Comparison of phage-mediated and conjugative transfer of staphylococcal plasmids *in vitro* and *in vivo*

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Summary. A strain of *Staphylococcus aureus* was constructed with which to compare transfer of resistance plasmids by the mechanisms of phage-mediated conjugation and conjugation. Transfer by each mechanism could be distinguished by the patterns of resistances transferred. Conjugation was favoured on dry absorbent surfaces, e.g., human skin, tissue and surgical gauze, whereas phage-mediated conjugation was favoured in fluids, e.g., milk and urine. The degree of hydration of the mating cells is postulated as one factor determining whether plasmids are transferred by phage-mediated conjugation or conjugation. Preliminary evidence indicates that topical creams and ointments affect the conjugative transfer of plasmids.

Introduction

The propensity of staphylococci to develop antibiotic resistance is thought to result from the acquisition of antibiotic-resistance determinants often borne on plasmids (Lacey, 1975). This raises questions regarding the mechanism of transfer and origin of the resistance determinants. Experiments *in vitro* with mixed-broth cultures have suggested that phage-mediated conjugation is a likely mechanism of transfer (Lacey, 1980*a* and 1984; Fouace, 1981; Witte, 1981). Recent evidence suggests that aminoglycoside-resistance plasmids may be transferred on the surface of membrane filters by a phage-independent method requiring close cell-to-cell contact. This mechanism has been termed conjugation (Archer and Johnston, 1983; Forbes and Schaberg, 1983; McDonnell *et al.*, 1983; Townsend *et al.*, 1985*b*). There being no formal proof of the absence of a phage genome, the two mechanisms have been regarded as possibly related manifestations of the same mechanism that should perhaps be referred to simply as conjugation (Lacey *et al.*, 1984). However, comparison of the transfer of conjugative and non-conjugative plasmids has not demonstrated the presence of a phage or phage genome that might explain the transfer of conjugative plasmids (Townsend *et al.*, 1985*a*).

Experiments *in vivo* have shown that resistance determinants can be transferred at high frequency between staphylococci in urine, serum and on the

surface of skin (Lacey, 1971; Naidoo and Noble, 1978; Jaffe *et al.*, 1980; Lacey and Lord, 1980; Naidoo, 1984). Little is known about the mechanisms of transfer operating in these conditions. One study has provided strong evidence for a conjugative mechanism occurring on human skin whereby non-conjugative plasmids were transferred by a process resembling mobilisation by a conjugative plasmid (Naidoo, 1984).

A previous study of the transfer of aminoglycoside-resistance plasmids in mixed cultures in the presence of different concentrations of polyethylene glycol (PEG) has demonstrated that conditions favouring transfer by a phage-mediated mechanism inhibited the transfer of plasmids by a phage-independent, conjugative mechanism and *vice versa* (Townsend *et al.*, 1985*b*). This paper extends this observation to a variety of conditions *in vitro* and *in vivo*.

Materials and methods

Plasmids and properties of S. aureus strains

Strain RN450 was plasmid-free, non-lysogenic (Novick and Bouanchaud, 1971) and sensitive to all antibacterial agents used. Donor strain WG4483 was constructed by successive transductions (Townsend *et al.*, 1985*b*) of plasmids pWG115, pWG3 and pE194 and PEG-mediated transfer of plasmid pWG615 to strain RN450; recipient strain WG2432 was RN450 with chromosomal mutations to novobiocin and rifampicin resistance; and strain WG3358 was strain WG2432 lysogenic for bacteriophage J (Townsend *et al.*, 1984). Plasmid

pWG615 (mol. wt $c. 28 \times 10^6$) was conjugative and encodes resistance to gentamicin, kanamycin, neomycin (Townsend *et al.*, 1985b) and ethidium bromide (Emslie *et al.*, 1985). Plasmid pWG115 (mol. wt 14.6×10^6) encoding resistance to trimethoprim, propamidine isethionate and ethidium bromide (Townsend *et al.*, 1984; Emslie *et al.*, 1985), plasmid pWG3 (mol. wt 2.7×10^6) encoding resistance to tetracycline (Townsend *et al.*, 1985b) and plasmid pE194 (mol. wt 2.3×10^6) encoding resistance to erythromycin (Iordanescu and Surdeanu, 1980) were non-conjugative plasmids.

Media and reagents

These have been described previously (Townsend *et al.*, 1985b). Ethidium bromide was purchased from Sigma. Macrogol Ointment (B.P.) and Cetomacrogol Cream Aqueous (A.P.F.) were prepared without preservatives.

Susceptibility testing

The methods employed were described by Townsend *et al.* (1985b). Resistance to ethidium bromide was tested on plates of Brain Heart Infusion Agar (BHIA) containing ethidium bromide 30 mg/L (Emslie *et al.*, 1985). Strain RN450 was an ethidium bromide-sensitive control strain.

Detection of lysogenic bacteriophages

This was determined after induction with mitomycin C or ultraviolet light (Townsend *et al.*, 1985b).

Transfer experiments

The methods followed those previously described by Townsend *et al.* (1985b). The donor strain WG4483 was grown for 18 h in Trypticase Soy Broth (TSB) containing propamidine isethionate (100 mg/L) and the recipient strain was grown for 18 h in TSB. For transfers in liquid media, 50 μ l of donor and recipient cultures were added to 5 ml of pooled human serum, pooled human urine (midstream), bovine milk, human saliva, surgical drainage fluid (known to be free of antibiotics), Brain Heart Infusion Broth (BHIB) or BHIB containing 0.01M CaCl_2 , 0.1M sodium citrate or various concentrations of PEG. The mixed cultures were shaken gently for 18 h at 37°C, washed in physiological saline and 100 μ l spread on to the surface of appropriate selective media. Separate control cultures of donor and recipient strains were treated in parallel.

For transfers on solid media, recipient culture (100 μ l) was spread on to pre-dried agar and allowed to dry before 100 μ l of donor culture were spread on to the agar. The solid medium was BHIA or BHIA containing 0.01M CaCl_2 or 0.1M sodium citrate. To determine the effect of different materials on transfer, after inoculation, one of

the following was placed on the surface of the agar: sterile lens tissue (Whatman; 30 mm square), sterile, 4-ply, surgical gauze (Nufold Gauze, Johnson and Johnson Pty Ltd, Sydney, Australia; 30 mm square), sterile surgical thread (black-braided, non-capillary, gauge 4, manufactured by Davis and Geck and supplied by Anax, Perth, Western Australia; 300 mm), teflon tape (PTFE tape, Eureka, Australia; 30 $\times$ 12 mm), a 0.45- μ m plain, white membrane filter (Millipore; 45-mm diameter) or human skin. For skin-transfer experiments *in vitro*, skin shavings collected from the dorsal and plantar surfaces of the feet of podiatry patients were stored desiccated.

In some experiments, skin was moistened by storage for 24 h in five times its weight of physiological saline. After incubation for 18 h at 37°C, growth was harvested with a glass rod and suspended in saline (10 ml), or the skin or article was vortexed in saline (10 ml). Bacteria were washed with saline, resuspended in saline (10 ml) and 100 μ l volumes inoculated on to appropriate selective media.

To test the effect of topical preparations on resistance transfer, Macrogol Ointment and Cetomacrogol Cream were spread thinly over half of a piece of tissue and the tissue placed on BHIA inoculated with mixed cultures. After incubation for 18 h, the tissue was removed and growth on the plate velvetreen-replicated to gentamicin-containing selective agar.

Skin-transfer experiments *in vivo* followed the method of Naidoo and Noble (1978). Permission for these experiments was obtained from the Human Ethics Committee of the Western Australian Institute of Technology.

Transfer was always controlled by parallel treatment of separate donor and recipient cultures and confirmed by comparison of the plasmid profile and antibacterial resistance of the transcient with those of donor and recipient strains. For experiments in which the transfer medium could not be sterilised, representative transcipients were checked for gram reaction, and catalase and coagulase production.

Selection of transcipients and transfer frequency

The selective medium (BHIA) contained novobiocin 5 mg/L and rifampicin 25 mg/L and one of the following: gentamicin 8 mg/L; tetracycline 5 mg/L; propamidine isethionate 100 mg/L; or erythromycin 5 mg/L.

Transfer frequency was calculated as the ratio of the number of transcipients to the number of donor cells after incubation of the mixture of donor and recipient cultures. Donor bacteria were enumerated on BHIA containing gentamicin 2 mg/L. Each frequency recorded was the average of two determinations. Co-transfer frequencies were determined after replication of at least 100 transcient colonies to other antibiotic-containing media.

Plasmid analysis

The methods for isolation of plasmid DNA and agarose-gel electrophoresis were described by Townsend *et al.* (1984).

Results

Properties of test strain WG4483

Strain WG4483 was analysed for plasmid DNA and four plasmid bands detected on agarose gels had mol. wts expected for plasmids pWG615, pWG115, pWG3 and pE194. There was no evidence for recombination between the plasmids. As expected, plasmid pWG115 was not maintained stably (Townsend *et al.*, 1984); consequently strain

WG4483 was always cultured in the presence of propamidine before transfer experiments. There was no evidence for incompatibility between these plasmids. Lysogenic phages were not detected after induction of strain WG4483 with mitomycin-C or ultraviolet light.

Phage-mediated plasmid transfer

Transfer of resistances between donor strain

Table. Transfer of resistance from strain WG4483 in different cultural conditions

Experiment no.	Transfer conditions	Recipient strain	Resistance selected	Frequency of transfer	Co-transfer of resistance: frequency (%)
1	BHIB + Ca ⁺⁺	WG3358	Gm	1 × 10 ⁻⁵	Pi: ND, Tc: ND, Em: ND
			Pi	2 × 10 ⁻⁶	Gm: ND, Tc: ND, Em: ND
			Tc	4 × 10 ⁻⁵	Gm: ND, Pi: ND, Em: 3
			Em	3 × 10 ⁻⁵	Gm: ND, Pi: ND, Tc: 1
2	BHIB + 40% PEG	WG2432	Gm	4 × 10 ⁻⁴	Pi: ND, Tc: 10, Em: 8
			Pi	1 × 10 ⁻⁷	Gm: 100, Tc: ND, Em: ND
			Tc	3 × 10 ⁻⁵	Gm: 79, Pi: ND, Em: 2
			Em	5 × 10 ⁻⁵	Gm: 74, Pi: ND, Tc: 8
3	Milk	WG3358	Gm	1 × 10 ⁻⁴	Pi: ND, Tc: ND, Em: ND
			Pi	1 × 10 ⁻⁵	Gm: ND, Tc: ND, Em: ND
			Tc	8 × 10 ⁻⁵	Gm: ND, Pi: ND, Em: 4
			Em	5 × 10 ⁻⁴	Gm: ND, Pi: ND, Tc: 1
4	Urine	WG3358	Gm	2 × 10 ⁻⁴	Pi: ND, Tc: ND, Em: ND
			Pi	3 × 10 ⁻⁵	Gm: ND, Tc: ND, Em: ND
			Tc	2 × 10 ⁻⁴	Gm: ND, Pi: ND, Em: 3
			Em	3 × 10 ⁻⁴	Gm: ND, Pi: ND, Tc: 1
5	BHIA + Ca ⁺⁺	WG3358	Gm	5 × 10 ⁻⁷	Pi: ND, Tc: ND, Em: ND
			Pi	1 × 10 ⁻⁷	Gm: ND, Tc: ND, Em: ND
			Tc	2 × 10 ⁻⁶	Gm: ND, Pi: ND, Em: ND
			Em	1 × 10 ⁻⁶	Gm: ND, Pi: ND, Tc: 1
6	BHIA + membrane filter	WG3358	Gm	5 × 10 ⁻⁷	Pi: ND, Tc: 2, Em: 1
			Pi	ND	—
			Tc	8 × 10 ⁻⁹	Gm: 91, Pi: ND, Em: 8
			Em	6 × 10 ⁻⁹	Gm: 87, Pi: ND, Tc: 11
7	BHIA + tissue	WG3358	Gm	5 × 10 ⁻⁶	Pi: ND, Tc: 2, Em: 1
			Pi	ND	—
			Tc	8 × 10 ⁻⁸	Gm: 93, Pi: ND, Em: 1
			Em	5 × 10 ⁻⁸	Gm: 90, Pi: ND, Tc: 5
8	BHIA + surgical gauze	WG3358	Gm	6 × 10 ⁻⁶	Pi: ND, Tc: 2, Em: 2
			Pi	ND	—
			Tc	9 × 10 ⁻⁸	Gm: 85, Pi: ND, Em: 2
			Em	8 × 10 ⁻⁸	Gm: 88, Pi: ND, Tc: 5
9	BHIA + surgical thread	WG3358	Gm	9 × 10 ⁻⁶	Pi: ND, Tc: 1, Em: 1
			Pi	ND	—
			Tc	1 × 10 ⁻⁷	Gm: 86, Pi: ND, Em: 1
			Em	7 × 10 ⁻⁸	Gm: 83, Pi: ND, Tc: 4
10	BHIA + dry skin	WG3358	Gm	3 × 10 ⁻⁵	Pi: ND, Tc: 2, Em: 1
			Pi	2 × 10 ⁻⁸	Gm: 100, Tc: ND, Em: ND
			Tc	2 × 10 ⁻⁶	Gm: 82, Pi: ND, Em: 1
			Em	2 × 10 ⁻⁶	Gm: 81, Pi: ND, Tc: 3
11	Human skin <i>in vivo</i>	WG3358	Gm	4 × 10 ⁻⁵	Pi: ND, Tc: 10, Em: 2
			Pi	5 × 10 ⁻⁹	Gm: 100, Tc: ND, Em: ND
			Tc	1 × 10 ⁻⁵	Gm: 63, Pi: ND, Em: 1
			Em	1 × 10 ⁻⁵	Gm: 80, Pi: ND, Tc: 3

Gm = gentamicin; Pi = propamidine isethionate; Tc = tetracycline; Em = erythromycin; ND = not detected.

WG4483 and recipient strain WG3358 was attempted in BHIB with Ca^{++} . Gentamicin, propamidine, tetracycline or erythromycin resistances were transferred (table). Both the resistance patterns themselves and the plasmid analysis of representative transcipts suggested that individual plasmids had been transferred. Tetracycline and erythromycin resistances were co-transferred occasionally and two plasmids with mol. wts corresponding to those of pWG3 and pE194, respectively, were detected in transcipts. There was no transfer of these resistances alone or together in BHIB without Ca^{++} or with citrate, nor in tests in which the non-lysogenic recipient strain WG2432 was substituted for strain WG3358.

PEG-mediated, conjugative plasmid transfer

In transfer experiments with the donor strain and the non-lysogenic strain WG2432 in BHIB with PEG, gentamicin resistance (along with resistances to kanamycin, neomycin and ethidium bromide, and a plasmid of mol. wt 28×10^6) appeared at the highest frequency and was transferred often with tetracycline or erythromycin resistances (table). Transcipts with tetracycline or erythromycin resistances were detected at lower frequencies; 74–79% of them carried the conjugative plasmid pWG615 (table). Some 3–6% of tetracycline- or erythromycin-resistant transcipts carried resistances of all three plasmids. Plasmid analysis of these transcipts revealed the presence of a single plasmid when only tetracycline or erythromycin resistance had been transferred and an additional plasmid of mol. wt 28×10^6 when gentamicin resistance was co-transferred. Transfer of propamidine resistance was not detected initially; low frequencies of transfer were detected, however, after strain WG4483 was stored routinely on agar slopes for several weeks and subcultured in the presence of propamidine to force maintenance of plasmid pWG115. The majority (98%) of these transcipts were resistant to gentamicin, kanamycin, neomycin, propamidine, ethidium bromide and trimethoprim; an occasional colony was resistant to gentamicin, kanamycin, propamidine and ethidium bromide but sensitive to neomycin and trimethoprim. Plasmid analysis of these propamidine-resistant transcipts revealed a single plasmid species, the mol. wt of which was greater than that of plasmid pWG115. These were the only examples in which the co-transfer of resistance determinants appeared to result from recombination between plasmids (table).

Effect of varying transfer conditions between those that favour phage-mediated and PEG-mediated conjugation

An experiment was designed to investigate the effect of varying the transfer conditions between those favouring phage-mediated conjugation and conjugation. This was done by mating strain WG4483 with strain WG3358 in BHIB with Ca^{++} and increasing the concentrations of PEG. Transfer and co-transfer of all resistances was determined at each concentration of PEG. The results are presented in fig. 1. As the concentration of PEG was increased from 0 to 8%, the transfer of all resistances was stimulated and the pattern of resistances transferred indicated that this was attributable to phage-mediated conjugation. At concentrations of PEG between 8 and 12%, nearly all transfer was abolished. From 20 to 40% PEG, the transfer of gentamicin resistance markedly increased and was followed by gradual increases in the transfer frequencies of tetracycline and erythromycin resistances. The pattern of resistances transferred was indicative of conjugation.

Fig. 1. Effect of PEG on the growth, turbidity and resistance-transfer properties of strain WG4483. Turbidity of washed, Δ , and unwashed, $\bullet$, bacteria. Transfer frequency of: gentamicin resistance, $\blacksquare$; tetracycline resistance, $\circ$; erythromycin resistance, $\triangle$; propamidine resistance, $\blacktriangle$; cotransfer frequency of gentamicin resistance among tetracycline-resistant transcipts, $\bullet$.

The turbidity of the control and test cultures was measured before and after the cells had been washed in saline. There was a sharp decline in turbidity of unwashed cells at 24% PEG but, after washing, all cultures showed high turbidity with a slight decrease as the PEG concentration increased. Donor viable counts were in the range $(5-10) \times 10^8$ cfu/ml (fig. 1). Phase-contrast microscopy of the unwashed cultures revealed significant aggregation of the cells at PEG concentrations of $\geq 24\%$. The changes in turbidity and cellular aggregation were detected easily with the naked eye.

Transfer of resistance in other conditions

Results of transfer experiments in milk, urine, pooled human serum, surgical-drainage fluid and saliva showed that urine and milk alone provided an environment for high-frequency transfer of resistance; tetracycline and erythromycin resistances were transferred at the highest frequencies (table). If urine was autoclaved before inoculation, a heavy precipitate formed and transfer of resistance was not detected.

Transfer of resistance on solid media

When strains WG4483 and WG3358 were cultivated together on the surface of solid media, only BHIA containing Ca^{++} promoted transfer but at lower frequencies of transfer than those obtained in BHIB with Ca^{++} , or in milk or urine (table).

Transfer on solid media was stimulated in the presence of a membrane filter; gentamicin-resistance was transferred at the highest frequency and, when tetracycline or erythromycin resistances were transferred, a high frequency of co-transfer for gentamicin resistance and the other resistances of plasmid pWG615 occurred. This pattern of resistance transfer was similar to that obtained in BHIB containing PEG 40%. Tissue, surgical gauze, surgical thread and dry skin shavings gave results similar to those obtained in the filter-membrane experiments except that transfer frequencies were higher (table).

As a further demonstration of the ability of tissue to stimulate transfer, instead of assaying organisms on the tissue for transcipts, the tissue was removed from BHIA and growth from the entire plate velveteen-replated to a gentamicin-containing selective agar. Gentamicin-resistant transcipts were detected only where the cultures had been in contact with the tissue (fig. 2).

All articles which stimulated transfer between mixed cultures on a BHIA plate were highly

absorbent. They may stimulate transfer by virtue of an ability to remove water from the cultures. This possibility was tested by repeating all transfer experiments but moistening the article with BHIB immediately before applying to the surface of the inoculated agar. Transfer was abolished on tissue, surgical gauze and surgical thread (results not shown); on skin, however, transfer frequencies marginally increased. Further examination of this effect revealed that dried skin shavings absorbed several times their own weight of water over an extended period of time. When the transfer experiment was repeated with skin that had absorbed moisture over a 24-h period, transfer of resistance was not detected. Transfer was not detected with non-absorbent articles such as teflon tape.

When topical preparations were tested for their effect on resistance transfer, the area of growth under the Macrogol Ointment showed a *c.* 10-fold increase in the transfer frequency of gentamicin resistance, whereas transfer was not detected in the area under the Cetomacrogol Cream (fig. 2). Tissue covered with Macrogol Ointment showed the same pattern of resistance transfer and co-transfer as that observed with tissue alone (results not shown).

Transfer of resistance on human skin in vivo

Gentamicin, tetracycline and erythromycin resistances were transferred, gentamicin resistance at the highest frequency. Tetracycline- or erythromycin-resistant transcipts exhibited a co-transfer frequency of 63–80% for gentamicin resistance along with the other resistances characteristic of plasmid pWG615 (table). Plasmid analysis of the co-transcipts demonstrated two plasmids with mol. wts corresponding to plasmid pWG615 and either plasmid pWG3 or plasmid pE194. Propamidine resistance was transferred at low frequency with 100% co-transfer of gentamicin resistance.

Discussion

The advantage of using strain WG4483 as a donor was that it allowed the use of several parameters to detect and differentiate transfer by phage-mediated conjugation and conjugation. The transfer experiments in BHIB with Ca^{++} or PEG defined parameters for distinguishing the two mechanisms that transfer resistance plasmids from strain WG4483: (i) phage-mediated conjugation required both Ca^{++} and a lysogenic recipient and transferred all plasmids, usually independently of each other; small plasmids were transferred at the highest frequencies and occasionally were co-trans-

Fig. 2. Transfer of gentamicin resistance on BHIA seeded with strains WG4483 and topical WG3358 with tissue and topical preparations applied: tissue *a* and *c*; tissue + Macrogol ointment, *b*; tissue + Cetomacrogol Cream Aqueous, *d*. After removal of tissue and topical preparations, BHIA plates were velveteen replicated to plates of gentamicin-containing selective medium (shown).

ferred, as expected for a phage-mediated mechanism in the light of the co-transduction of similar small staphylococcal plasmids (Iordanescu, 1977); (ii) conjugation required PEG and transferred the conjugative plasmid pWG615 at the highest frequency with evidence for high-frequency mobilisation of the small plasmids. Plasmid pWG115 had been included in strain WG4483 as an example of a non-conjugative plasmid that could not be mobilised (Townsend *et al.*, 1985*b*). However, the resistances of plasmid pWG115 were transferred by conjugation although this appeared to have

occurred after recombination between plasmids pWG615 and pWG115, possibly as a consequence of continued subculture of strain WG4483 in propamidine. This observation requires further analysis but the results do provide another parameter for distinguishing phage-mediated conjugation and conjugation, i.e., the former method allowed transfer of plasmid pWG115 independently of other plasmids but the latter mechanism transferred this plasmid at low frequency and recombined with the conjugative plasmid pWG615.

Transfer experiments in BHIB with increasing concentrations of PEG confirmed preliminary results (Townsend *et al.*, 1985b) that phage-mediated conjugation and conjugation operate in different cultural conditions *in vitro*. PEG concentrations > 16% inhibit and possibly abolish transfer by the phage-mediated mechanism. Furthermore, the changes in transfer frequencies could not be attributed to any inhibition of growth by PEG because the viable counts showed only a marginal decrease with increased concentrations of PEG. However, the addition of PEG produced a dramatic and sharp drop in turbidity at PEG concentrations > 12%, with a minimum turbidity at 24%. Phase-contrast microscopy indicated that the decrease in turbidity resulted from an increase in cell aggregation. This aggregation correlated with the commencement of conjugative transfer and provided the basis for the proposed role of PEG in conjugation (Townsend *et al.*, 1985b).

The stimulation of phage-mediated conjugation at PEG concentrations between 0 and 8% had not been observed previously (Townsend *et al.*, 1985b). It probably resulted from the use of smaller increments in PEG concentration. Witte (1981) has reported a similar stimulation of plasmid transfer between mixed cultures in the presence of 4% PEG. The low concentrations of PEG may produce a slight reduction in the size of the exclusion volumes of mating cells (Townsend *et al.*, 1985b) allowing easier access to cell-wall receptor sites for the phage-mediated mechanism.

Staphylococci inhabit body surfaces that vary from the moist conditions of mucosal linings, body tissues and exudates to dry areas of exposed skin, the latter being the likeliest body site for exchange of genetic information. Clinical isolates of staphylococci, when seeded on to the surface of human skin, appeared to transfer conjugative plasmids and mobilise small, non-conjugative plasmids at much higher frequencies than attainable in broth cultures (Naidoo, 1984). The results presented here provide a simple but surprising answer to the question of which factor favours conjugation *in vitro* and *in vivo*. It appears that highly absorbent articles including human skin may stimulate conjugative transfer by removing water from the cells. On the other hand, phage-mediated conjugation was favoured in fluids with free Ca^{++} . By way of confirmation, heat-sterilised urine formed an insoluble precipitate, probably comprising calcium phosphate, that removed Ca^{++} and abolished phage-mediated transfer.

These results can be interpreted with reference to the postulated role of PEG in stimulating conjuga-

tive transfer. The conjugative transfer observed on absorbent surfaces could be visualised as occurring by a temporary loss of water from the cell's surface, a sudden collapse of the hydrated layer around the cells with consequent aggregation of recipient cells by hydrophobic interactions. On skin, the conditions may be so sufficiently dehydrated that the cells are always suited to conjugative transfer. These same conditions inhibit phage-mediated conjugation. Several possible reasons may be advanced for this inhibition including: (i) prevention of phage adsorption by altering the conformation of the cell-wall receptor or phage-binding site; (ii) promotion of hydrophobic interactions between cells in preference to ionic ones involving divalent cations; and (iii) agglutination and precipitation of free virus particles, if these are the agents of transfer. These deliberations refer only to the formation of mating pairs. The mechanisms by which plasmids are actually transferred are unknown.

Conjugative transfer of plasmids on absorbent surfaces may be clinically significant as exudates from the body absorbed by such materials as tissues, gauze, bandages and surgical thread may be a site for genetic exchange between staphylococci. These and other topical preparations need to be evaluated for their ability to stimulate or inhibit transfer of resistance by phage-mediated and conjugative-transfer mechanisms, especially those topical preparations that contain antibacterial agents. The results of the preliminary experiment suggest that PEG-based ointments stimulate conjugative transfer whereas creams based on the less hydrophilic cetomacrogol inhibit it.

The elucidation of the cultural conditions favouring each transfer mechanism requires a re-evaluation of previous experiments. For example, transfer of neomycin resistance in broth cultures and on skin was attributed to a phage-mediated mechanism (Lacey, 1971). However, the results presented here suggest that the transfer on skin may result from conjugation. Surveys of coagulase-negative staphylococci and animal isolates of *S. aureus* found little or no evidence for transferable resistance to human isolates of *S. aureus*, raising doubts about these organisms as a source of antibiotic resistance. However, the experiments were performed under conditions that allowed transfer by a phage-mediated mechanism only (Lacey, 1980b; Fawcett *et al.*, 1981).

Recent reports of resistance transfer from coagulase-negative staphylococci and streptococci to *S. aureus* involve conjugative plasmids (Schaberg *et al.*, 1982; Archer and Johnston, 1983; Forbes and Schaberg, 1983; McDonnell *et al.*, 1983). Thus,

conjugation may be the route by which resistance determinants are transferred interspecifically and intergenerically to *S. aureus* either on conjugative plasmids or on non-conjugative plasmids that have been mobilised. This would appear to be a more appropriate mechanism for interspecific transfer of resistance determinants because the coagulase-negative staphylococci inhabit the drier regions of the body surface where the conjugative mechanism is operable but a phage-mediated mechanism would be inhibited. Because phages of staphylococci are

generally species-specific, the phage-mediated mechanism is more suited to intraspecific transfer of resistance determinants in moist areas of the body.

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REFERENCES

- Archer G L, Johnston J L 1983 Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. *Antimicrobial Agents and Chemotherapy* **24**:70–77.
- Emslie K R, Townsend D E, Bolton S, Grubb W B 1985 Two distinct resistance determinants to nucleic acid-binding compounds in *Staphylococcus aureus*? *FEMS Microbiology Letters* **27**:61–64.
- Fawcett C J, Lord V L, Lacey R W 1981 Failure of coagulase-negative staphylococci to transfer antibiotic resistance to *Staphylococcus aureus* 1030 in mixed cultures. *Journal of General Microbiology* **126**:507–509.
- Forbes B A, Schaberg D R 1983 Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugative exchange of resistance. *Journal of Bacteriology* **153**:627–634.
- Fouace J 1981 Mixed cultures of *Staphylococcus aureus*: some observations concerning transfer of antibiotic resistance. *Annales de Microbiologie (Institut Pasteur)* **132B**:375–386.
- Iordanescu S 1977 Relationships between cotransducible plasmids in *Staphylococcus aureus*. *Journal of Bacteriology* **129**:71–75.
- Iordanescu S, Surdeanu M 1980 New incompatibility groups for *Staphylococcus aureus* plasmids. *Plasmid* **4**:256–260.
- Jaffe H W, Sweeney H M, Nathan C, Weinstein R A, Kabins S A, Cohen S 1980 Identity and interspecific transfer of gentamicin-resistance plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Journal of Infectious Diseases* **141**:738–747.
- Lacey R W 1971 High-frequency transfer of neomycin resistance between naturally occurring strains of *Staphylococcus aureus*. *Journal of Medical Microbiology* **4**:73–84.
- Lacey R W 1975 Antibiotic resistance plasmids of *Staphylococcus aureus* and their clinical importance. *Bacteriological Reviews* **39**:1–32.
- Lacey R W 1980a Evidence for two mechanisms of plasmid transfer in mixed cultures of *Staphylococcus aureus*. *Journal of General Microbiology* **119**:423–435.
- Lacey R W 1980b Rarity of gene transfer between animal and human isolates of *Staphylococcus aureus* in vitro. *Journal of General Microbiology* **119**:437–442.
- Lacey R W 1984 Antibiotic resistance in *Staphylococcus aureus* and streptococci. *British Medical Bulletin* **40**:77–83.
- Lacey R W, Lord V L 1980 Transfer of gentamicin resistance between cultures of *Staphylococcus aureus* in nutrient broth, serum and urine. *Journal of Medical Microbiology* **13**:411–421.
- Lacey R W, Keyworth N, Lincoln C 1984 Staphylococci in the U.K.: a review. *Journal of Antimicrobial Chemotherapy* **14** (Suppl. D):19–25.
- McDonnell R W, Sweeney H M, Cohen S 1983 Conjugational transfer of gentamicin resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrobial Agents and Chemotherapy* **23**:151–160.
- Naidoo J 1984 Interspecific co-transfer of antibiotic resistance plasmids in staphylococci in vivo. *Journal of Hygiene* **93**:59–66.
- Naidoo J, Noble W C 1978 Transfer of gentamicin resistance between strains of *Staphylococcus aureus* on skin. *Journal of General Microbiology* **107**:391–393.
- Novick R P, Bouanchaud D 1971 Extrachromosomal nature of drug resistance in *Staphylococcus aureus*. *Annals of the New York Academy of Science* **182**:279–294.
- Schaberg D R, Clewell D B, Glatzer L 1982 Conjugative transfer of R-plasmids from *Streptococcus faecalis* to *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **22**:204–207.
- Townsend D E, Ashdown N, Greed L C, Grubb W B 1984 Transposition of gentamicin resistance to staphylococcal plasmids encoding resistance to cationic agents. *Journal of Antimicrobial Chemotherapy* **14**:115–124.
- Townsend D E, Ashdown N, Annear D I, Grubb W B 1985a A conjugative plasmid encoding production of a diffusible pigment and resistance to aminoglycosides and macrolides in *Staphylococcus aureus*. *Australian Journal of Experimental Biology and Medical Science* **63**:513–586.
- Townsend D E, Bolton S, Ashdown N, Grubb W B 1985b Transfer of plasmid-borne aminoglycoside-resistance determinants in staphylococci. *Journal of Medical Microbiology* **20**:169–186.
- Witte W 1981 Transfer of drug-resistance plasmids in mixed cultures of staphylococci. In: Jeljaszewicz J (ed) *Staphylococci and staphylococcal infections*, Gustav Fischer Verlag, Stuttgart, (Zentralblatt für Bakteriologie Parasitenkunde, Infektionskrankheiten und Hygiene, Suppl. **10**) pp 543–545.