. **Novick, R. P.** 1967. Properties of a cryptic high frequency transducing phage in *S. aureus*. Virology **33**:155–166.
40. **Novick, R. P., E. Murphy, T. J. Gryczan, E. Baron, and I. Edelman.** 1979. Penicillinase plasmids of *Staphylococcus aureus*: restriction-deletion maps. Plasmid **2**:109–129.
41. **O'Reilly, M., G. Dougan, T. J. Foster, and J. P. Arbuthnott.** 1981. Plasmid in epidermolytic strains of *Staphylococcus aureus*. J. Gen. Microbiol. **124**:99–107.
42. **Pattee, P. A., and D. S. Neveln.** 1975. Transformation analysis of three linkage groups in *Staphylococcus aureus*. J. Bacteriol. **124**:201–211.
43. **Portnoy, D. A., S. L. Moseley, and S. Falkow.** 1981. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect. Immun. **31**: 775–782.
44. **Privitera, G., G. Botta, and M. Sebald.** 1981. Macrolide, lincosamide, streptogramin and tetracycline transferable resistance in the *Bacteroides fragilis* group. J. Antimicrob. Chemother. **8**(Suppl. D):87–94.
45. **Pulverer, G., J. Pillich, and P. B. Heczko.** 1976. Activity of staphylococcal phages against staphylococci of various cell wall structure, p. 153–157. *In* J. Jeljaszewicz (ed.), Staphylococci and staphylococcal diseases. Gustav Fischer Verlag KG, Stuttgart, Federal Republic of Germany.
46. **Rountree, P. M.** 1949. The serological differentiation of staphylococcal bacteriophages. J. Gen. Microbiol. **3**:164–173.
47. **Rountree, P. M.** 1951. The role of certain electrolytes in the adsorption of staphylococcal bacteriophages. **5**:673–680.
48. **Schwesinger, M. D., and R. P. Novick.** 1975. Prophage-dependent plasmid integration in *Staphylococcus aureus*. J. Bacteriol. **123**:724–738.
49. **Shoemaker, N. B., M. D. Smith, and W. R. Guild.** 1979.

Organization and transfer of heterologous chloramphenicol and tetracycline resistance genes in pneumococcus. J. Bacteriol. **139**:432–441.

50. **Shoemaker, N. B., M. D. Smith, and W. R. Guild.** 1980. DNase-resistant transfer of chromosomal *cat* and *tet* insertions by filter mating in *Pneumococcus*. Plasmid **3**:80–87.
51. **Smith, C. J., S. M. Markowitz, and F. L. Macrina.** 1981. Transferable tetracycline resistance in *Clostridium difficile*. Antimicrob. Agents Chemother. **19**:997–1003.
52. **Smith, C. J., R. A. Welch, and F. L. Macrina.** 1982. Two independent conjugal transfer systems operating in *Bacteroides fragilis* V479–1. J. Bacteriol. **151**:281–287.
53. **Townsend, D. E., N. Ashdown, L. C. Greed, and W. B. Grubb.** 1984. Transposition of gentamicin resistance to staphylococcal plasmids encoding resistance to cationic agents. J. Antimicrob. Chemother. **14**:115–124.
54. **Townsend, D. E., N. Ashdown, M. Momoh, and W. B. Grubb.** 1985. Distribution of plasmid-borne resistance to nucleic acid binding compounds in methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **15**:417–434.
55. **Witte, W., and K. Dünnhaupt.** 1984. Occurrence of a nonplasmid-located determinant for gentamicin resistance in strains of *Staphylococcus aureus*. J. Hyg. **93**:1–8.
56. **Wüst, J., and U. Hardegger.** 183. Transferable resistance to clindamycin, erythromycin, and tetracycline in *Clostridium difficile*. Antimicrob. Agents Chemother. **23**:784–786.