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Methicillin-Resistant Strains of *Staphylococcus aureus* Phage Type 92

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Methicillin-resistant (Mec^r) strains of *Staphylococcus aureus* received for phage typing from several hospitals in New York City were resistant to the international set of typing phages but susceptible to experimental phage 92. Subsequently, strains of type 92 were detected in two outbreaks with Mec^r strains in two other locations in the United States. In all instances, type 92 was predominant among the Mec^r strains isolated in each hospital. With the exception of one strain, the methicillin resistance of the Mec^r strains investigated was homogeneous. In most instances, isolates from the same hospital were closely similar in their antibiotic resistance patterns. The strains isolated in New York City could be divided into three groups by the host range of their lysogenic phages and by antigenic structure. Transduction experiments indicated that the transfer of chromosomal tetracycline resistance from Mec^r strains into a strain susceptible to several international typing phages renders the latter nontypable. However, the acceptor strain remains susceptible to experimental phages 92 and 88. Transduction of methicillin resistance had no effect on the phage susceptibility of the acceptor strain. It is possible that the presence of chromosomal tetracycline resistance is a determining factor in the phage susceptibility of Mec^r strains isolated in New York City.

Although penicillinase-resistant penicillins are widely used in the treatment of staphylococcal infections, there are marked differences in the frequency of isolation of methicillin-resistant (Mec^r) strains in different countries (4, 6, 16, 18, 20). The differences in the geographical distribution of Mec^r strains cannot be explained solely by differences in the therapeutic use of penicillinase-resistant penicillins. Additional factors which could affect the distribution of Mec^r strains of *S. aureus* include their very limited host range for the genetic transfer of methicillin resistance (5, 9) and the relatively slower growth rate of some multiply resistant strains (10). Generally, Mec^r strains are lysed by only a few phages, and they often display common traits such as tetracycline and streptomycin resistance. These shared characteristics of Mec^r strains led Lacey and Grinsted (11) to the hypothesis that all Mec^r strains of *S. aureus* isolated in England are of common origin. A similar hypothesis was proposed by Kayser et al. (7) for strains isolated in Switzerland.

In the United States, Mec^r strains of *S. aureus* have been isolated infrequently (16), although several outbreaks with Mec^r strains have been described. Since 1974 we have investigated a large number of closely similar Mec^r strains that were isolated in several hospitals in New York City, Hartford, Conn., and St. Paul, Minn. Some of these strains have been described (8, 19; P. Nicholas, G. Pringle, and M. Malowany, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Ill., Abstr. no. 144, 1976; K. B. Crossley, D. M. Loesch, and B. J. Landesman, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 423, 1977; R. A. Recco, S. Schaefler, and Y. L. Gladstone, Abstr. Clin. Res. **23**:622A, 1976). All strains investigated were resistant to the international set of *S. aureus* typing phages (3, 14), but were susceptible to experimental phage 92 and, in varying degree, to experimental phages 88, 89, and 90. Similarities in antibiotic resistance spectra and phage susceptibility appear to indicate a common origin for these strains. However, a more detailed analysis, using such parameters as the lytic spectrum of temperate phages harbored by Mec^r strains and serological characteristics, indicated that these strains are a more heterogeneous group.

MATERIALS AND METHODS

Strains and nomenclature. The Mec^r *S. aureus* strains were isolated at the following hospitals in New York City: Elmhurst Hospital (69 strains), New York

V.A. Hospital (5 strains), New York Hospital (2 strains), Mount Sinai Hospital (5 strains), Coney Island Hospital (43 strains), and Montefiore Hospital (1 strain). In addition, we investigated strains isolated at Hartford Hospital, Hartford, Conn. (7 strains) and Ramsey-St. Paul Hospital, St. Paul, Minn. (8 strains). Results obtained with the strains selected for a more detailed investigation are shown in Tables 1 and 2, where the origin of each strain is also indicated. The Mec[r] strains had the typical biochemical characteristics of *S. aureus*. With the exception of strain VIII, all strains tested were lipase positive.

Antibiotic susceptibility testing. Antibiotic susceptibility testing was performed by using the Kirby-Bauer method (2). Minimal inhibitory concentrations were determined by plate dilution using the Steers replicator (23).

The ratio of methicillin-resistant cells in the populations of Mec[r] strains was tested by plating 10^{-2} to 10^{-9} dilutions of overnight cultures on LB medium (22) containing 12.5 μg of methicillin per ml and LB medium without antibiotic. A 0.1-ml sample of each dilution was spread on each of six plates without antibiotic and six plates with antibiotic. Each set of plates was divided in two groups, one for incubation at 30°C and one at 37°C.

Bacteriophages. Susceptibility was determined by using the international set of *Staphylococcus* typing phages at routine test dilution (RTD) and 100 × RTD dilutions (3, 14). In addition we used a set of 18 experimental phages isolated in our laboratory (S. Schaefler, J. Rybak, and D. Jones, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C121, p. 47). Phage 88 was obtained from R. W. Lacey, and phages 89 and 92 came from A. W. Jackson.

The induction of phages harbored by Mec[r] strains was carried out as described previously (22) using 1 μg of mitomycin C per ml. The induced phages were propagated on strain RN 450, which harbors no detectable lysogenic phage (13). In some instances the phages were also isolated and propagated on indicator strains PS 6 and PS 83A. Plaque titers of the phage preparations were determined by plaque counts on Novick phage medium (12) and expressed in plaque-forming units (PFU). Preliminary determinations of phage titers were carried out by spotting 10-fold dilutions of the phages on Novick medium with a 3-mm-diameter loop and flooding with a 1/20 dilution of an overnight culture of the indicator strain.

Lysogenic derivatives of strain RN 450 with phages originating from Mec[r] strains were selected from plates of Novick phage agar containing 10^8 to 10^9 PFU of phage per ml, flooded with an overnight culture of strain RN 450. Resistant colonies were reisolated and tested for lysogeny by induction with mitomycin C.

Transduction experiments. Transduction of tetracycline resistance was performed as previously described (22). Transduction of methicillin resistance was performed as described by Cohen and Sweeny (5). The transducing phage 88 was propagated on Mec[r] donor strains; in most instances, phage preparations of 4×10^{10} PFU/ml were obtained. The phage preparations were irradiated for 45 s with a General Electric bactericidal lamp at a distance of 20 cm, resulting in a 30 to 40% decrease of the plaque count. Selection was made on beef heart infusion agar with 12.5 μg of methicillin per ml and LB agar with 2.5 μg of tetracycline per ml.

RESULTS

Antibiotic susceptibility. The Mec[r] strains of *S. aureus* were tested for their susceptibility to cephalothin, chloramphenicol, clindamycin, erythromycin, gentamicin, kanamycin, methicillin, minocycline, novobiocin, oxacillin, penicillin, tetracycline, and vancomycin. Susceptibility to methicillin and oxacillin was tested at 30 and 37°C. All strains tested were resistant to methicillin and oxacillin. The Mec[r] strains of phage type 92 were resistant to erythromycin, penicillin, and tetracycline. With the exception of the strains isolated at Ramsey-St. Paul Hospital, all strains tested were resistant or of intermediate resistance to minocycline. The strains isolated at Ramsey-St. Paul Hospital yielded minocycline-resistant mutants. It appears, therefore, that the Mec[r] strains of phage type 92 possess the chromosomal tetracycline- and minocycline-resistant marker (22).

The strains isolated at Elmhurst Hospital (except one strain), New York V.A. Hospital, and New York Hospital were resistant to chloramphenicol, clindamycin, and kanamycin. The strains isolated at Mount Sinai Hospital were resistant to clindamycin and kanamycin, but susceptible or of intermediate resistance to chloramphenicol, whereas the strains isolated at Coney Island and Montefiore Hospitals were susceptible to all three antibiotics. The strains isolated at Ramsey-St. Paul Hospital were resistant to kanamycin and clindamycin but sensitive to chloramphenicol. The latter strains were also resistant to amikacin, and one strain was resistant to gentamicin. It appears, therefore, that strains of type 92 have some common traits, such as erythromycin resistance, a similar degree of methicillin resistance, and the presence of chromosomal tetracycline resistance. As for resistance to chloramphenicol, clindamycin, and kanamycin, the patterns seem to depend on the hospital from which the strains were isolated. The only hospital with a more heterogenous population of strains appears to be Hartford Hospital (8).

The testing of methicillin susceptibility by the disk diffusion method at 30 and 37°C, as well as the plate count of cells grown in the presence and absence of methicillin, indicate that, in contrast to most of Mec[r] strains described in the literature (1, 5, 24), the majority of strains of type 92 possess homogeneous methicillin resistance. With the exception of strain VIII, isolated at Montefiore Hospital, the strains tested

showed no differences in the inhibition zones at 30 and 37°C (1).

The determination of the ratio of methicillin-resistant cells in the bacterial cultures was carried out by plating 0.1 ml of 10^{-2} to 10^{-8} dilutions of overnight cultures on plates with and without methicillin (12.5 μg/ml) as described in Materials and Methods. Twelve Mec^r strains isolated at five different hospitals were tested. With the exception of strain VIII, all strains tested gave a similar colony count at 30 and 37°C on plates with and without methicillin. At 37°C, strain VIII gave 3×10^4 colonies per ml on LB medium with methicillin and 4×10^9 colonies per ml on LB medium without methicillin. At 30°C, however, the strain gave 8×10^8 colonies in the presence of methicillin and 3×10^9 colonies in the absence of methicillin. From plates with methicillin we isolated homogeneous resistant Mec^r mutants of strain VIII which gave a similar colony count on plates with and without methicillin.

Bacteriophage susceptibility. All strains tested were susceptible to phage 92. Most were susceptible to some extent to phage 88, and some were also susceptible to phages 89 and 90. All Mec^r strains of type 92 were resistant to the international *Staphylococcus* typing phages at RTD and 100 × RTD concentrations.

A survey of strains of type 92 among strains received for phage typing by the Bureau of Laboratories, New York City, indicated that most strains were methicillin susceptible but possessed chromosomal tetracycline and minocycline resistance.

Since the Mec^r strains of *S. aureus* were of very similar phage susceptibility, we attempted their differentiation by testing the host spectrum of lysogenic phages carried by these strains. Lysogenic phages harbored by Mec^r strains isolated at different hospitals were induced with mytomycin C (22). All phage preparations were tested with strain RN 450, which does not harbor detectable lysogenic phages, thus eliminating potential interference with the phages introduced from Mec^r strains. The titers obtained with phages derived from strains isolated at Coney Island Hospital were between 2×10^9 and 8×10^9 PFU/ml. The phage titers obtained by the induction of strains from other hospitals were generally lower, with a range of 5×10^6 to 8×10^8 PFU/ml. By using the spotting method, the strains isolated at Coney Island gave near-confluent lysis of the RN 450 indicator cultures at dilutions of 10^{-6} to 10^{-7}, whereas strains isolated at other hospitals gave near-confluent lysis at dilutions of 10^{-4} to 10^{-5}. In subsequent tests for host range, all phages were tested by the spot method at dilutions of 10^{-2} to 10^{-7}. Controls by plaque counts gave results consistent with those obtained by the spot method.

After repeated single-plaque isolation, the phages harbored by Mec^r strains were propagated on strain RN 450. Lysogenic derivatives of strain RN 450 were obtained by the selection of colonies resistant to phages propagated on this strain as outlined in Materials and Methods. The lysogenic derivatives were induced with mitomycin C, and the phages were then tested for titer and host range. With strain RN 450 as indicator, the titers of induced phages of different origin were very similar (4×10^9 to 7×10^9 PFU/ml). In some instances the phages induced in Mec^r strains were propagated on strains PS 6 and PS 83A, which were also lysogenized in a manner similar to strain RN 450.

In a first series of experiments, phages obtained by the induction of the original strains and the corresponding lysogenic derivatives of strain RN 450 were tested with the propagating strains for *Staphylococcus* international typing phages and the propagating strains of a set of experimental phages used in our laboratory.

Most indicator strains tested were resistant to the above phages. The results obtained with strains susceptible to phages induced in Mec^r strains and the corresponding lysogenic derivatives of strain RN 450 are summarized in Table 1.

Phages obtained from strains isolated at Elmhurst, New York, New York V.A., and Mount Sinai Hospitals lysed indicator strains RN 450, PS 6, and PS 85 but not PS 54, PS 83A, 26, and 41 (group A; Table 1). Similar results were obtained with phages obtained from other strains isolated at the above hospitals. Phages obtained by the induction of lysogenic derivatives of strain RN 450 had a host range similar to the phages induced in the corresponding original strains.

The phages obtained by the induction of the Mec^r strains 209 and 130, as well as of other strains isolated at Coney Island Hospital, lysed all indicator strains (group B). However, the phages obtained by the induction of the corresponding lysogenic derivatives of strain RN 450 did not lyse indicator strains PS 6 and PS 85, whereas phages obtained by the lysogenization of indicator strains PS 6 lysed strains PS 6, PS 83A, and PS 85, but not the other indicator strains. This seems to indicate that strains of group B isolated at Coney Island Hospital harbor at least two lysogenic phages with a different host range.

Strain VIII, isolated at Montefiore Hospital, yielded a phage preparation which lysed the same indicator strains as the phages obtained from strains of group A, but also lysed the indicator strain 26 (group C). The strains isolated at

TABLE 1. *Lysis of indicator strains by phages induced in Mec strains and lysogenic derivatives of strain RN 450*

Induced phage[a]	Indicator strain[b]						
	RN 450	PS6	PS54	PS83A	PS85	26	41
1975 Elm.[c]	L	L	R	R	L	R	R
1975/RN 450[d]	L	L	R	R	L	R	R
1991 Elm.[c]	L	L	R	R	L	R	R
1991/RN 450[d]	L	L	R	R	L	R	R
147 V.A.[c]	L	L±	R	R	L	R	R
147/RN 450[d]	L	L	R	R	L	R	R
148 V.A.[c]	L	L	R	R	L	R	R
148/RN 450[d]	L	L	R	R	L	R	R
2350 M.S.[c]	L	L	R	R	L	R	R
2350/RN 450[d]	L	L	R	R	L	R	R
2995 N.Y.H.[c]	L	L	R	R	L	R	R
2995/RN 450[d]	L	L	R	R	L	R	R
209 C.I.[c]	L	L	L	L	L	L	L
209/RN 450[d]	L	R	L	L	R	L	L
130 C.I.[c]	L	L	L	L	L	L	L
130/RN 450[d]	L	R	L	L	R	L	L
198 C.I.[c]	L	L	L	L	L	L	L
198/RN 450[d]	L	R	L	L	R	L	L
VIII Mont.[c]	L	L	R	R	L±	L	R
VIII/RN 450[d]	L	L	R	R	L	L	R
6B Hart.[c]	L	L	L	L	L	R	L
6B/RN 450[d]	L	L	L	L	L	R	L
3C Hart.[c]	L	L	L	L	L	L	L
3C/RN 450[d]	L	L	L	L	L	L	L

[a] Sources of strains: Elm., Elmhurst Hospital (M. Malowany); V.A., New York V. A. Hospital (J. Rahal, Jr.); M.S., Mount Sinai Hospital (E. Bottone); N.Y.H., New York Cornell Hospital (M. Houghton); C.I., Coney Island Hospital (J. Gladstone); Mont., Montefiore Hospital (J. Singer); Hart., Hartford Hospital, Hartford, Conn. (R. Bartlett).

[b] R, Resistant to the phage preparation. L, Confluent lysis at a phage dilution of 10^{-2} or higher by spotting with a 3-mm-diameter loop; L±, Isolates plaques at a phage dilution of 10^{-2}.

[c] Phage preparations obtained by the induction with mitomycin C of Mec strains.

[d] Phage preparations obtained by the induction of strain RN 450 lysogenized with phage originating from an Mec strain; the first figure indicates the origin of the phage.

Hartford Hospital appear to be more heterogeneous in the host range of their lyogenic phages.

In further experiments, Mec^r strains isolated at different hospitals were used as indicators with the phage preparations tested the same as those in Table 1. Phage preparations obtained by the induction of strains isolated at Elmhurst, New York V.A., Mount Sinai, and New York Hospitals (group A) did not lyse the Mec^r strains isolated at Coney Island Hospital, but lysed strain VIII isolated at Montefiore Hospital (Table 2). Phages obtained by the induction of strains 309, 130, and 198 isolated at Coney Island Hospital (group B) lysed the strains of group A and also strain VIII. All Mec^r strains tested were resistant to the phage induced in strain VIII (group C). The results obtained with phages obtained from other strains isolated at the above hospitals were similar to those summarized in Table 2. However, the strains isolated at Hartford Hospital yielded phage preparations with different host ranges.

There was less uniformity in each group in the host range of phages obtained by the lysogenization of strain RN 450. These differences could be due either to the presence of several phages in the original Mec^r strains or to restriction and modification phenomena.

Serological data. A preliminary serological investigation of 13 strains of type 92 isolated in New York City was undertaken through the courtesy of Jay Cohen at the Center for Disease Control, Atlanta, Ga. Strains isolated at Elmhurst, New York V.A., New York, and Mount Sinai Hospitals (group A) were agglutinated by sera a, b, and k, and some were also agglutinated by sera c_p and h. Strains isolated at Coney Island Hospital (group B) were not typable; strain VIII (group C) was of serotype c_1. These preliminary data appear consistent with the data on the host range of lysogenic phages carried by these strains.

Transduction of tetracycline and methicillin resistance. All Mec^r strains of type 92

TABLE 2. *Lysis of Mec strains by phages from Mec strains*[a]

Induced phage	Indicator strain												
	1975	1991	147	148	2350	2995	209	130	198	VIII	6B	3C	RN 450
1975 Elm.	R	R	R	R	R	R	R	R	R	L	R	R	L
1975/RN 450	R	R	R	R	L	R	R	R	R	L	R	L	L
1991 Elm.	R	R	R	R	R	R	R	R	R	L	R	R	L
1991/RN 450	R	R	R	R	L	R	R	R	R	L	R	L	L
147 V.A.	R	R	R	R	R	R	R	R	R	L	R	R	L
147/RN 450	R	R	R	R	R	R	R	R	R	L±	R	L	L
148 V.A.	R	R	R	R	R	R	R	R	R	L	R	R	L
148/RN 450	R	R	R	R	R	R	R	R	R	L±	R	L	L
2350 M.S.	R	R	R	R	R	R	R	R	R	L±	R	R	L
2350/RN 450	R	R	R	R	R	R	R	R	R	L±	R	R	L
2995 N.Y.H.	R	R	R	R	R	R	R	R	R	L±	R	R	L
2995/RN 450	R	R	R	R	R	R	R	R	R	L±	R	R	L
209 C.I.	L	L	L	L	L	L	R	R	R	L	R	R	L
209/RN 450	L	L	L	L	L	L	R	R	R	L	R	R	L
130 C.I.	L	L	L	L	L	L	R	R	R	L	R	R	L
130/RN 450	L	L	L	L	L	L	R	R	R	L	R	R	L
198 C.I.	L	L	L	L	L	L	R	R	R	L	R	R	L
198/RN 450	L	L	L	L	L	L	R	R	R	L	R	R	L
VIII Mont.	R	R	R	R	R	R	R	R	R	R	R	R	L
VIII/RN 450	R	R	R	R	R	R	R	R	R	R	R	R	L
6B Hart.	R	R	R	R	R	L±	R	R	R	L	R	L	L
6B/RN 450	L	L	L	L	L±	L	R	R	R	L	R	L	L
3C Hart.	R	R	R	R	L	L	L±	R	R	L	R	R	L
3C/RN 450	R	L	R	R	L	L	L±	R	R	L	R	R	L

[a] Nomenclature of the phage preparations and indicator strains is as indicated in Table 1. R, Resistant to the phage preparation. L, Confluent lysis at a phage dilution of 10^{-2} or higher by spotting with a 3-mm-diameter loop. L±, Isolates plagues at a phage dilution of 10^{-2}.

were tetracycline resistant and showed also a low degree of minocycline resistance (minimum inhibitory concentration, 1.56 to 6.2 μg/ml). The presence of minocycline resistance is indicative of the chromosomal location of the tetracycline resistance marker (22). Genetic transfer was attempted with phages induced in Mec^r strains and phage preparations obtained by propagation of phage 88 on Mec^r strains. The acceptor strain was strain 8325 P_{524}, used by Cohen and Sweeny (5) as an acceptor for methicillin resistance. We obtained a low rate of transduction of tetracycline resistance (2×10^{-8} to 4×10^{-8}/PFU) with preparations obtained by the propagation of phage 88 on strains 130 C.I., 206 C.I., and 180 Elm. We also obtained transduction of tetracycline resistance with phages obtained by the induction of strains 130 and 206. The transduction of tetracycline resistance was accompanied by the transduction of a low level of minocycline resistance, indicating the probable chromosomal location of the tetracycline resistance marker.

Prior experiments indicated that the acquisition of chromosomal tetracycline resistance is accompanied by loss of susceptibility to several typing phages such as 6, 47, or 75 and retention of susceptibility to phages 83A and especially 85 (21, 22). The determination of the phage susceptibility of tetracycline-resistant transductants obtained with transducing phages derived from the three Mec^r donor strains indicated that the transductants lost their susceptibility to international typing phages 6, 42E, 53, 54, 75, 83A, and 85, but retained susceptibility to experimental phages 89 and 92 and, to a lesser degree, to phages 88 and 90. Two transductants from strain 130 retained a low degree of susceptibility to phage 85. Testing at 100 × RTD concentration of the phages gave similar results as at the RTD concentration. It appears therefore that introduction of the chromosomal tetracycline marker from Mec^r strains of phage 92 into strain 8325 P_{524} resulted in a change of phage susceptibility, the transductants becoming similar, but not identical, to the donor Mec^r strains.

In a second series of experiments, both the original acceptor strain 8325 P_{524} and tetracycline-resistant transductants were used as acceptors for methicillin resistance. Transduction into the acceptor strain occurred at a very low rate, with negative results in some experiments. The Mec^r transductants retained the phage susceptibility of the acceptor strain 8825 P_{524}. The transduction experiments were repeated by using tetracycline-resistant transductants from strains 130 C.I. and 180 Elm. into strain 8325

P_{524} as the acceptor. The presence of the chromosomal tetracycline marker did not result in a detectable increase in the rate of transfer of methicillin resistance or in additional changes in phage susceptibility.

DISCUSSION

Since 1974, during a period of more than 3 years, Mec^r *S. aureus* strains of experimental phage type 92 apparently have been the predominant group of methicillin-resistant strains isolated in hospitals in New York City. However, strains of type 92 can be also found among methicillin-susceptible staphylococci, and most strains of type 92 tested in our laboratory possessed chromosomal tetracycline resistance. Mec^r strains of type 92 were also isolated in Connecticut and Minnesota, indicating a relatively wide geographical distribution of these strains. In all instances the strains of type 92 were the predominant type of Mec^r strain in each hospital.

In contrast to the majority of Mec^r *Staphylococcus* strains described in the literature (1, 24), with the exception of strain VIII, all Mec^r strains tested possess homogeneous methicillin resistance, resulting in similar zone sizes at 30 and 37°C. Strain VIII was also the only strain found to be lipase negative. Other common characteristics of Mec^r strains of type 92 are erythromycin resistance and chromosomal tetracycline resistance.

The strains of type 92 isolated in New York City were divided by the host range of their lysogenic phages into groups A, B, and C. These strains differ also in their susceptibility to chloramphenicol, clindamycin, and kanamycin. Preliminary serological data are consistent with this subdivision. At each of the hospitals involved, only strains belonging to a single subgroup were isolated. However, the strains isolated at Hartford Hospital appear to be a more heterogeneous group. The strains isolated at Ramsey-St. Paul Hospital differ from the other strains of type 92 by their amikacin resistance and lower minocycline resistance.

It was assumed for strains isolated in England (11) and Switzerland (7) that Mec^r strains of *S. aureus* isolated in these countries have derived from a single strain, with the differences in some of their characteristics resulting from selective pressure and genetic exchange with other *Staphylococci*. Some common characteristics of Mec^r strains of type 92, and especially the homogeneous nature of their methicillin resistance, could indicate a common origin of these strains, whereas other characteristics, such as differences in antigenic structure, could indicate a separate evolution.

An alternative possibility is based on our prior observations (22) that transfer of chromosomal tetracycline resistance into strain RN 450 renders the strain resistant to phages to which it was initially susceptible, such as typing phages 29, 80, 6, 47, 53, and 77, but does not affect susceptibility to phages 83A and especially 85. These transductants into strain RN 450 also retained susceptibility to phages 88, 89, and 92 (S. Schaefler, unpublished data). The transfer of chromosomal tetracycline resistance from strains of type 92 into strain RN 450 resulted in a more pronounced loss of susceptibility, the transductants becoming resistant to all international typing phages but retaining susceptibility to experimental phages 88, 89, and 92. It is, therefore, possible that strains of different origin acquired phage type 92 after transfer of chromosomal tetracycline resistance.

An evolution somewhat similar to that outlined above may have occurred in Denmark (4, 20), where changes in both antibiotic and phage susceptibility of a large number of cultures isolated mainly from blood cultures were investigated. The appearance of tetracycline and streptomycin resistance was accompanied by loss of susceptibility to most typing phages of group III, with the exception of the 83A/84/85 complex. A further evolution toward multiple resistance, including methicillin, was accompanied by the loss of susceptibility to phages of the 83A/84/85 complex and appearance of susceptibility to phage 89. We found that the propagating strain for phage 89 as well as other multiply resistant strains isolated in Denmark are minocycline resistant and therefore probably possess chromosomal tetracycline resistance.

The two hypotheses are not mutually exclusive, since the limitation of genetic transfer of methicillin resistance to a small number of strains may restrict the potential evolution of methicillin resistance to a very small number of evolutionary pedigrees.

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