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Minocycline Resistance in *Staphylococcus aureus*: Effect on Phage Susceptibility

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Tetracycline-resistant strains of *Staphylococcus aureus* are minocycline sensitive, with the exception of strains susceptible to phages of the 83A/84/85 complex and some methicillin-resistant strains of other phage types. Strains of the 83A/84/85 complex yield mutants with increased minocycline resistance. Transduction of minocycline resistance into the susceptible strain RN 450 was obtained with donor strains possessing either markers for both extrachromosomal tetracycline resistance *(tet)* and chromosomal tetracycline + minocycline resistance (*tmn* R), or only for chromosomal *tmn* R resistance. The chromosomal marker was differentiated from the extrachromosomal marker by the lack of detectable extrachromosomal deoxyribonucleic acid after transfer into strain RN 450, transfer into a *rec*$^+$ strain, lack of transfer into *rec*$^-$ acceptor strain, and cotransduction with chromosomal determinants for guanine biosynthesis. Both chromosomal and extrachromosomal tetracycline resistance can be induced by tetracycline. Induction by tetracycline of chromosomal tetracycline resistance resulted in simultaneous induction of minocycline resistance. The mutation toward increased minocycline resistance (*tmn* → *tmn* R) is a regulatory mutation toward constitutivity or semiconstitutivity. Constitutive resistance is dominant in *tmn* R/*tet* diploids. Transfer of the *tet* marker does not affect the phage susceptibility of the acceptor strain. The *tmn* R marker, originating from donor strains of the 83A/84/85 complex, renders strain RN 450 resistant to several typing phages, with the exception of phages of the 83A/84/85 complex. This could possibly account for the phage typing patterns of minocycline-resistant staphylococci.

In most instances tetracycline resistance in *Staphylococcus aureus* and gram-negative bacteria is extrachromosomal (21, 25). Chromosomal resistance has been reported for a methicillin-resistant strain of *S. aureus* (15) and *Escherichia coli* (8). The mechanism of tetracycline resistance in *S. aureus* and *E. coli* is still not fully understood. A decrease in the accumulation of tetracycline by resistant cells appears to be an important element in the mechanism of resistance (11, 12, 30), but the role of other factors, such as the appearance of a new protein in resistant cells, has still to be elucidated (2, 5, 20).

Minocycline (7-dimethylamino-6-deoxy-6-demethyltetracycline) is a semisynthetic derivative of tetracycline to which most tetracycline-resistant strains of *S. aureus* are susceptible (10, 16). Experiments of Kuck and Forbes (17) with *E. coli* and Sompolinsky and Krausz (33) with *S. aureus* indicate that tetracycline-resistant cells which retain their susceptibility to minocycline show a decreased accumulation of tetracycline but retain the ability to accumulate minocycline. Resistance to both tetracycline and minocycline determined by some R factors (29, 31) is accompanied by a decrease in the accumulation of both tetracycline and minocycline (17).

Phair and Carleton (27) found that *S. aureus* strains of phage group III, although susceptible to minocycline, show a lower degree of susceptibility than strains of phage groups I and II. A survey of a large number of *S. aureus* strains carried out in our laboratory indicated a lower susceptibility to minocycline of strains of phage types 83A/85, 84, 85, and 84/85 of phage group III (83A/84/85 complex) than strains of other phage types. Most tetracycline-resistant strains susceptible to phages of the 83A/84/85 complex yielded mutants of increased resistance to minocycline. Transduction experiments with minocycline-resistant mutants indicated the simultaneous presence of two types of tetracycline resistance markers, one for tetracycline resistance without simultaneous minocycline

resistance and another for both tetracycline and minocycline resistance (S. Schaefler and W. Francois, Abstr. Int. Congr. Bacteriol., 2nd, Jerusalem, Abstr. 358, p. 30, 1973). Further investigation indicated that the genetic marker for tetracycline resistance associated with minocycline resistance is chromosomal (S. Schaefler, Int. Congr. Chemother., 9th, London, Abstr. M 484, 1975). Chromosomal location of the minocycline resistance determinant was also shown by Asheshov (1), who found minocycline-resistant strains also among tetracycline-resistant strains of other phage types than 83A/84/85 complex. The present investigation describes some genetic and biochemical characteristics of minocycline-resistant strains and the effect of minocycline resistance on susceptibility to several typing phages.

MATERIALS AND METHODS

Staphylococcus aureus strains used in the described experiments were obtained from the following sources: Phage Typing Unit, Bureau of Laboratories, New York City Department of Health; Center for Disease Control, Atlanta, Ga.; H. O. Halander; H. Lacey, M. Musher, R. Novick, and S. Seligman. Donor and acceptor strains and their derivatives are described in Table 2. The nomenclature of the genotypes for tetracycline and minocycline resistance and the corresponding phenotypes are described in Table 1. The purine-requiring mutants RN 450 G-3 and RN 450 G-9 (Table 2) require guanine, which cannot be replaced by adenine.

Media. LB medium (20 g of tryptone, Difco Laboratories, Detroit, Mich.; 10 g of yeast extract; 5 g of NaCl; 1,000 ml of water brought to pH 7.0 with NaOH) was used for cultivation. Supplemented with antibiotics, LB medium was used for transduction and susceptibility testing. Phage susceptibility was tested with Novick's phage medium (23).

Antibiotic susceptibility testing. Screening for tetracycline and minocycline resistance was carried out by plate dilution (34). Strains found resistant to minocycline were retested by tube dilution with an inoculum of 10^6 cells/ml. Susceptibility to other antibiotics was tested by the Kirby-Bauer method (3).

Bacteriophages and transduction. Transducing phages were obtained either by the induction of temperate phages with mitomycin C (32) or by phage propagation on susceptible donor strains. For phage induction, mitomycin C was added at a final concentration of 1 μg/ml to cultures of approximately 3×10^7 cells/ml in LB broth. Further incubation for 3 to 5 h usually resulted in the full lysis of the culture. Phage propagation was carried out by the overlay method using Novick's phage agar (23). Plates showing almost confluent lysis were extracted at room temperature with 5 ml of phage buffer (23). After centrifugation and filtration through a Millipore filter (0.45-μm pore size), preparations of 2×10^9 to 6×10^9 plaque-forming units (PFU) were obtained.

Phage susceptibility was tested by the overlay method using Novick's phage medium and expressed as PFU. Phage typing with the international set of phages (4, 26) was carried out in the Phage Typing Unit of the Bureau of Laboratories, Department of Health, New York City, N.Y.

For transduction experiments acceptor cells in the mid-stationary phase were mixed with the phage preparation at a ratio of 0.1 PFU/bacterial cell and maintained for 20 min at 37 C in LB broth containing 3×10^{-3} M $CaCl_2$. Selection was made on LB agar with 2.5 μg of tetracycline per ml or 2 μg of minocycline per ml after an incubation of 48 h at 37 C. Selection for penicillinase activity was made on CY medium (23) with 5×10^{-5} $CdCl_2$.

Purine-dependent mutants. Purine-dependent mutants of strain RN 450 were obtained by mutagenesis with ethyl methanesulfonate and selection

TABLE 1. *Genotypes and phenotypes for tetracycline and minocycline resistance*[a]

Genotype	Phenotype	Genetic location	Tetracycline susceptibility (range, μg/ml)	Minocycline susceptibility (range, μg/ml)	Regulation	Effect on phage susceptibility[b]
tmn	Tmn	chromosomal	R (12–25)	I (0.78–3.1)	low constitutive or inducible[c]	yes
tmn R	Tmn^R	chromosomal	R (25–100)	R (3.1–25)	high constitutive or semiconstitutive	yes
tet	Tc	plasmidic	R (50–100)	S (0.04–0.39)	inducible[d]	no
tet C	Tc^c	plasmidic	R (150)	S (0.19)	semiconstitutive	no

[a] The characteristics of the genotypes and phenotypes were determined after transfer by transduction into strain RN 450. Abbreviations: R, resistant; I, intermediate resistance; S, susceptible.

[b] Decrease of susceptibility to typing phages 6, 47, 53, 54, 75, 80, and phage P 11.

[c] Tetracycline induces resistance to both tetracycline and minocycline.

[d] Tetracycline induces resistance to tetracycline but not to minocycline.

TABLE 2. *Bacterial strains*[a]

No.	Strain	Pertinent genetic marker	Lysogenic for phage	Phage type	Origin of derivation
1.	PS 84	*tet, tmn*	184	84/85	a
2.	PS 84 *tmn* R	*tet, tmn* R	184	84/85	mutagenesis of 1.
3.	C 10	*tet, tmn*	10	84	b
4.	C 40	*tet, tmn, met*	40	84/85	c
5.	11164	*tmn* R, *met*	11164	84/85	d
6.	467	*tmn R, met*	?	42E	a
7.	C 32		nonlysogenic[f]	84	b
8.	RN 450		nonlysogenic[g]	6/47/53/75/83A/84/85	e
9.	RN 450 G-3	*pur* 3[1]	nonlysogenic	6/47/53/75/83A/84/85	mutagenesis of 8.
10.	RN 450 G-9	*pur* 9[1]	nonlysogenic	6/47/53/75/83A/84/85	mutagenesis of 8.
11.	C 32/84/1	*tmn* R	184	84/85	transduction 2. × C 32
12.	RN 450/84/48	*tet*	nonlysogenic[h]	6/47/75/83A/84/85	transduction 2. × RN 450
13.	RN 450/84/19	*tet*	184	6/47/53/83A/84/85	transduction 2. × RN 450
14.	RN 450/84/116	*tmn* R	184	84/85	transduction 11. × RN 450
15.	RN 450/84/116/1	*tmn* R	nonlysogenic	83A/84/85	transduction 14. × RN 450
16.	RN 450/84/116/48	*tet, tmn* R	nonlysogenic	83A/84/85	transduction 14. × RN 450/84/48
17.	RN 450/40/ T 5	*tet*	nonlysogenic	6/47/53/75/83A/84/85	transduction 4. × RN 450
18.	RN 450/40/ T 5 C	*tet* C	nonlysogenic	6/47/53/75/83A/84/85	mutagenesis of 17.
19.	RN 450/40/ M 2	*tmn*	nonlysogenic	84/85	transduction 4. × RN 450
20.	RN 450/40/ M 2 C	*tmn* R	nonlysogenic	84/85	mutagenesis of 19.
21.	RN 450/11164/5	*tmn* R	nonlysogenic	84/85	transduction 5. × RN 450
22.	RN 450/467/8	*tmn* R	nonlysogenic	6/47/53/83A/84/85	transduction with phage 80 6. × RN 450

[a] Sources of strains: (a) Phage Typing Unit, Bureau of Laboratories, New York City Department of Health; (b) Center for Disease Control, Atlanta, Ga.; (c) H. O. Hallander; (d) R. W. Lacey; (e) R. Novick. Criteria for lack of lysogeny: (f) strain C 32, lack of detectable lysis after induction with mitomycin C and lack of detectable plaques after testing the filtrate with indicator strains for international typing phages and indicator strains for experimental phages for nontypable strains (S. Schaefler, J. Rybak, and D. Jones, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C121, p. 47); (g) strain RN 450 was obtained by curing strain 8825 of three temperate phages (24); (h) transductant RN 450/84/48 retains the susceptibility to phage 184 of the acceptor strain RN 450 and shows no detectable lysis upon induction with mitomycin C. The same criteria were applied to other transductants of strain RN 450; (i) the phage type was determined at routine test dilution with the international set of *Staphylococcus* phages (4, 26).

on AOAC synthetic medium (32) supplemented with 0.2% glucose, 1 μg of thiamine per ml, 2 μg of nicotinamide per ml, 0.01 μg of adenine per ml, and 0.02 μg of guanosine per ml. Small colonies were tested for purine requirements on AOAC medium without purines and on AOAC medium with 1 μg of adenine per ml and 2 μg of guanosine per ml. Auxotrophic mutants were tested for guanine and adenine requirements and classified as mutants requiring guanine, mutants requiring adenine, and mutants requiring guanine + adenine.

Growth response to tetracycline. Growth was tested with cells grown in LB broth by following absorbance changes at 590 nm. Overnight cultures diluted to 4×10^7 to 7×10^7 cells/ml were grown in the absence of tetracycline or in the presence of tetracycline or its derivatives in concentrations of 0.05 to 0.5 μg/ml. After incubation by aeration for 30 min at 37 C, tetracycline was added to final concentrations of 10 to 40 μg/ml and minocycline to final concentrations of 2.5 to 20 μg/ml. The growth response of induced and uninduced cells was followed for 270 min.

Determination of extrachromosomal deoxyribonucleic acid. Extrachromosomal deoxyribonucleic acid (DNA) was determined in cesium chloride-ethidium bromide buoyant density gradients and sucrose gradients by the method described by Novick and Bouanchaud (25), with cell lysates prepared by the method of Clewell and Helsinki (9).

RESULTS

Minocycline resistance. Data on tetracycline and minocycline resistance of *S. aureus* strains isolated in 1971, 1972, and 1973 at different hospitals in New York City are given in Table 3. The strains were grouped according to their phage susceptibility (4, 26). Strains classified as group III include strains susceptible to phages of group III other than 83A/84/85 but may also include in their lytic spectrum phages of the 83A/84/85 complex. Strains classified as 83A/84/85 complex are susceptible only to phages 83A, 84, or 85 singly or in combination. Of the 621 strains of group I, 132 (22%) were tetracycline resistant but minocycline sensitive, and similarly no minocycline-resistant strains were found among the 17 tetracycline-resistant strains of group II (Table 3). Among the 52 tetracycline-resistant strains of group III, two strains of type 42 E showed a low level of resistance to minocycline. Both strains were methicillin resistant.

The group of strains susceptible to phages of the 83A/84/85 complex included a relatively high ratio of tetracycline-resistant strains (52%), and 49 of the 51 tetracycline-resistant strains of this group showed lower susceptibility to minocycline than the tetracycline-resistant strains of other phage types. The minimal inhibitory concentration (MIC) for these strains was 0.78 to 3.1 μg of minocycline per ml as compared with 0.04 to 0.39 μg of minocycline per ml for tetracycline-resistant strains of other phage groups. These strains yielded minocycline-resistant mutants after incubation on LB agar with 5 μg of minocycline per ml, with a 4- to 12-fold increase in minocycline resistance (Table 3). The rate of mutation was increased by mutagenesis with ethyl methanesulfonate. The mutation toward higher minocycline resistance was accompanied by an increase in tetracycline resistance, from an MIC of 25 to 50 μg/ml for the parental strains to 50 to 100 μg/ml for the mutants.

The testing of additional strains susceptible to phages of the 83A/84/85 complex gave results similar to those with strains isolated in New York City. This included 32 strains isolated from different parts of the United States and received from the Center for Disease Control, Atlanta, Ga., five strains isolated in Sweden, and three strains isolated in England. All tetracycline-resistant strains yielded minocycline-resistant mutants.

Among strains of phage types other than 83A/84/85, minocycline resistance was detected mostly among methicillin-resistant strains. This included strains of group III susceptible to phage 77 and strains nontypable with the international set of phages. Among the nontypable strains isolated from several hospitals in New York City, the majority could be typed with phage 92 (S. Schaefler, J. Rybak, and D. Jones, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C121, p. 47). Among the methicillin-resistant strains described by Minuth et al. (22) we found both minocycline-resistant and minocycline-susceptible strains. In most instances methicillin-resistant strains showed a relatively low minocycline resistance (MIC, 1.6 to 6.4 μg/ml), and only 6 out of 38 strains tested yielded mutants of higher minocycline resistance. It appears, therefore, that in *S. aureus* minocycline resistance can be detected mainly in two groups of strains: (i) tetracycline-resistant strains of the 83A/84/85 complex, including methicillin-resistant and methicillin-susceptible strains, and (ii) methicillin-resistant strains of other phage types. The present paper deals with strains belonging to the first group.

Transduction of resistance characteristics. Transduction experiments with phages obtained by induction with mitomycin C of phages carried by strains of 83A/84/85 complex resulted in transfer to strain RN 450 of minocycline and tetracycline resistance from four different strains (Table 4). Most experiments were

TABLE 3. *Tetracycline and minocycline resistance of S. aureus strains*

Phage group	No. of strains	Tetracycline-resistant, minocycline-sensitive strains (no.)[a]	Tetracycline-resistant yielding minocycline-resistant mutants (no.)[b]	Tetracycline- and minocycline-resistant strains (no.)
I (incl. 80/81)	621	132	0	0
II	83	17	0	0
III (except 83A/84/85 complex)	235	52	0	2[c]
83A/84/85 complex	97	2	49	0

[a] Tetracycline-resistant strains of high susceptibility to minocycline (MIC, 0.04 to 0.09 μg/ml). No minocycline-resistant mutants could be obtained spontaneously or by mutangenesis.

[b] Tetracycline-resistant mutants of intermediate resistance to minocycline (MIC, 0.78 to 3.1 μg/ml) yield minocycline-resistant mutants (MIC, 3.1 to 25 μg/ml) spontaneously or after mutagenesis.

[c] Minocycline-resistant strains of type 42 E (MIC, 6.2 μg/ml).

TABLE 4. *Transduction of tetracycline and tetracycline + minocycline resistance into strain RN 450*

Donor strain	Phage	No. of resistant transductants		No. of transductants/PFU	
		Tetracycline (Tc)	Tetracycline + minocycline (Tmn^R)	Tc	Tmn^R
PS 84 Tmn^R	184[a]	864	152	6×10^{-7}	10^{-7}
C 10 Tmn^R	10[a]	138	24	5×10^{-8}	1.5×10^{-8}
C 40 Tmn^R	40[a]	115	36	9×10^{-8}	3×10^{-8}
11164 Tmn^R	11164[a]		658		4×10^{-6}
467 Tmn^R	80[b]		242		2×10^{-7}
450/84/19[c]	184[a]	425		6×10^{-7}	
450/84/116[c]	184[a]		1,095		4×10^{-2}
450/84/48[d]	184[b]	98		6×10^{-8}	
450/84/54[d]	184[b]		32		4×10^{-9}

[a] Phages obtained by induction by mitomycin C.
[b] Phages obtained by propagation on the donor strain.
[c] Lysogenic transductants obtained by transduction PS 84 Tmn^R × RN 450 or C 32 /84/1 × RN 450.
[d] Nonlysogenic transductants obtained by transduction PS 84 Tmn^R × RN 450. Transductants were obtained by selection on LB medium with 2.5 μg of tetracycline per ml and tested for minocycline resistance. Transductants resistant to tetracycline and susceptible to minocycline were classified as phenotype Tc. Transductants resistant to both antibiotics were classified as phenotype Tmn^R.

carried out with the phage 184 obtained by induction of a minocycline-resistant mutant (Tmr^R) of strain PS 84. By selection for tetracycline resistance the transduction from the Tmr^R mutant of strain PS 84 yielded two distinct types of transductants: (i) 82 to 84% of the transductants were of phenotype Tc (tetracycline resistant and minocycline sensitive) and (ii) 16 to 18% were of phenotype Tmn^R (tetracycline and minocycline resistant). The second type of transductants was also obtained by selection on LB agar with 2 μg of minocycline per ml. Phage preparations obtained by induction with mitomycin C of minocycline-resistant mutants of the strains C 10 and C 40 gave the two types of transductants, whereas the phage preparation obtained by the induction of strain 11164 and by propagation of phage 80 on strain 467 yielded only transductants of the Tmn^R phenotype (Table 4). When phage 184 was induced in wild-type cells of strain PS 84, transductants of the phenotypes Tc and Tmn (low minocycline resistance, Table 1) were obtained. The transductants of low minocycline resistance yielded mutants of the Tmn^R phenotype. We could not obtain minocycline-resistant mutants from transductants of the Tc phenotype.

Most transductants of Tc and Tmn^R phenotypes were lysogenic for phage 184. Further transduction experiments were carried out with lysogenic Tc and Tmn^R transductants into strain RN 450 as donors and the wild-type strain RN 450 as acceptor. The transductants lysogenic for phage 184 were induced with mitomycin C, and the phage preparations were tested for transducing activity. The results depended on the phenotype of the lysogenic transductant. Transductants of the Tc phenotype yielded phage preparations which transferred Tc resistance at a frequency of 6×10^{-7} to 8×10^{-7} PFU (450/84/19, Table 4). Similar transducing frequencies were obtained with 28 different lysogenic transductants of this phenotype.

In contrast to transductants of the Tc phenotype, the lysogenic transductants of the Tmn^R phenotype showed marked differences in their transducing activities. Most phage lysates obtained by the induction of Tmn^R transductants had lytic titers of 5×10^8 to 2×10^9 PFU/ml but showed no detectable transducing activity for minocycline resistance. Lysogenic Tmn^R transductants containing also the penicillinase plasmid yielded phage preparations which transduced the penicillinase plasmid at a frequency of 8×10^{-6} to 6×10^{-7}/PFU but did not transduce minocycline resistance. Only 4 out of 54 lysogenic Tmn^R transductants into strain RN 450 yielded phage preparations which transferred Tmn resistance at a frequency of 10^{-4} to 10^{-5}/PFU. The lysogenic transductants with transducing activity dissociated into colonies yielding transducing phages and colonies yielding nontransducing phages. Transductants into strain C 32 showed characteristics similar to those of transductants into strain RN 450.

When phage 184 was propagated on nonlysogenic transductants (450/84/48 Tc and 450/84/54 Tmn^R, Table 4), the resulting phage preparations transferred at low frequency the Tc and

Tmn^R markers. Transductants containing simultaneously both markers were obtained by introducing first the Tc marker (Table 4, 450/84/48) and then the Tmn^R marker by a second transduction with strain 450/84/116 as donor, strain 450/84/48 as acceptor, and selection for minocycline resistance (450/84/116/48, Table 2).

High-frequency transduction of minocycline resistance. A stable lysogenic minocycline-resistant transductant of strain RN 450 (RN 450/84/116) was used in most transduction experiments (Tables 4 and 5). This lysogenic strain was obtained by first transducing minocycline resistance from strain PS 84 into strain C 32, followed by a second transduction of minocycline resistance from strain C 32 into strain RN 450. Selection was then made for stability and high-transducing activity. The resulting lysogenic transductant (116) yielded upon induction phage preparations of relatively low lytic titer (5×10^5 to 2×10^6 PFU/ml) with a high transducing activity (10^{-2} to 4×10^{-2}/PFU). In contrast to the predominantly lysogenic transductants obtained with phage lysates of the strain PS 84, the majority of the transductants obtained with phage 116 were nonlysogenic.

Location of the *tet* and *tmn* markers. The location of the two markers was tested by: (i) transfer into rec^+ and rec^- acceptor strains, (ii) determination of the presence of extrachromosomal DNA by the use of cesium chloride-ethidium bromide and sucrose centrifugation gradients, and (iii) cotransduction with chromosomal markers. The results obtained with the rec^+ and rec^- recipient strains are summarized in Table 5. Tetracycline resistance *(tet)* from strain PS 84 and from the lysogenic transductants 450/84/19, 450/C 10/8, and 450/C 40/5 was transferred in both rec^+ and rec^- strains. Tetracycline + minocycline resistance (*tmn* R) from strains PS 84 and 11164 and from the lysogenic transductants 450/84/116, 450/C103, 450/C40/12, and 450/467/3 was transferred only to the rec^+ strain, thus indicating the probable chromosomal nature of the *tmn* locus. Similar results were obtained by the transfer of the *tmn* R locus from strains 88 and 478.

TABLE 5. *Transfer of tetracycline and tetracycline + minocycline resistance into rec^+ and rec^- strains*[a]

Donor strain	Phage	Marker transferred	No. of transductants into:	
			864 rec^+	1030 rec^-
84 Tmn^R	184[b]	*tet*	483	42
84 Tmn^R	184[b]	*tmn* R	82	0
11164 Tmn^R	11164[b]	*tmn* R	928	0
450/84/19 Tc	184[b]	*tet*	731	85
450/84/116 Tmn^R	184[b]	*tmn* R	1,095	0
450/C10/8 Tc	184[c]	*tet*	134	17
450/C10/3 Tmn^R	184[c]	*tmn* R	141	0
450/C40/5 Tc	184[c]	*tet*	174	19
450/C40/12 Tmn^R	184[c]	*tmn* R	189	0
450/467/3 Tmn^R	P 11[c]	*tmn* R	768	0

[a] Selection for tetracycline resistance was carried out on LB medium with 2.5 μg of tetracycline per ml and subsequent testing for tetracycline and minocycline resistance.

[b] Transducing phage obtained by induction with mitomycin C.

[c] Transducing phage obtained by propagation on donor strain.

The results of cesium chloride-ethidium bromide buoyant equilibrium centrifugation and sedimentation in neutral 10 to 30% sucrose gradients are shown in Fig. 1 and 2. The [^{3}H]thymine-labeled lysate of a nonlysogenic *tet* transductant into strain RN 450 (Fig. 1B, 2B) showed a peak similar to the peak obtained with DNA from the ^{14}C-labeled plasmidic Tc-resistant control strain RN 1304. The cosedimentation with the ^{14}C-labeled DNA of the *tet* plasmid from strain RN 1304 indicated the presence in the *tet* transductant from strain PS 84 of a 20S DNA molecule corresponding to a molecular weight of 2.66 Mdal (25). However, transductants of the genotype *tmn* R from strains PS 84, C 10, C 40, and 467 showed no detectable extrachromosomal peak with both cesium chloride-ethidium bromide (Fig. 1A, C, D) and sucrose (Fig. 2A, C, D) gradients. It appears, therefore, that *tmn* resistance cannot be correlated with the presence of circular covalently closed DNA duplexes.

Association of the *tmn* marker with genetic determinants of guanine biosynthesis. Different auxotrophic markers were introduced by ethyl methanesulfonate mutagenesis into strain RN 450 in an attempt to map the *tmn* marker on the *S. aureus* chromosome. Transduction experiments were carried out with six purine-requiring mutants of strain RN 450 (genotypes *pur*) as acceptors. Three of the mutants required guanine, one required adenine, and two required adenine and guanine. The requirement for guanine cannot be replaced by adenine or vice versa. Preliminary data obtained with the above mutants indicated that the *tmn* R marker derived from strain PS 84 and from the high-frequency transducing transductant 450/84/116 was cotransduced with the markers *pur*-3 and *pur*-9, which determine guanine biosynthesis, at rates of 51% for *pur*-3 and 16% for *pur*-9 when selection is made on synthetic medium without guanine, and 84 and

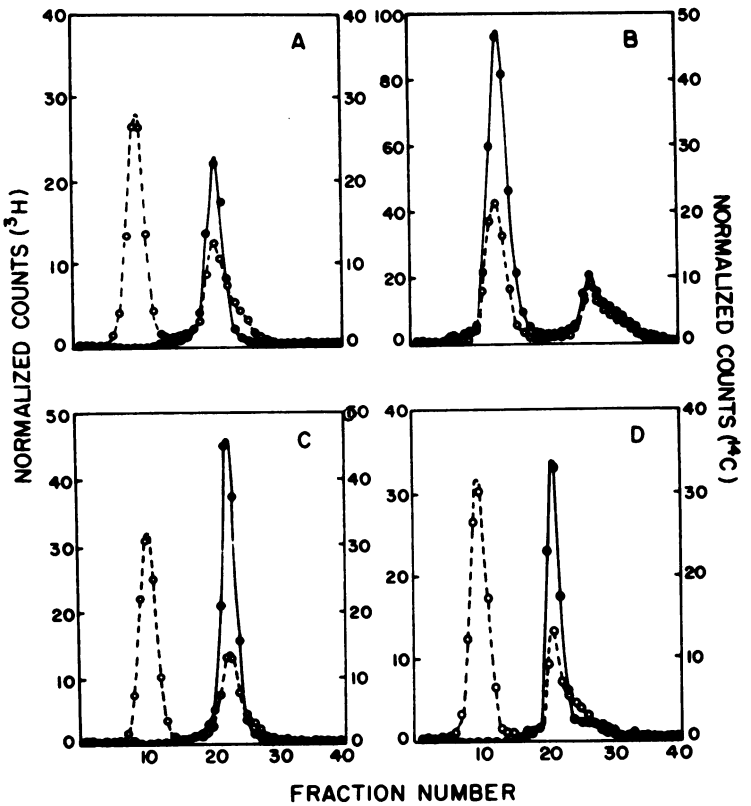

FIG. 1. *Cesium chloride-ethidium bromide buoyant density gradients. Symbols: (—○—○—○—) ^{14}C-labeled control strain, RN 1304; (—●—●—●—) ^{3}H-labeled transductants into strain RN 450. (A) PS 84 × RN 450, genotype tmn R; (B) PS 84 × RN 450, genotype tet; (C) C 40 × RN 450, genotype tmn. (D) 467 × RN 450, genotype tmn R. Each cleared lysate (9, 25) was mixed with saturated cesium chloride to give a final density of 1.54 and ethidium bromide was added to give a final concentration of 100 μg/ml. The mixture was centrifuged at 20 C for 36 h at 42,000 rpm in a Spinco Ti 50 rotor. The resulting gradient was collected by dripping onto Whatman 2.4-cm GF/A filters which were then washed twice in 5% trichloracetic acid and twice in 95% ethanol and then dried and counted with a standard toluene PPO scintillation mixture in a Beckman L 200 scintillation spectrometer. The counts for each isotope have been normalized to total incorporation for that isotope. The density of the gradient decreases from fraction 1 to fraction 40. The peak observed at fractions 6 to 12 represents extrachromosomal DNA, determined by the tet marker of the control strain RN 1304 (A, B, C, D) and of the transductant of genotype tet from the cross PS 84 × RN 450 (B). The peak at fractions 22-26 represents chromosomal DNA.*

34%, respectively, when selection is made for tetracycline resistance. Similar data were obtained with the *tmn* marker transferred by phage lysates obtained by induction of strains C 40 and 11164. The high-frequency transducing phage 450/84/116 transfers the *pur*-3 and *pur*-9 markers at a similar frequency as the *tmn* marker, whereas *pur* markers which are not cotransduced with the *tmn* resistance marker are transduced at a lower frequency (10^{-3} to 10^{-4}/PFU).

In contrast to the *tmn* chromosomal marker no cotransduction could be detected between the *pur*-3 and *pur*-9 markers and extrachromosomal *tet* markers derived from strains PS 84, C 10, and C 40.

Transfer of Tc and Tmn resistance into different acceptor strains. A large number of *S. aureus* strains of different phage types were tested for their susceptibility to phage 184. Strains found susceptible were used as acceptors for the transfer of tetracycline and tetracycline + minocycline resistance. The selection was carried out by: (i) plating on 2.5 μg of tetracycline per ml and testing the transductants for minocycline resistance and (ii) direct selection on plates with 2 μg of minocycline per ml. Both methods gave consistent results in the

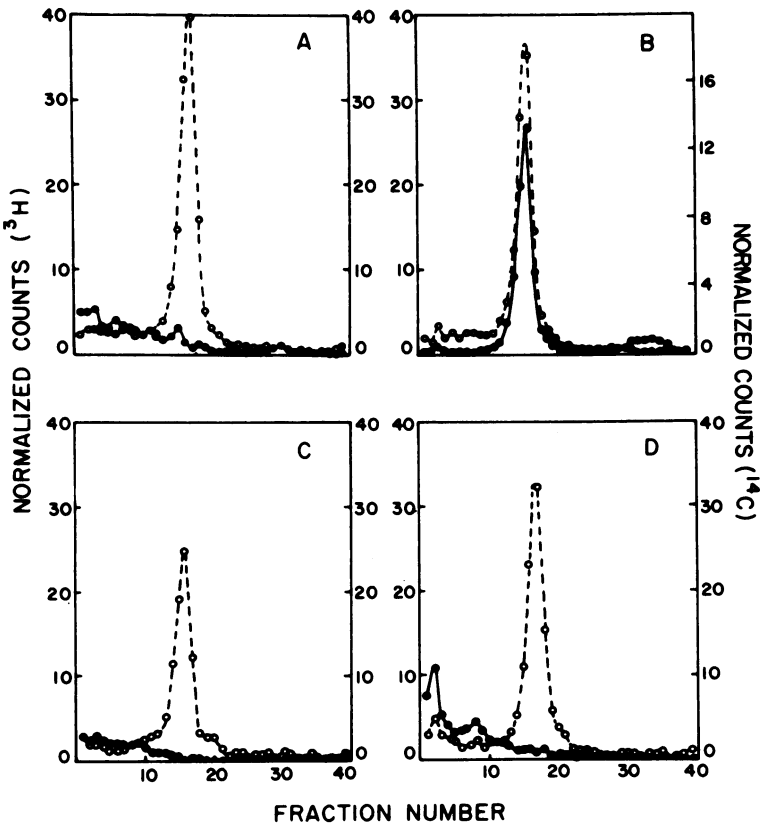

FIG. 2. *Sucrose density gradients. Symbols: (– ○– ○– ○–) ^{14}C-labeled control strain RN 1304; (– ●– ●– ●–) ^{3}H-labeled transductants into strain Rn 450. (A) PS 84 × RN 450, genotype tmn^R; (B) PS 84 × RN 84, genotype tet; (C) C 40 × RN 450, genotype tmn^R; (D) 567 × RN 450, genotype tmn^R. The sucrose gradients (5 ml, 10 to 30%, pH 7.0) contained 1 M NaCl and 50 mM EDTA and were layered with 0.3 ml of a cleared mixed lysate. Centrifugation was at 45,000 rpm for 5 h at 20 C in an SW 50.1 rotor. Fractionation of gradients and counting of radioisotopes were the same as for cesium chloride gradients (Fig. 1). The density of the gradient decreases from fraction 1 to fraction 40. The peak observed between fractions 13 and 17 represents extrachromosomal DNA determined by the tet marker of the control strain RN 1340 (A, B, C, D) and of the tranductant of genotype tet from the cross PS 84 × 450 (B).*

screening of minocycline-resistant transductants. Phage preparations were obtained by induction with mitomycin C of strain PS 84 Tmn^R harboring the *tmn* R and *tet* genes and the lysogenic transductants 450/84/19 *tet* and 450/84/116 *tmn* R. The acceptor strains can be divided in two groups: type A, acceptors for both the *tet* and *tmn* R markers from both strain PS 84 and the lysogenic transductants, and type B, acceptors only for the extrachromosomal *tet* marker (Table 6). There is no direct relationship between acceptor function and the phage type of the acceptor strain, although most acceptor strains of type A were of phage group III. It appears that the main difference between the A and B type acceptor strains consists in the ability of the former to integrate the chromosomal *tmn* R marker, a situation similar to that found with the *rec*$^+$ and *rec*$^-$ acceptor strains.

Induction of tetracycline and minocycline resistance. Induction of resistance was determined with transductants into strain RN 450 of genotypes *tet*, *tmn*, and *tmn* R from strains PS 84, C 10, and C 40 and transductants of genotype *tmn* R from strains 478 and 11164. Transductants simultaneously harboring both markers (genotype *tet-tmn* R) were obtained by successive selection for tetracycline and minocycline resistance. The presence of inducible and constitutive resistance was investigated by growth response to the challenge by high concentration of tetracycline or minocycline after previous incubation in the presence or absence of small concentrations of tetracycline or mino-

TABLE 6. *Transduction of tetracycline and tetracycline + minocycline resistance by phage 184 in different acceptor strains*[a]

Acceptor strain	Phage 184 induced in strain				Type of transfer
	84 Tmn R		450/84/19	450/84/116	
	tet	*tmn R*	*tet*	*tmnR*	
RN 450	346	48	254	1,048	A
C 32	232	41	241	823	A
6719	282	22	295	486	A
6732	184	20	198	342	A
6742	251	35	228	796	A
6759	221	27	236	568	A
6654	158	0	183	0	B
6656	239	0	243	0	B
6729	85	0	132	0	B
6788	351	0	318	0	B
6912	136	0	185	0	B
7136	124	0	176	0	B

[a] Phage preparations obtained by induction with mitomycin C of strains PS 84 Tmn^R, 450/84/19, and 450/84/116 were used as donors for the *tet* and *tmn*^{R} markers. Selection was carried out on LB medium with 2.5 μg of tetracycline per ml. The transductants were tested for tetracycline and tetracycline + minocycline resistance and assigned the genotypes *tet* and *tmn*^{R}.

cycline acting as inducers (Fig. 3, 4).

Data on the induction of transductants of the genotypes *tet*, *tmn* R, and *tet/tmn* R obtained by transfer of the resistance markers from strain PS 84 into strain RN 450 are shown in Fig. 3. The transductant 84/48 *tet*, when grown in the absence of inducer or in the presence of 0.1 μg of minocycline per ml, shows no subsequent growth after challenge with 40 μg of tetracycline per ml, whereas induction by 1 μg of tetracycline per ml resulted in subsequent growth in the presence of 40 μg of tetracycline per ml. Very low concentrations of minocycline (0.002 μg/ml) produced a weak induction of tetracycline resistance. Induction of tetracycline resistance did not produce an increase in minocycline resistance. Resistance determined by the chromosomal *tmn* R marker (84/116) was constitutive, with cells grown in the absence of inducer showing a rate of growth similar to that of cells induced by tetracycline. In contrast to the *tet* transductant the *tmn* R transductant was also resistant to minocycline. The wild-type *tmn* marker determined low constitutive resistance to tetracycline and minocycline. The double transductant 84/48/116 *tet/tmn* R was constitutive, with a higher growth rate in the presence of 40 μg of tetracycline per ml than the constitutive chromosomal transductant *tmn* R. It appears, therefore, that the effect of the simultaneous presence of the *tet* and *tmn* markers is synergistic and that the constitutive resistance determined by the *tmn* R marker is dominant over the inducible resistance determined by the *tet* marker. The minocycline-resistant mutant of the donor strain PS 84 which also contains the chromosomal and extrachromosomal markers *tet/tmn* R showed a growth response to tetracycline similar to that found with the double transductant 84/48/116.

In contrast to transductants from strain PS 84 both *tet* and *tmn* transductants from strain C 40 were inducible, with the mutation toward the *tmn* R genotype resulting in semiconstitutive minocycline resistance (Fig. 4). The resistance determined by the *tmn* R locus derived from strain C 40 is lower than that derived from strain PS 84 (MIC of 6.2 μg/ml for minocycline and 25 μg/ml for tetracycline). Data obtained with transductants from strain C 40 are given in Fig. 4. Induction of the transductant 40/T5 of the genotype *tet* resulted in resistance to tetracycline but not to minocycline. In contrast to the transductant of *tet* genotype, induction by tetracycline of the transductant 40/M2 of genotype *tmn* resulted in resistance to both tetracycline and minocycline. The mutation *tmn* to *tmn* R (40/M2C) produced semiconstitutive resistance to both tetracycline and minocycline, the constitutive resistance to tetracycline and minocycline being increased by induction with tetracycline but not by minocycline.

In order to determine if an increase in minocycline resistance can be obtained by the isolation of constitutive mutants affecting the extrachromosomal *tet* resistance, the transductant 40/T5 was subject to ethyl methanesulfonate mutagenesis and plated on LB medium with 150 μg of tetracycline per ml. The resulting mutant 40/T5C showed an increase in the resistance to tetracycline from an MIC of 75 μg/ml to an MIC of 150 μg/ml. There was only a slight decrease in the susceptibility to minocycline. Induction by tetracycline increased further the resistance to tetracycline, but not to minocycline (Fig. 4).

Transductants from strain C 10 into strain RN 450 were similar to those from strain C 40, whereas transductants from strains 11164 and 478 were semiconstitutive. The Tmn^R mutants of the donor strains C 10 and C 40 were semiconstitutive, indicating dominance of the chromosomal tetracycline + minocycline resistance.

Induction curves for 32 different wild-type strains of *S. aureus* of low minocycline resistance and their minocycline-resistant mutants

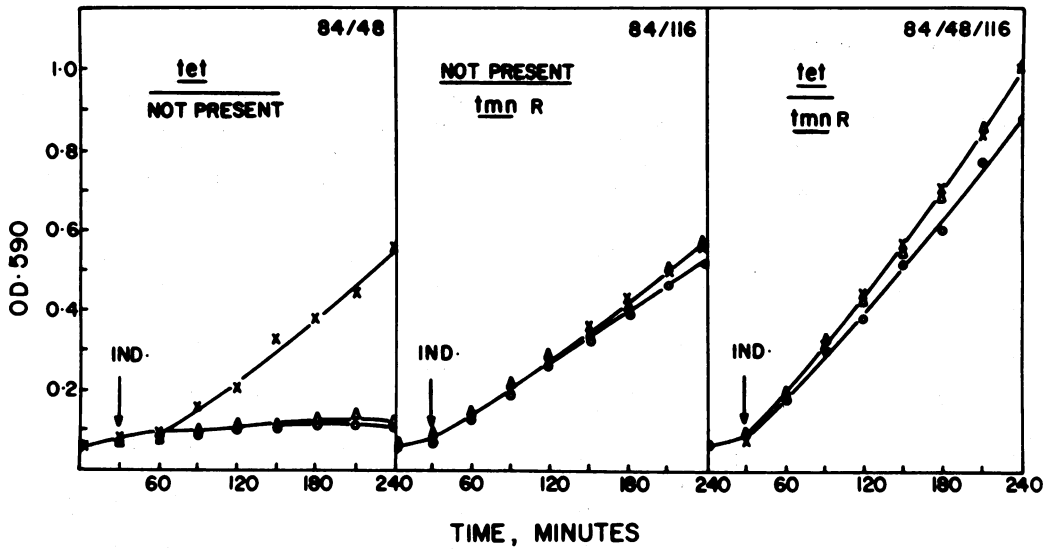

FIG. 3. *Inducible and constitutive resistance determined by the tet and tmn^R genes, introduced from strain PS 84 into strain RN 450. Strains and genotypes: genotypes of the strains were expressed by the presence or absence of both chromosomal or extrachromosomal markers, extrachromosomal/chromosomal. "Not present" for the extrachromosomal marker indicates the absence of the tet plasmid. Not present for chromosomal marker is indicated by lack of minocycline resistance. 84/48 is PS 84 × RN 450, selection for Tc. 84/116 is PS 84 × RN 450, selection for Tmn^R. 84/48/116 is 84/118 × 84/48 (double transductant), selection for Tmn^R. Nine milliliters of overnight cultures diluted to a final optical density of 0.05 to 0.06 at 590 nm was introduced into nephelometric flasks containing 1 ml of broth (control), LB broth with tetracycline to a final concentration of 1 μg/ml, and LB broth with minocycline to a final concentration of 0.1 μg/ml (inducers). After incubation by aeration for 30 min, in the absence and presence of inducers tetracycline was added to a final concentration of 40 μg/ml (challenge). Changes in optical densities were tested at intervals of 30 min. Symbols: (Δ–Δ–Δ–Δ–Δ–Δ) no inducer added; (×–×–×–×–×) induced with 1 μg of tetracycline per ml; (O–O–O–O–O) induced with 0.1 μg of minocycline per ml.*

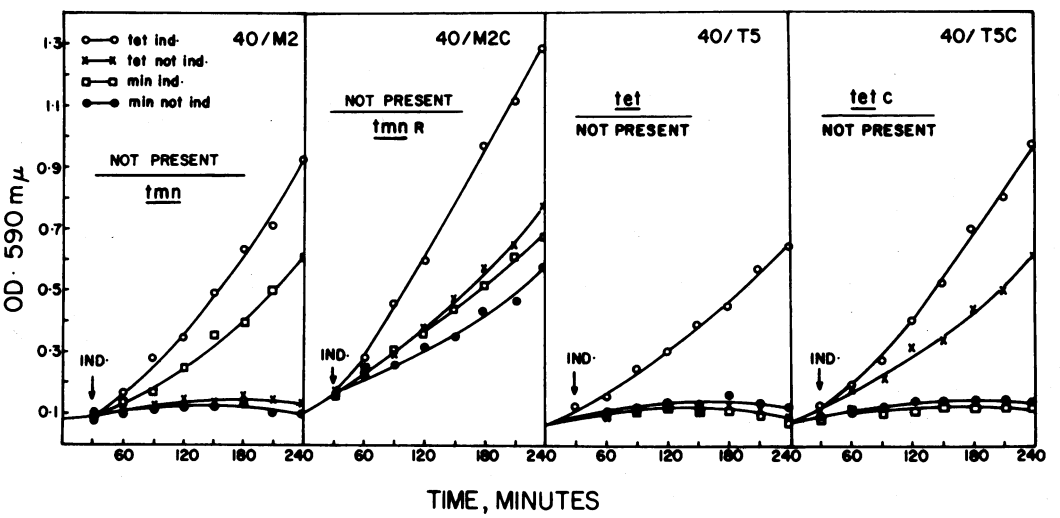

FIG. 4. *Inducible and constitutive resistance determined by the tet, tet C, tmn, and tmn^R genes transferred from strain C 40 into strain RN 450. Symbols for genotypes of extrachromosomal and chromosomal resistance are the same as for Fig. 3. Strains: 40/M2 is C 40 × RN 450, selection for Tmn; 40/M2 C is mutant of 40/M2, selection for Tmn^R; 40/T5 is C 40 × RN 450, selection for Tc; 40/T5C is Tc^C mutant of 40/tC (Table 1). The conditions for induction were the same as in Fig. 3, except that induction was carried out only by tetracycline, and induced and noninduced cells were challenged with both tetracycline and minocycline. Symbols: (O–O–O–O–O–O–O) induced with 0.5 μg of tetracycline per ml and challenged with 20 μg of tetracycline per ml; (×–×–×–×–×–×) not induced, challenged with 20 μg of tetracycline per ml; (□–□–□–□–□–□–□) induced with 0.5 μg of tetracycline per ml and challenged with 5 μg of minocycline per ml; (●–●–●–●–●–●–●) not induced, challenged with 5 μg of minocycline per ml.*

TABLE 7. *Effect of tetracycline and tetracycline + minocycline resistance on phage susceptibility of transductants from strain 84 into strain RN 450*

Phage	RN 450 (wild type)	Transductants into strain RN 450		
		48 *tet*	128 *tmn*	116/1 *tmn*^{R}
184	2×10^{9a}	4×10^9	10^9	3×10^9
6	5×10^8	2×10^8	2×10^3	10^3
47	4×10^9	5×10^9	3×10^4	5×10^4
53	8×10^8	3×10^8	2×10^2	6×10^2
54	5×10^7	8×10^7	4×10^3	8×10^2
75	7×10^7	4×10^7	0[b]	0
80	5×10^6	7×10^6	3×10^2	4×10^2
83 A	4×10^9	2×10^9	2×10^8	8×10^7
84	2×10^9	10^9	3×10^9	4×10^9
85	8×10^8	6×10^8	2×10^9	3×10^9
P11	4×10^9	3×10^9	3×10^3	5×10^3

[a] Lytic titer with strain RN 450 at 37 C (PFU per milliliter).

[b] A lytic titer of 2×10^2 is observed when the testing is carried out at 43 C.

indicated for the wild-type strains a low constitutive or inducible tetracycline and minocycline resistance and constitutive or semiconstitutive resistance for their minocycline-resistant mutants.

Effect of minocycline resistance on phage susceptibility. The effect on phage susceptibility of the transfer of the *tet*, *tmn*, and *tmn* R genotypes was tested with nonlysogenic transductants of strain RN 450 (Table 7). This strain retains essentially the phage susceptibility pattern of the parental type strain PS 47 but became susceptible to phage P 11 for which strain PS 47 was lysogenic. Phage susceptibility of strain RN 450 and of the transductants was tested with the typing phages 6, 29, 47, 53, 54, 75, 80, 83A, and 85 and also with the phages P 11 *vir* and 184. Lytic titers were determined with the wild-type strain RN 450 and nonlysogenic transductants of the genotypes *tet*, *tmn*, and *tmn* R. There was no detectable change in phage susceptibility after transfer from strain PS 84 into strain RN 450 of the extrachromosomal *tet* locus. The transfer of the chromosomal *tmn* and *tmn* R markers resulted in a marked decrease in susceptibility to phages 6, 47, 53, 54, 75, 80, and P 11, a less pronounced decrease of the susceptibility to phage 83A, and the retention of the initial susceptibility to phages 84, 85, and 184. By using the routine phage typing method (4), it was found that the introduction of minocycline resistance resulted in a change of the phage type of the parental strain RN 450 from 6/47/53/75/83A/84/85 to 83A/84/85 (Table 2). Lysogenization with phage 184 resulted in a further change to type 84/85. The decrease in phage titers is also accompanied by a pronounced decrease in the size of the plaques. Titration at 43 C resulted in a slight increase in the titers obtained with phages 28, 53, 80, and P 11 and also in an increase in the plaque size, with the titers remaining, however, well below those obtained with wild-type cells.

Results very similar to those obtained with transductants from strain PS 84 were also obtained after transfer of the *tmn* and *tmn* R markers from strains C 10, C 40, and 11164. With transductants from strains C 10 and C 40, the decrease of phage susceptibility was more pronounced and the effect of growth at 43 C was less than with transductants from strain PS 84. Transfer of the *tmn* R marker from strain PS 84 into the acceptor strains C 32, 6759, 6978, and 783 resulted in a variable degree of decrease in susceptibility to phages 53, 80, and P 11. A marked increase in the susceptibility to phage 85 was observed with *tmn* R transductants in strains C 32 and 6978. The introduction of the *tmn* R marker from strains PS 84 into strain C 32 resulted in a change from resistant to phage 85 to a lytic titer of 2×10^{-4} and with strain 6978 from 8×10^{-4} to 3×10^{-9}.

In contrast to *tmn* R transductants obtained with donor strains susceptible to phages of the 83A/84/85, no detectable change in phage susceptibility was observed with *tmn* R transductants into strain RN 450 from two donor strains of phage type 42 E. Similar results were reported by Chopra et al. (7) after transfer of chromosomal tetracycline resistance from a methicillin-resistant strain of phage type 53/77/84/88.

We obtained a mutant of phage P 11 designated P 11 Mn, which formed large plaques on a minocycline-resistant transductant from strain C 10 into strain RN 450. In contrast to the wild-type phage, the mutant phage gave similar titers with wild-type cells of strain RN 450 and *tmn* R transductants derived from strains PS 84, C 10, C 40, and 11164. To determine whether naturally occurring strains of the Tmn phenotype show an increased susceptibility to the mutant phage, preparations of phage P 11 and P 11 Mn adjusted to a titer of 2×10^9 PFU/ml with strain RN 450 were tested with 32 wild-type strains of the Tmn phenotype susceptible to phages of the 83A/84/85 complex and 43 Tc-resistant control strains of phage groups I, II, and III. No differences in the phage titers obtained with the two phages could be detected with any of the control strains. Among the strains of the 83A/84/85 complex, seven showed

a low susceptibility to the wild-type phage P 11 (titers of 10^3 to 10^6 PFU/ml). Of these strains, five were of higher susceptibility to phage P 11 Mn (titers of 8×10^7 to 2×10^9 PFU/ml). Three strains resistant to phage P 11 were susceptible to phage P 11 Mn.

DISCUSSION

Minocycline resistance in *S. aureus* is usually of low level (0.78 to 25 μg/ml) and was found mostly in two groups of strains: tetracycline-resistant strains of the 83A/84/85 complex, where it was found among methicillin-resistant and methicillin-susceptible strains, and methicillin-resistant strains of other phage types. Some methicillin-resistant strains of *S. aureus*, however, were minocycline susceptible (22).

In all tested strains minocycline resistance *(tmn)* appears to be chromosomal. This is indicated by transfer experiments where there is no transfer to *rec*⁻ acceptor strains and the data obtained by buoyant density centrifugation indicating no plasmid DNA. Cotransduction data with chromosomal *pur*-3 and *pur*-9 markers are consistent with this hypothesis. The differences in the host range in the phage transfer of the *tet* and *tmn* markers are also probably due to the requirement of chromosomal integration of the minocycline resistance marker. In wild-type strains PS 84, C 10, and C 40 the chromosomal *tmn* marker was found together with the extrachromosomal *tet* marker, whereas in strain 11164 of type 84/85 and two strains of type 42E only the *tmn* marker was found. The genetic analysis of a larger number of strains is required for an understanding of the natural diploidy of tetracycline and tetracycline + minocycline resistance.

The chromosomal transductants of genotypes *tmn* and *tmn* R from strain PS 84, C 10, and C 40 into strain RN 450 differ in several respects from extrachromosomal *tet* transductants derived from the same strains. This involves differences in the transducing activity of induced phages, regulation of resistance, and the effect on phage susceptibility.

With lysogenic transductants from strain PS 84 into strain RN 450, it was found that, whereas lysogenic *tet* transductants yielded transducing phage preparations that showed no significant differences in their transducing activity, most *tmn* transductants yielded phage preparations with no detectable transducing activity for the *tmn* marker. Lysogenic transductants that yielded transducing phages for the *tmn* R marker dissociated into active and inactive colonies. Upon induction both types of colonies yielded phage preparations of similar titers. There are still insufficient experimental data to explain the heterogeneity of the *tmn* lysogenic transductants. A possible explanation of the lack of transducing activity of phage preparations obtained by the induction of *tmn* transductants could be the existance of a crossing-over between the *tmn* locus enclosed in the phage genome and the bacterial chromosome of the acceptor strain without the simultaneous attachment of the phage particle.

The high-frequency transducing derivative of phage 184 (116) is characterized by a low-lytic titer and a high-frequency transducing activity for the *tmn*, *pur*-3, and *pur*-9 markers. The same phage also transfers the purine markers *pur*-11, *pur*-26, *pur*-28, *pur*-31 at frequencies of 10^{-3} to 10^{-4}. It appears, therefore, that phage 116 is preferentially attached to the *tmn*-*pur*-3-*pur*-9 region but can also pick up other sites of the chromosome. A still unsolved problem is whether the lytic titer (10^{-5} to 10^{-6}) of the phage preparation reflects the actual number of phage particles present in the induced phage preparations or the majority of the phage particles are defective and unable to form plaques on the indicator strain used. The complete lysis after mitomycin C induction of the transductant 84 × RN 450/116, at a cell density of approximately 2×10^8 cells/ml and similar to that of transductants which yield high-titer phage preparations, appears to support the defective phage hypothesis.

The regulations of chromosomal tetracycline + minocycline resistance differ in two respects from the extrachromosomal tetracycline resistance (11, 33). In inducible *tmn* transductants of the type obtained by transfer from strain C 40 (Fig. 4), tetracycline induces both tetracycline and minocycline resistance, whereas with inducible *tet* transductants induction by tetracycline results only in tetracycline resistance with the induced cells preserving their susceptibility to minocycline. Strains of low minocycline resistance yield mutants of higher resistance which are constitutive or semiconstitutive for both tetracycline and minocycline resistance. No such mutants could be obtained spontaneously or by mutagenesis from cells harboring the extrachromosomal *tet* resistance marker. The cause of the difference in resistance spectrum is not known, although differences in the accumulation of the two compounds could be at least partly responsible (17, 33). Our inability to obtain minocycline-resistant mutants from a large number of *S. aureus* strains of the Tc phenotype could be explained by the requirement for a double mutation, a

mutation resulting in a change in the specificity of the resistance and the regulatory mutation toward constitutivity or semiconstitutivity. This probably accounts for the minocycline susceptibility of constitutive mutants of transductants of the *tet* genotype. The dominance of the chromosomal resistance over the extrachromosomal resistance and the synergistic activity of the two markers indicate an interaction of the two types of resistance whose mechanism has still to be established.

A characteristic feature of minocycline resistance in *S. aureus* is its limitation to strains of the 83A/84/85 complex and methicillin-resistant strains of other phage types. An important element in the limitation of minocycline resistance to strains of the 83A/84/85 complex is probably the change in phage susceptibility resulting from the genetic transfer of minocycline resistance. Both the loss of susceptibility to phages of group III such as 6, 53, and 77 and the increase in susceptibility to phage 85 could account for the limitation of minocycline resistance to strains of the 83A/84/85 complex. Data found by Danish investigators (6, 14) indicate that the loss of susceptibility to phages of group III, other than the 83A/84/85 complex, occurred simultaneously with the appearance of tetracycline resistance, often accompanied by methicillin resistance. The narrower host range of the transfer of minocycline resistance as compared with tetracycline resistance could be an additional factor in the limitation of the natural distribution of minocycline resistance.

The transfer of chromosomal minocycline resistance from two methicillin-resistant strains of type 42 E was not accompanied by changes in phage susceptibility. Similar results were obtained by Chopra et al. (7) after transfer of constitutive, chromosomal, tetracycline resistance from a strain of phage group III. No information is available on the minocycline susceptibility of this strain. The presence of the *tmn* marker in most methicillin-resistant strains tested is difficult to explain. A possible explanation could be the origin of methicillin-resistant strains from a small number of strains or from even a single strain as suggested by Lacey and Grinsted (19). The phage 92 susceptibility of methicillin-resistant strains isolated in New York City (S. Schaefler, J. Rybak, and D. Jones, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C121, p. 47) appears to indicate a common origin of these strains.

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