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Interspecific co-transfer of antibiotic resistance plasmids in staphylococci *in vivo*

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SUMMARY

The co-transfer of plasmid-borne genes governing resistance to gentamicin, tetracycline, erythromycin and chloramphenicol has been demonstrated on human and mouse skin. Two different gentamicin resistance plasmids have been studied in detail; both appear to have the ability to mobilize *in vivo* otherwise non-transferable resistance plasmids from coagulase-negative to coagulase-positive staphylococci. This emphasises the role of the skin in maintaining a pool of resistance genes available to pathogenic staphylococci.

INTRODUCTION

There has long been concern at the increasing antibiotic resistance of *Staphylococcus aureus* and at the way in which resistance develops or is transferred. Lacey (1975) suggested that the coagulase-negative staphylococci which form part of the normal skin flora may be the source of resistance genes available to *S. aureus*. There have been several reports of the transfer of plasmids governing gentamicin resistance from coagulase-negative staphylococci to *S. aureus in vitro* (Forbes & Schaberg, 1983; McDonnell, Sweeney & Cohen, 1983) and *in vivo* (Jaffe *et al.* 1980; Naidoo & Noble, 1981). In all these reports a conjugational type of transfer mechanism has been implicated. McDonnell *et al.* (1983) reported that their conjugational plasmid could mobilize other plasmids *in vitro*.

This paper reports the mobilization and co-transfer of individual plasmids governing tetracycline, erythromycin and chloramphenicol resistances from *S. epidermidis* and *S. hominis* to *S. aureus in vivo* on human and mouse skin.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. Recipient strains were made resistant to either rifampicin (rif) or streptomycin (strep) by inoculating cultures on gradient antibiotic plates.

Media. Oxoid blood agar base (CM55) was used throughout with the addition of either rifampicin 80 μg/ml, streptomycin 60 μg/ml, neomycin 15 μg/ml, gentamicin 10 μg/ml, tetracycline 20 μg/ml, erythromycin 20 μg/ml or chloramphenicol 20 μg/ml for the selective plates. CY broth (Novick, 1963) with additional 0·01 M-$CaCl_2$ was the fluid medium.

Table 1. *Strains of staphylococci used*

Strain	Characteristics	Source	Plasmid designations
J724	Pc^r Tc^r Em^r Nm^r Cm^r Gm^r *S. epidermidis*	Hospital isolate	pJ7241 (Pc^r Gm^r), pJ7242 (Tc^r), pJ7243 (Em^r), pJ7244 (Cm^r)
J836	Pc^r Tc^r Em^r Nm^r Gm^r *S. hominis*	Hospital isolate	pJ8361 (Gm^r), pJ8362 (Tc^r), Em^r/Nm^r *
B111	Phage-type 3A/53/85, antibiotic sensitive *S. aureus*	Non-hospital clinical isolate	
80CR5	Phage-type Gps 1, 11, 111, Pc^r *S. aureus*	Engel *et al.* (1980)	
M1	Phage-type 79, antibiotic sensitive *S. aureus*	Hospital isolate	

Pc, penicillin; Tc, tetracycline; Em, erythromycin; Nm, neomycin; Cm, chloramphenicol; Gm, gentamicin.

* Genes governing resistance to Em and Nm in this strain were always transferred simultaneously but no plasmid DNA could be demonstrated for these determinants.

Plasmid transfer. In vitro transfer was carried out in mixed broth culture (10^9 c.f.u./ml) or aliquots of the same cultures were drawn through filters and the filters incubated on agar plates as described previously (Naidoo & Noble, 1981). *In vivo* plasmid transfer was carried out on hairless/obese mice or on the human forearm (Naidoo & Noble, 1978).

Isolation of plasmid DNA. Cultures grown on selective antibiotic plates were used for the isolation of plasmid DNA. Cleared lysates were prepared using lysostaphin (Sigma Chemical Co.) at a final concentration of 100 μg/ml followed by detergent (1·2% Brij 58, 0·5% sodium deoxycholate, 0·02 M-EDTA pH 8·4) lysis of protoplasts. After centrifugation the cleared lysate was deproteinated with protease (Sigma Chemical Co.) and then made 0·3 M in sodium acetate. Two volumes of ice-cold ethanol were added and the DNA precipitated overnight at −20 °C. Precipitated DNA was collected by centrifugation and dissolved in 100–150 μl TES buffer (0·05 M-TRIS, 0·005 M-EDTA, 0·05 M-NaCl). Gels were run at 35 V overnight and then stained in a solution of ethidium bromide (5 μg/ml) for 30 min. DNA bands were visualized with a long wave u.v. light transilluminator (C-62, Ultra-Violet Products Inc.) and photographed on Polaroid 665 film.

RESULTS

Table 2 shows in detail the frequencies for plasmid transfer from *S. epidermidis* J724 to *S. aureus* recipient 80CR5rif *in vitro* and *in vivo*; similar results were obtained for recipient B111rif. The gentamicin resistance (Gm^r) plasmid of J724 also bears the genes for penicillinase production. Transfer of this plasmid occurs on mouse skin at rates 10–100-fold greater than on filters and transfer rates for broth were lower than filters by a similar amount. The apparently poor transfer rates for the tetracycline resistance (Tc^r) and erythromycin resistance (Em^r) plasmids pJ7242 and pJ7243 *in vivo* may be an artifact due to the comparatively smaller number of organisms recovered from the mice (10^6 to 10^7 c.f.j./ml) compared to filters (10^9 c.f.u./ml). However when the transfer of either pJ7242 or

Table 2. In vivo *and* in vitro *transfer of resistance plasmids from* S. epidermidis *J*724 *to* S. aureus 80*CR*5*rif*

		Transfer frequency*			
		pJ7241	pJ7242	pJ7243	pJ7244
Broth	6 h	2×10^{-9}	2×10^{-10}	0†	3×10^{-8}
Filter	6 h	7×10^{-7}	2×10^{-8}	1×10^{-7}	1×10^{-6}
Mouse 1	6 h	6×10^{-4}	1×10^{-6}	3×10^{-5}	2×10^{-4}
Mouse 2	6 h	5×10^{-5}	1×10^{-6}	3×10^{-6}	3×10^{-5}
Mouse 3	6 h	3×10^{-4}	8×10^{-7}	3×10^{-7}	4×10^{-4}
Mouse 4	6 h	2×10^{-5}	0	2×10^{-6}	3×10^{-5}
Mouse 5	6 h	6×10^{-6}	0	0	3×10^{-6}
Mouse 6	6 h	8×10^{-6}	0	0	5×10^{-6}
Mouse 7	6 h	9×10^{-6}	0	1×10^{-6}	2×10^{-5}
Mouse 8	6 h	5×10^{-6}	0	9×10^{-7}	2×10^{-6}

* Number of transconjugants per final number of recipient cells.
† $< 10^{-10}$.

Table 3. In vivo *and* in vitro *transfer of resistance plasmids from* S. hominis *J*836 *to* S. aureus *B*111*rif*

		Transfer frequency*		
		pJ8361	pJ8362	Em^r/Nm^r
Broth	6 h	6×10^{-10}	0†	6×10^{-10}
Filter	6 h	5×10^{-7}	6×10^{-8}	7×10^{-8}
Mouse 1	6 h	5×10^{-8}	0	0
Mouse 2	6 h	1×10^{-6}	8×10^{-8}	0
Mouse 3	6 h	3×10^{-7}	5×10^{-8}	0
Mouse 4	6 h	2×10^{-7}	2×10^{-7}	0
Mouse 5	6 h	1×10^{-5}	3×10^{-7}	0
Mouse 6	6 h	2×10^{-6}	2×10^{-7}	0
Mouse 7	6 h	1×10^{-5}	1×10^{-6}	0
Mouse 8	6 h	2×10^{-7}	0	0

* Number of transconjugants per final number of recipient.
† $< 10^{-10}$.

pJ7243 was detected on mouse skin, the transfer frequency was always higher than that on filters or in broth. The chloramphenicol resistance (Cm^r) plasmid pJ7244 was transferred to *S. aureus* at about the same rate as the Gm^r plasmid pJ7241. Although the data shows that variability in the rate of transfer on different mice often occurred, transfer on skin, when detected, was consistently more efficient than on filters or in broth. Control cultures were always set up inoculating the donor and recipient strains singly on mice, in broth or on filters. Although some *S. epidermidis* donor cells did sometimes grow on selective plates no selectively resistant *S. aureus* transconjugant colonies were ever detected in the controls.

Plasmid transfer from *S. hominis* (J836) to *S. aureus* is shown in Table 3. The plasmid determining gentamicin resistance in *S. hominis* J836 was structurally different from the Gm^r plasmid of the *S. epidermidis* strain by restriction endonuclease cleavage (unpublished data) and did not determine penicillinase

Table 4. *Co-transfer of plasmids from* S. epidermidis *J724 to* S. aureus

S. aureus recipient	Transfer site	Selective agent	Percentage isolates with unselected plasmid (mediating resistance shown) pJ7241 (Gm)	pJ7242 (Tc)	pJ7243 (Em)	pJ7244 (Cm)	No. isolates tested
B111rif	Filter	Gm	(43)	0	0	55	402
		Tc	94	(1)	25	87	249
		Em	92	2	(1)	61	475
		Cm	77	2	5	(20)	187
	Mouse skin	Gm	(47)	0	7	49	283
		Tc	—	—	—	—	0
		Em	83	8	(0)	66	12
		Cm	54	1	4	(42)	598
80CR5rif	Filter	Gm	(72)	0	8	22	483
		Tc	64	(17)	5	36	159
		Em	97	1	(2·5)	16	196
		Cm	81	1	6	(17)	121
	Mouse skin	Gm	(67)	1	8	26	508
		Tc	100	(0)	20	60	5
		Em	95	1	(2)	53	87
		Cm	77	2	7	(22)	449

Figures in parentheses indicate isolates bearing selected plasmid alone.

production. Determinants for erythromycin resistance and neomycin resistance were always simultaneously transferred from this *S. hominis* strain but no plasmid has been detected governing these linked resistance markers. It may be that in this strain the genes for erythromycin and neomycin resistance are carried on a transposable element; an erythromycin resistance transposon has been described in *S. aureus* (Novick *et al.* 1979). Both plasmids pJ8361 and pJ8362 were transferred at about the same frequency or higher on mouse skin compared to the same cultures on filters. No Tc^r transconjugants were recovered from mice 1 and 8 but again this may have been due to the lower recovery of cells from skin rather than plasmid transfer not occurring at all. The simultaneous transfer of erythromycin and neomycin resistance determinants was only ever detected in broth culture or on filters at low frequency.

Co-transfer of plasmids

Phage-typing and antibiotic sensitivity testing was carried out on the transconjugants produced by the *in vitro* and *in vivo* mating of the coagulase-negative strains with *S. aureus* B111rif and 80CR5rif. When individual transconjugant colonies were examined it was frequently found that unselected antibiotic resistance plasmids had also been transferred. Table 4 shows the co-transfer of plasmids from *S. epidermidis* J724 to *S. aureus* recipients. For example with recipient B111rif after filter mating, transconjugant colonies selected on gentamicin agar had also acquired the erythromycin and chloramphenicol resistance determinants. Forty-three percent of the 402 transconjugants picked from gentamicin antibiotic plates were gentamicin resistant only and contained only the Gm^r plasmid pJ7241, none

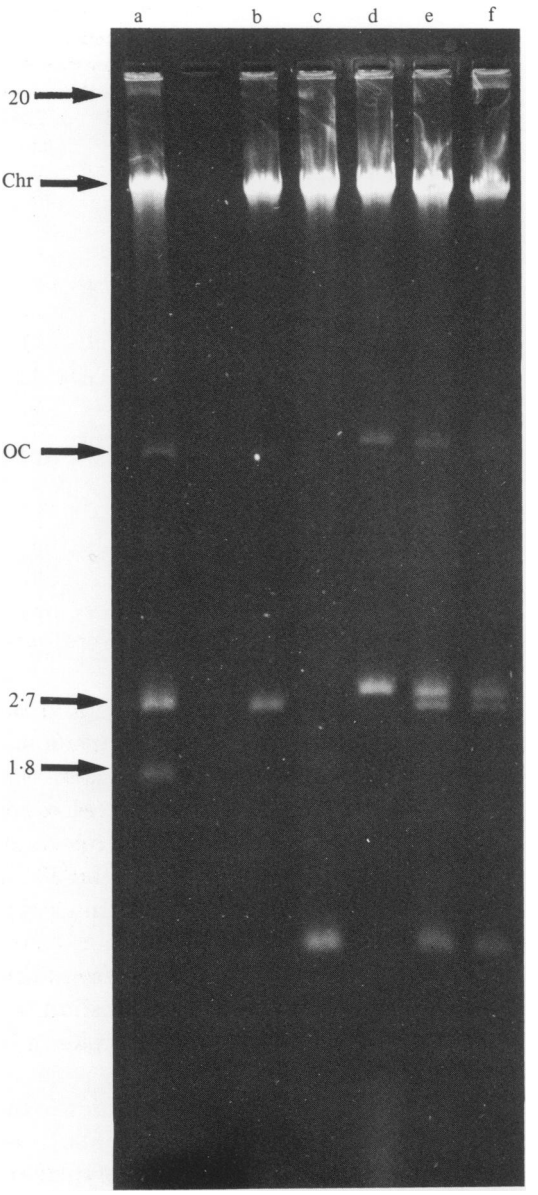

Fig. 1. Agarose gel (0·8%) electrophoresis of DNA from *S. aureus* B111rif after acquisition of *S. epidermidis* J724 plasmids. Lane a, standard *S. aureus* plasmids pI524 (Pc^r 20 Md), pT169 (Tc^r 2·7 Md), pE194 (Em^r 1·8 Md). Chromosomal (Chr) DNA and open-circular (OC) DNA is also present: Lane b, Tc^r transconjugant containing pJ7242: Lane c, Em^r transconjugant containing pJ7243: Lane d, Cm^r transconjugant containing pJ7244: Lane e, Tc^r Em^r Cm^r transconjugant containing pJ7242, pJ7243, pJ7244: Lane f, Pc^r Gm^r Tc^r Em^r Cm^r transconjugant containing pJ7241, pJ7242, pJ7243, pJ7244.

Table 5. *Mobilization of plasmids* in vitro *and* in vivo

Plasmids[a]	Site of transfer	Transfer frequency to *S. aureus* M1 strep. pJ7242	pJ7243	pJ7244
From *S. epidermidis* J724				
pJ7242, pJ7243, pJ7244	Filter 6 h	0*	1×10^{-9}	0*
pJ7242, pJ7243, pJ7244 + pJ7241	Filter 6 h	9×10^{-9}	4×10^{-7}	3×10^{-7}
pJ7242, pJ7243, pJ7244	Mice[b] 6 h	0	0	0
pJ7242, pJ7243, pJ7244 + pJ7241	Mice[b] 6 h	9×10^{-8}	2×10^{-6}	4×10^{-6}
pJ7242, pJ7243, pJ7244	Human 1, R. forearm	0	0	0
pJ7242, pJ7243, pJ7244 + pJ7241	Human 1, L. forearm	4×10^{-6}	5×10^{-5}	5×10^{-5}
pJ7242, pJ7243, pJ7244	Human 2, R. forearm	0	0	0
pJ7242, pJ7243, pJ7244 + pJ7241	Human 2, L. forearm	4×10^{-8}	3×10^{-6}	5×10^{-6}
		pJ8362		
From *S. hominis* J836				
pJ8362	Filter 6 h	0*		
pJ8362 + pJ8361	Filter 6 h	3×10^{-7}		
pJ8362	Mice[b] 6 h	0		
pJ8362 + pJ8361	Mice[b] 6 h	2×10^{-6}		
pJ8362	Human 1, R. forearm	0		
pJ8362 + pJ8361	Human 1, L. forearm	3×10^{-6}		
pJ8362	Human 2, R. forearm	0		
pJ8362 + pJ8361	Human 2, L. forearm	7×10^{-6}		

[a] Borne by donor B111rif; [b] Mean of four mice.
* $< 10^{-10}$.

contained the Tc^r plasmid, 5% had acquired the Em^r plasmid pJ7243 and 55% the Cm^r plasmid pJ7244. In contrast, more than 90% of the transconjugants selected on either tetracycline or erythromycin agar also contained the Gm^r plasmid. Twenty percent of the 187 chloramphenicol resistant transconjugants contained the Cm^r plasmid only, 2 and 5% contained the Tc^r and Em^r plasmids respectively, and 77% contained the Gm^r plasmid. In general, co-transfer of plasmids *in vivo* proceeded at the same rates as *in vitro*.

Agarose gel electrophoresis of the plasmid DNA from the transconjugants revealed that although some of the plasmids, e.g. pJ7241 and pJ7242 were co-transferred at high frequency into the *S. aureus* recipient, once acquired they existed as separate elements. In some instances all four resistance plasmids were acquired simultaneously but they existed individually in the new host (Fig. 1).

With *S. hominis* J836 the co-transfer of plasmids pJ8361 and pJ8362 governing gentamicin and tetracycline resistances respectively, resembled that of the *S. epidermidis* strain (data not shown). The linked erythromycin and neomycin resistance determinants were never co-transferred with either pJ8361 or pJ8362.

Plasmid mobilization

The fact that the two Gm^r plasmids pJ7241 and pJ8361 were acquired by the tetracycline, erythromycin and chloramphenicol resistant transconjugants at such high frequency suggested that these two plasmids were involved in the mobilization of the other resistance plasmids. McDonnell *et al.* (1983) demonstrated the mobilization of otherwise not independently transferable plasmids by their Gm^r

plasmid pSH8. However this mobilization effect was demonstrated *in vitro* on filters. We attempted to determine whether the *S. epidermidis* and *S. hominis* plasmids pJ7241 and pJ8361 could mobilize the other resistance plasmids *in vivo* on mouse skin and human skin. Table 5 shows the effects of plasmids pJ7241 and pJ8361 on the transfer of the other antibiotic resistance plasmids *in vivo* and *in vitro*. The donor strain containing the coagulase-negative staphylococcal plasmids was *S. aureus* B111rif and the recipient was *S. aureus* M1 which was made streptomycin resistant. The mixed cultures were incubated on filters or occluded on mice as previously and in addition inoculated and occluded on the forearm skin of volunteers for 6 h. In general the three *S. epidermidis* plasmids, pJ7242, pJ7243 and pJ7244 fail to transfer or are transferred at extremely low frequency in both the *in vivo* and *in vitro* situations unless the Gm^r plasmid pJ7241 is present. In the case of the filter matings the lowest frequency of transfer detectable was 1×10^{-10} and *in vivo* on skin 1×10^{-8}. The data shows that the presence of pJ7241 promotes the transfer of all three resistance plasmids pJ7242, pJ7243 and pJ7244, on filters and on mouse skin and human skin. Similarly the *S. hominis* gentamicin resistance plasmid pJ8361 promoted the transfer of the Tc^r plasmid pJ8362. Both the *S. epidermidis* plasmids and *S. hominis* plasmids once mobilized by the Gm^r plasmids were again transferred at higher frequency *in vivo* on skin compared to incubation on filters.

DISCUSSION

Many hospital strains of *S. aureus* are multiply antibiotic resistant, the resistances being plasmid encoded. The natural reservoir of these resistance plasmids is thought to be the coagulase-negative staphylococci of the normal skin flora. The transfer of plasmids determining gentamicin resistance from coagulase-negative staphylococci to *S. aureus* has been described (Jaffe *et al.* 1980; Naidoo & Noble, 1981; McDonnell *et al.* 1983). There have also been reports of the natural existence of similar plasmids in these organisms (Groves, 1979; Cohen, Wong & Falkow, 1982) indicating that the different staphylococcal species do not exist in genetic isolation. It is now well established that conjugation is a mechanism of plasmid transfer in the staphylococci (Forbes & Schaberg, 1983; McDonnell *et al.* 1983) but there has been no formal proof that this mechanism operates in the natural environment. This paper demonstrates conjugation *in vivo* and the co-transfer and mobilization of otherwise non-transferable plasmids by two coagulase-negative Gm^r plasmids.

The transfer of plasmids mediating resistances to tetracycline, erythromycin and chloramphenicol was facilitated on skin compared to incubation on filters. Further, mobilization of these plasmids was also better achieved on skin compared to filters suggesting that cell-to-cell contact was not the only promoting factor for transfer on skin. Work is now continuing to determine the factors on skin which enhance plasmid transfer in this way.

The Gm^r conjugative plasmids for *S. epidermidis* J724 and *S. hominis* J836 are quite different as shown by restriction endonuclease cleavage maps. Strain J724 was isolated in the U.S.A. whereas J836 was an isolate from the skin of a patient at St John's Hospital, London. The distribution of such conjugative elements in the coagulase-negative staphylococcal skin flora and the fact that skin promotes

the operation of such elements, provides the potential for the spread of varieties of otherwise non-transferable plasmids between the coagulase-negative species and pathogenic *S. aureus*. The therapeutic use of gentamicin may therefore encourage the selection and transfer of genes determining other antibiotic resistances even though these exist as separate plasmids in the host staphylococci.

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