ATTEMPTED INTERSPECIFIC TRANSDUCTION OF ANTIBIOTIC RESISTANCE IN STAPHYLOCOCCI

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THE coagulase-negative staphylococci isolated from patients in hospital are commonly resistant to antibiotics (e.g., Corse and Williams, 1968), and it was of some interest to see whether resistance-determining factors could be transferred from such strains to *Staphylococcus aureus*. This paper reports a number of unsuccessful attempts.

METHODS

Phages were propagated by the methods described by Dean *et al.* (1973), and the number of plaque-forming units (pfu) was determined after spotting 0.01 cm³ of 10-fold dilutions on to the phage-typing agar plates (prepared for us by Oxoid Ltd) previously seeded with a 6-hour culture of the propagating strain.

Bacterial counts were determined from optical density readings with the help of standard curves.

In all attempted transduction experiments, cocci were used at a count estimated to be 2×10^7 colony-forming units per cm³ and phages at 1.4×10^7 pfu per cm³, giving a multiplicity of infection of 0.7.

Despite a very large number of attempts it did not prove possible to find more than very few phages that lyse both coagulase-positive and coagulase-negative staphylococci. Trials were made (1) with the polyvalent K phage (no. NCTC7814; Krueger, 1932); (2) by the mixed culture technique described by Lacey (1972); (3) by the use of culture supernates, as described by Yu and Baldwin (1970), and (4) with phage 155 from our typing set (Dean *et al.*, 1973) which lyses a staphylococcus no. M6499 (Mortensen, 1969) that is also sensitive to phages active on coagulase-positive staphylococci.

RESULTS

Phage K was propagated on two *S. epidermidis* strains (nos. 304 and 885) that were resistant to tetracycline 10 µg per ml. A portion (1.0-ml) of each phage filtrate was incubated with an equal volume of a 18-hour broth culture of each of four strains of *S. aureus*, all sensitive to tetracycline, in the presence of 0.04% calcium chloride, for 30 min. at 37°C. The mixture was centrifuged, and the deposit suspended in 2 ml of nutrient broth containing 0.02M sodium citrate and incubated for 2 hours at 37°C. The mixture was again centrifuged and the deposit suspended in 2.0 ml of nutrient broth; 0.1-ml volumes were seeded on to nutrient-agar plates containing tetracycline 10 µg per ml and incubated for 48 hours at 37°C. No tetracycline-resistant colonies developed.

We used the same method in an attempt to transduce streptomycin resistance from a strain of *S. aureus* to one of *S. epidermidis*, employing a preparation of the K phage grown on streptomycin-resistant *S. aureus*. No resistant *S. epidermidis* colonies developed.

Subsequent attempts at intraspecific transduction with the K phage were also unsuccessful.

The same tetracycline-resistant *S. epidermidis* strains and streptomycin-resistant mutants of the tetracycline-sensitive *S. aureus* strains were grown together in nutrient broth (Lacey, 1972) for 18 hours and then subcultured on to nutrient agar containing tetracycline 10 µg per ml and streptomycin 10 µg per ml and incubated for 48 hours. No colonies developed.

Next we used two novobiocin-resistant, coagulase-negative strains (nos. A111 and A115) of the biotype referred to as "*S.a*" in our previous paper (Dean *et al.* 1973); the cocci were

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incubated at 37°C for 4 hours in broth containing mitomycin C 0.5 µg per ml. The broth was spun and the supernate spotted on the nutrient agar previously seeded with strains (nos. 13596 and 14077) of *S. aureus* that were novobiocin sensitive and lysed by the supernate from the mitomycin broth. Following the transduction methods described by Yu and Baldwin (1970), we incubated the recipient strains together with the lytic supernates. The mixtures were centrifuged and the deposits subcultured to nutrient agar containing novobiocin 0.9 µg per ml, and incubated overnight at 30°C. The results of a typical experiment are shown in the table; there was a very high frequency of spontaneous mutation to novobiocin resistance and no evidence of transduced resistance.

TABLE
Attempted transfer of novobiocin resistance by lytic supernates of coagulase-negative staphylococci to Staphylococcus aureus

"Recipient" strain: <i>S. aureus</i> number	Supernate of "donor" culture of coagulase-negative staphylococcus number	Number of novobiocin-resistant mutants (cfu per ml)
13596	...*	890
13596	A111	880
13596	A115	890
14077	...	190
14077	A111	70
14077	A115	70

* Control: lytic supernate not added.

Lastly we used phage 155 from our typing set grown on each of several coagulase-negative strains that were resistant to various antibiotics. These phage preparations were used in attempts to transduce resistance into the strain M6499 (Mortensen, 1969) which is sensitive to these *S. epidermidis* phages and, although coagulase-negative, has biochemical characters (including deoxyribonuclease production) that indicate that it is closely related to *S. aureus*. No resistant colonies were obtained. Phage 155 had previously been shown (Dean, unpublished) to be capable of transducing tetracycline resistance at 10 µg per ml between coagulase-negative staphylococci.

SUMMARY

Various methods were used in attempts to transduce antibiotic resistance from coagulase-negative to coagulase-positive staphylococci, but none was successful.

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