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Recipient Characteristics in the Transduction of Methicillin Resistance in *Staphylococcus epidermidis*

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Methicillin resistance (Mec^r) was transduced into a methicillin-susceptible variant of the Mec^r donor *Staphylococcus epidermidis* BS10. UV irradiation of phage stimulated Mec^r transduction frequency. If loss of Cd and Hg ion resistance occurred in this recipient, or if the three markers Mec^r, Cd^r, and Hg^r were co-eliminated from the donor, neither strain acted as a recipient for Mec^r.

Methicillin resistance (Mec^r), found in more than 30% of clinically significant isolates of *Staphylococcus epidermidis* (15), presents therapeutic problems in the treatment of infection (12, 21). Resistance is often of the heterogeneous type, with about 1 in 10^5 cells expressing resistance at 37°C, while a majority express resistance at lower temperatures such as 30°C (18). Resistance is unstable under a number of conditions, particularly following storage of cultures at room temperature (2). Mec^r strains of *S. aureus* produce a new, low-affinity penicillin-binding protein (2′ or 2a) (9, 14, 17, 20), and changes in peptidoglycan synthesis occur (22). A penicillin-binding protein 2′ has been shown in Mec^r *S. epidermidis* (20). Transduction of Mec^r has not been described in *S. epidermidis*. In *S. aureus*, recipient effectiveness for Mec^r is associated with phage lysogeny and carriage of a penicillinase plasmid (6) or with beta-lactamase production (19). Although Mec^r in *S. aureus* is unstable (7, 8), *mec* has been mapped by transformation in the chromosome (10). A control locus for the expression of Mec^r has been identified at another chromosomal site in *S. aureus* (5).

Strain BS107 (Table 1), used as a donor in transduction, was derived from BS10, which produced a proportion of large colonies when grown on nutrient agar containing 4 μg of methicillin per ml. This large-colony form was retained during subculture on methicillin-containing medium. Strains BS10 and BS107 had similar MICs of about 512 μg/ml at 30°C, but BS10 showed reduced bacterial growth between 16 and 128 μg of methicillin per ml, a phenomenon known as optimal-zone activity (1), which was not shown by BS107. Heterogeneity of resistance was more marked in BS10 than in BS107 at 37°C. In other respects, the strains were very similar. Both acted as donors for Mec^r, although with either strain as the donor, transductants showed optimal-zone type resistance with MICs of about 512 μg/ml at 30°C. About 1 to 2% of colonies of BS10, screened after storage at room temperature in nutrient broth or on nutrient agar, were Mec^s (e.g., BS102), and about half of these Mec^s colonies had also lost resistance to Cd and mercuric Hg ions (e.g., BS105). Under similar conditions, about 1% of colonies of BS102 showed loss of Cd and Hg resistance (BS103 and BS104). Loss of tetracycline resistance (Tc^r) or chloramphenicol resistance (Cm^r) occurred spontaneously or after growth at 43°C. Reverse mutation to Mec^r was not detected in Mec^s cultures when more than 10^{10} colonies were screened. Transduction was based on the method previously described (13) with phage input ratios of 0.1 to 0.5. Tryptic soy broth (Difco Laboratories) was used for broth cultures solidified with 1% (wt/vol) agar (Oxoid no. 1) (TSA). For selection of transductants, 0.5-ml volumes of the transduction mixture were mixed with 3 ml of tryptic soy broth containing 0.3% (wt/vol) agar at 46°C and overlaid onto TSA plates containing 4 μg of methicillin per ml and 5 mM trisodium citrate. Disk susceptibility tests and strain identification were previously described (13).

Donor strains BS10 (13) and BS107 can transduce plasmid and chromosomal resistance genes to AS15, but Mec^r is not transduced. However, with strain BS102 as the recipient, methicillin resistance was transduced. BS102 differs from the donor only in its loss of methicillin resistance and selection of rifampin resistance as an additional marker. With φ108 (kindly provided by C. P. A. van Boven) grown on BS107 (φ108/BS107) as the donor, transduction frequencies of 3.3×10^{-8} to 6.2×10^{-9}/PFU were obtained. Similar results occurred with BS10 as the donor. Phage 155 (provided by the Staphylococcal Reference Unit, Colindale, London, England) also transduced Mec^r in this system. With UV doses of 0 to 8 min, maximum transduction of 10^{-6} to 10^{-7}/PFU occurred at 3 to 4 min of UV irradiation, corresponding to about 10% phage survival. This method is used to distinguish between plasmid and chromosomal genes, as chromosomal, but not plasmid, markers show enhanced transduction frequencies (3). This effect has been shown in *S. epidermidis* (16), indicating the presence of homology between the Mec-transducing DNA fragment and the recipient and suggesting that Mec^r may be chromosomal in the transductant. Transformation was excluded by treatment of phage for 10 min before transduction with 50 μg of DNase I (grade II; Boehringer Mannheim) in a final concentration of 50 mM magnesium chloride ($MgCl_2 \cdot 6H_2O$) (11). Mec^r transduction frequencies were 3.3×10^{-6} with treated phage and 3.8×10^{-6} with untreated phage.

As the ineffective recipient AS15 is nonlysogenic and penicillin susceptible, we tested the effect of these factors by lysogenization of AS15 (AS151) with phage derived from a filtered culture supernatant of BS10 followed by introduction of Pc^r into AS151 with phage φ108/BS10 as the donor. However, no Mec^r transductants were recovered ($<10^{-10}$/PFU) with either of two lysogenic Pc^r recipient strains, AS213, in which Pc^r is linked to phage modification and restriction, or AS214, which is Pc^r alone (13). The host range of the supernatant phage was similar for BS10 and the lysogenized clone AS151. Quantitative differences could be

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TABLE 1. Strains of *S. epidermidis*

Strain	Phenotype and derivation[a]
BS10	Pc Tc Cm Sm Mec Cd Hg ly$^+$
BS107	Culture derived from a large colony obtained when BS10 was grown on agar containing 4 μg of methicillin per ml.
BS102	Mec^s loss mutant of BS10 following storage at room temperature. Rifampin resistance selected.
BS103	Cd-, Hg-, and Tc-sensitive variant of BS102.
BS104	Cd- and Hg-sensitive variant of BS102.
BS105	Loss of Mec, Cd, and Hg from strain BS10 following storage at room temperature. Novobiocin resistance selected.
BS106	Same Mec^s Cd^s Hg^s strain as BS105. Novobiocin resistance selected. Tc-sensitive variant.
AS15	Nm ly$^-$
AS151	AS15 lysogenized with phage from BS10.
AS213	Pc^r transductant of strain AS151 from φ108 grown on BS10 as the donor. The transductant was also Mod$^+$ Res$^+$ for phage 82 (provided by C. P. A. van Boven).
AS214	Pc^r transductant of strain AS151 from φ108 grown on BS10 as the donor. The transductant remained Mod$^-$ Res$^-$ for phage 82.

[a] Resistance to: benzylpenicillin (Pc), chloramphenicol (Cm), methicillin (Mec), neomycin (Nm), streptomycin (Sm), tetracycline (Tc), cadmium (Cd) or mercuric (Hg) ions, lysogeny (ly), phage modification (Mod), and restriction (Res).

accounted for by the Mod$^+$ Res$^+$ character of BS10 and suggests that BS10 carries one phage.

No role has been found in Mec transduction for the lysogenic phage or the plasmid markers Tc^r and Cm^r. Loss of detectable phage following UV irradiation (4) in both donor (BS107) and recipient (BS102) followed by independent loss of Tc^r and Cm^r plasmids had no effect on Mec transduction frequencies. As loss of penicillinase production in BS10 and its derivatives has not been detected, we took an alternative approach using the beta-lactamase inhibitor clavulanic acid. Stewart and Rosenblum (19) used this method to demonstrate a role for beta-lactamase in recipient effectiveness in *S. aureus*. With BS102 as the recipient, clavulanic acid was included in the methicillin agar for transductant recovery. The numbers of Mec^r transductants recovered were 409, 273, 202, and 158 at 0, 1.0, 2.0, and 4.0 μg of clavulanic acid per ml, respectively. Similar results were obtained in repeat experiments, with some reduction in numbers occurring at 0.5 μg/ml. In a reconstruction experiment, an established Mec^r strain (BS10) was not inhibited at these concentrations, although inhibition occurred at >4 μg/ml. With viable counts, both the donor (BS10) and recipient (BS102) were recovered on 4 μg/ml and lower concentrations of clavulanic acid, although colony size for BS102 was reduced at 4 μg/ml. For comparison, the effect of clavulanic acid on transduction of chloramphenicol resistance (Cm^r) was tested with φ108/BS107 and BS102 Cm^s Tc^s as the recipient. No effect on Cm^r transduction occurred at 2 μg of clavulanic acid per ml or less, but at 4 μg/ml the numbers recovered were reduced by 33% from 780 to 525. These results suggest that reduced numbers of Mec^r transductants at ≤2 μg of clavulanic acid per ml are unlikely to be due to inhibition of growth of the recipient strains. If 3 to 4 h of growth was allowed before selection for Mec^r, the number of transductants doubled, and the effect of clavulanic acid on transductant numbers was lost. The presence of clavulanic acid (2 μg/ml) in the medium during phenotypic expression produced the same result, suggesting, overall, that beta-lactamase plays a minor role in recovery of newly formed transductants.

As several ineffective recipient strains for Mec^r were Cd and Hg susceptible, we tested a number of Mec-susceptible strains which had also lost Cd^r and Hg^r. With BS105 as the recipient, no Mec^r transduction was achieved with frequencies $<4.8 \times 10^{-9}$ to $<9.4 \times 10^{-10}$. In one experiment, the recipient was also tetracycline susceptible. Similarly, BS103, which is tetracycline susceptible, and BS104 did not act as recipients for Mec^r at frequencies of $<2.5 \times 10^{-10}$ and $<3.8 \times 10^{-10}$. Attempts to transduce Cd^r into several of these nonrecipient strains were unsuccessful. With φ108/BS107 as the donor and selection by overlay with 10 μg of cadmium sulfate ($3CdSO_4 \cdot 8H_2O$) per ml, no Cd^r transductants were recovered with Cd^s strains AS15, AS213, or AS214 or Cd^s loss variants BS103 or BS106 as recipients. Conditions included UV-irradiated phage for 0 or 3 min and growth for phenotypic expression of 0 or 24 h. Transduction frequencies were $<6.6 \times 10^{-9}$ to $<3.8 \times 10^{-10}$. Strains BS103 and BS106 are effective recipients for the Tc^r plasmid. AS15 and derivatives are recipient to a range of plasmid and chromosomal markers (13; unpublished observations). Transformation is an alternative method of transfer to consider if the reason for lack of transductants is the size of the Cd-associated locus.

To explain the results obtained, in which strains which had lost Mec^r alone, but not Mec^r, Cd^r, and Hg^r, acted as recipients, it was proposed that two loci required for the Mec^r phenotype occur in BS10, both of which are unstable, and one of which is linked to Cd and Hg resistance. To test this possibility, we used strains BS102 (Mec^s) and BS105 or BS106 (Mec^s Cd^s Hg^s) alternately as the donor and recipient in transduction crosses. Neither combination produced Mec recombinants at frequencies $<10^{-10}$/PFU, which does not support the retention of a complementary Mec locus in both types of strains which have lost the Mec phenotype. An alternative explanation is that loss of Cd and Hg with Mec^r is associated with loss of the entire Mec locus, while loss of Mec^r alone results in retention of a locus required for reintroduction of Mec^r by transduction.

LITERATURE CITED

1. **Annear, D. I.** 1972. An optimal zone of methicillin activity with *Staphylococcus epidermidis*. Med. J. Aust. **59**:413–414.
2. **Annear, D. I., and W. B. Grubb.** 1973. Unstable resistance to methicillin in *Staphylococcus epidermidis*. J. Med. Microbiol. **6**:393–398.
3. **Arber, W.** 1960. Transduction of chromosomal genes and episomes in *Escherichia coli*. Virology **11**:273–288.
4. **Asheshov, E. H., and J. E. Rippon.** 1959. Changes in typing pattern of phage-type 80 staphylococci. J. Gen. Microbiol. **20**:634–643.
5. **Berger-Bächi, B.** 1983. Insertional inactivation of staphylococcal methicillin resistance by Tn*551*. J. Bacteriol. **154**:479–487.
6. **Cohen, S., and H. M. Sweeney.** 1973. Effect of the prophage and penicillinase plasmid of the recipient strain upon the transduction and the stability of methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. **116**:803–811.
7. **Dornbusch, K., H. O. Hallander, and F. Löfquist.** 1969. Extrachromosomal control of methicillin resistance and toxin production in *Staphylococcus aureus*. J. Bacteriol. **98**:351–358.
8. **Grubb, W. B., and D. I. Annear.** 1972. Spontaneous loss of methicillin resistance in *Staphylococcus aureus* at room temperature. Lancet **ii**:1257.
9. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphy-*

lococcus aureus. J. Bacteriol. **158**:513–516.
10. **Kuhl, S. A., P. A. Pattee, and J. N. Baldwin.** 1978. Chromosomal map location of the methicillin resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **135**:460–465.
11. **Lindberg, M., J. E. Sjöström, and T. Johansson.** 1972. Transformation of chromosomal and plasmid characters in *Staphylococcus aureus*. J. Bacteriol. **109**:844–847.
12. **Lowy, F. D., and S. M. Hammer.** 1983. *Staphylococcus epidermidis* infections. Ann. Intern. Med. **99**:834–859.
13. **Poston, S. M., and T. J. Palmer.** 1977. Transduction of penicillinase production and other antibiotic-resistance markers in *Staphylococcus epidermidis*. J. Gen. Microbiol. **103**:235–242.
14. **Reynolds, P. E., and D. F. J. Brown.** 1985. Penicillin-binding proteins of β-lactam-resistant strains of *Staphylococcus aureus*: effect of growth conditions. FEBS Lett. **192**:28–32.
15. **Richardson, J. F., and R. R. Marples.** 1982. Changing resistance to antimicrobial drugs, and resistance typing in clinically significant strains of *Staphylococcus epidermidis*. J. Med. Microbiol. **15**:475–484.
16. **Rosendorf, L. L., and F. H. Kayser.** 1974. Transduction and plasmid deoxyribonucleic acid analysis in a multiply antibiotic-resistant strain of *Staphylococcus epidermidis*. J. Bacteriol. **120**:679–686.
17. **Rossi, L., E. Tonin, Y. R. Cheng, and R. Fontana.** 1985. Regulation of penicillin-binding protein activity: description of a methicillin-inducible penicillin-binding-protein in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **27**:828–831.
18. **Sabath, L. D.** 1977. Chemical and physical factors influencing methicillin resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis*. J. Antimicrob. Chemother. **3**(Suppl. C):47–51.
19. **Stewart, G. C., and E. D. Rosenblum.** 1980. Transduction of methicillin resistance in *Staphylococcus aureus*: recipient effectiveness and beta-lactamase production. Antimicrob. Agents Chemother. **18**:424–432.
20. **Ubukata, K., N. Yamashita, and M. Konno.** 1985. Occurrence of a β-lactam-inducible penicillin-binding protein in methicillin-resistant *Staphylococci*. Antimicrob. Agents Chemother. **27**:851–857.
21. **Wade, J. C., S. C. Schimpff, K. A. Newman, and P. H. Wiernik.** 1982. *Staphylococcus epidermidis*. An increasing cause of infection in patients with granulocytopaenia. Ann. Intern. Med. **97**:503–508.
22. **Wyke, A. W., J. B. Ward, and M. V. Hayes.** 1982. Synthesis of peptidoglycan in vivo in methicillin-resistant *Staphylococcus aureus*. Eur. J. Biochem. **127**:553–558.