J. Hyg., Camb. (1981), **86**, 183
Printed in Great Britain

Transfer of gentamicin resistance between coagulase-negative and coagulase-positive staphylococci on skin

By JAY NAIDOO and W. C. NOBLE

Department of Bacteriology, Institute of Dermatology, Homerton Grove, London, E9 6BX

(*Received* 16*th October* 1980)

SUMMARY

The transfer of gentamicin resistance between a coagulase-negative *S. hominis* strain and various coagulase-positive *S. aureus* strains on human and murine skin in the absence of a selective agent is described. Transfer occurs at higher frequency on skin than in broth. Skin transfer may account for the apparently explosive occurrence of gentamicin resistant staphylococci in hospitals.

INTRODUCTION

Naidoo and Noble (1978*a*) reported the experimental transmission of gentamicin resistance between two strains of *Staphylococcus aureus* on human skin and on mouse skin. The strains were isolated from a patient admitted to St John's Hospital for Diseases of the Skin and had been observed to become resistant to gentamicin (apparently as a result of natural plasmid transfer) during topical therapy with an ointment containing gentamicin. The source of the original plasmid was not determined but an '*S. epidermidis*' strain with labile gentamicin resistance was isolated from the patient at the same time as the *S. aureus* strains and was referred to in the description of the ward observations (Naidoo and Noble, 1978*b*). The transmission of resistance between this coagulase-negative strain and the coagulase-positive strain was briefly described at the Fourth Conference on Staphylococci and Staphylococcal Infection in Warsaw 1979; this report describes the observations in full.

METHODS

Staphylococci. Details of strains are shown in Table 1. The coagulase-negative strain was identified as *Staphylococcus hominis* by Dr R. R. Marples of the Central Public Health Laboratory, Colindale. *S. aureus* strain S130 was isolated during studies of cross-infection in 1960, used in a study of staphylococcal virulence reported in 1966 (Noble, 1966) and maintained in a freeze-dried state since that time. Phage propagating strains were made resistant to rifampicin by inoculating

0022-1724/81/8 0059-1981 $01.00

Table 1. *Characteristics of staphylococci used or isolated*

Strain	Source	Phage typing pattern	Resistance pattern*	Gentamicin MIC μg/ml
S. hominis†	Patient Mrs B	—	PTNEFG	> 32
S. hominis‡	Cured variant	—	PTNEF	< 0·5
S. aureus§	Patient Mrs B	NT	PTESM	< 0·5
S. aureus PS47	NCTC 8325	47/75/77/84/85	R	< 0·5
	Transferant	47/75/77/84/85	RG	> 32
S. aureus PS54	NCTC 8329	47/53/54/75/77/84/85	R	< 0·5
	Transferant	47/53/54/75/77/84/85	RG	> 32
S. aureus PS80	NCTC 9789	80/81	PR	< 0·5
	Transferant	80/81	PRG	> 32
S. aureus PS83A	NCTC 10039	6/47/53/83A/84/85	R	< 0·5
	Transferant	6/47/53/83A/84/85	RG	> 32
S. aureus S130	Patient (1960)	53/77/84/85	PTS	< 0·5
	Transferant	53/77/84/85	PTSG	> 32

* Resistance pattern: P, penicillin; T, tetracycline; N, neomycin; E, erythromycin; F, fusidic acid; S, streptomycin; M, methicillin; R, rifampicin; G, gentamicin.

† *S. epidermidis* of Naidoo and Noble (1978b)
‡ Ethidium bromide cured variant
§ Strain 3 of Naidoo and Noble (1978a)

cultures serially on to gradient plates containing rifampicin until good growth was obtained on media containing 50 μg/ml.

Media. Oxoid blood agar base was used throughout as described previously (Naidoo & Noble, 1978*a*). CY broth (Novick, 1963) was the fluid medium.

Plasmid transfer. In vivo plasmid transfer was carried out on hairless/obese mice or on the human forearm (Naidoo & Noble, 1978*a*).

In vitro transfer was studied by inoculating the strains at high density (*c.* 10^{10} c.f.u./ml) in CY broth and incubating these at 37 °C for 6 h, or by drawing such cultures through Millipore filters (0·45 μm pore size) the filters being incubated on blood agar base medium at 37 °C for 6 h and the organisms recovered by shaking the filter in broth. Aliquots of broth culture or of filter fluid were then inoculated onto agar containing two antibiotics for recovery of transferants.

The effect of adding $CaCl_2$ (0·01 M) or DNAase (Sigma) (100 μg/ml) to broth culture was also studied. All mouse experiments were made in duplicate and the results pooled.

RESULTS

Table 2 shows that it was possible to transfer resistance in mixed culture from the *S. hominis* strain to *S. aureus* NT strain on human and murine skin and in broth. The reverse transfer was also demonstrated. The protocol for a single experiment is shown in Table 3. Resistance transfer was also possible from *S. hominis* to three *S. aureus* phage propagating strains (PS47, PS54, PS83A) *in vivo* and *in vitro* and from these strains to a wild type *S. aureus* (S130). Although it was not possible to obtain transfer to *S. aureus* PS80 *in vivo*, *in vitro* transfer

Table 2. *Results of transfer experiments*

Donor	Recipient	Site of transfer	Frequency of transfer*
(*a*) *S. hominis*	*S. aureus* NT	Human arm female	$7:10^4$
		male	$16:10^4$
		Mouse back	$6:10^3$
(*b*) *S. hominis*	*S. aureus* NT	Human arm female	$0:10^5$
		male	$1:10^5$
		Mouse back	$5:10^5$
		Broth	$16:10^9$
(*c*) *S. hominis*	*S. aureus* NT	Mouse back	$1:10^5$
		Broth	$2:10^8$
(*d*) *S. aureus* NT	*S. hominis*†	Mouse back	$3:10^5$
(*e*) *S. aureus* NT	*S. hominis*†	Mouse back	$3:10^7$
(*f*) *S. hominis*	*S. aureus* PS83A	Human arm female	$32:10^8$
		male	$32:10^8$
		Broth	$13:10^8$
(*g*) *S. hominis*	*S. aureus* PS83A	Mouse back	$2:10^6$
		Broth	$2:10^7$
(*h*) *S. hominis*	*S. aureus* PS47	Mouse back	$2:10^7$
		Broth	$2:10^7$
(*i*) *S. hominis*	*S. aureus* PS54	Mouse back	$12:10^7$
		Broth	$3:10^{10}$
(*j*) *S. aureus* PS83A (transferant)	*S. aureus* S130	Mouse back	$26:10^6$
		Broth	$3:10^9$
(*k*) *S. aureus* PS47 (transferant)	*S. aureus* S130	Mouse back	$38:10^6$
		Broth	$28:10^9$
(*l*) *S. aureus* PS54 (transferant)	*S. aureus* S130	Mouse back	$1:10^7$
		Broth	$3:10^8$
(*m*) *S. aureus* PS80 (transferant)	*S. aureus* S130	Mouse back	$12:10^7$
		Broth	$0:10^{10}$

* Transfer frequency ($7:10^4$) is given as the number of transferants (7) in a given population of recipients (10^4).

† Cured variant obtained by ethidium bromide treatment of the original culture.

was achieved and this transferant was also able to transfer resistance to the wild type strain S130.

The addition of $CaCl_2$ enhanced transfer of resistance from *S. hominis* to *S. aureus* PS47 and PS83A by about eight- to tenfold but not to *S. aureus* PS54 where it was equal. Since this might indicate phage action, attempts were made to transfer resistance by culture supernatants and by Mitomycin-C induced lysates of *S. hominis* but without result. Transfer on filters generally occurred at a lower frequency than in broth suggesting that the stabilizing effect of a solid surface was not the sole skin factor assisting transfer. The effect of DNAase was generally to reduce the apparent rate of transfer by about fivefold.

DISCUSSION

We have shown previously that gentamicin resistance can be transferred between strains of *S. aureus* on human and murine skin under conditions resembling those that occur during treatment of skin patients but without the use of antibiotics as a selective agent (Naidoo & Noble, 1978*a*). We have also described the

Table 3. *Protocol for one experiment* (*i*). *Transfer of gentamicin resistance from* S. hominis *to* S. aureus *PS*54

Site	Cultures used	Total staphylococci recovered cfu/ml	Total RG resistant colonies recovered*		Transfer frequency
Mouse 1A	*S. hominis*	3×10^6	0	0	0
1B	*S. hominis*	5×10^6	0	0	0
Mouse 2A	*S. aureus* PS54	1×10^7	0	0	0
2B	*S. aureus* PS54	3×10^7	0	0	0
Mouse 3A	*S. hominis* + *S. aureus* PS54	15×10^7	26	31	$12:10^7$
3B	*S. hominis* + *S. aureus* PS54	11×10^7	106	142	
Broth 1	*S. hominis*	2×10^9	0	0	0
Broth 2	*S. aureus* PS54	19×10^9	0	0	0
Broth 3	*S. hominis*+ *S. aureus* PS54	10×10^9	2	4	$3:10^{10}$

* Cells were recovered from mice in 2·5 ml. Aliquots of 0·5 ml were inoculated onto selective agar for recovery of transferants. Cells from broth culture were inoculated as 1·0 ml aliquots on selective agar which contained 50 μg/ml rifampicin and 10 μg/ml gentamicin.

apparent transfer of resistance in patients undergoing therapy (Naidoo & Noble, 1976*b*). This paper extends the observations by suggesting that a coagulase-negative coccus, *S. hominis*, isolated from the patient's skin at the same time as the resistant *S. aureus* strains, could have been the source of the original resistance, though clearly all may have acquired resistance from some other source.

The plasmid from the *S. hominis* strain is freely transferable to an *S. aureus* strain isolated during the original outbreak. In addition it could be transferred to three of four standard strains of *S. aureus* and from these to another wild *S. aureus*. Such natural transfer could explain the apparently explosive appearance of gentamicin resistance (Wyatt *et al.* 1977).

We have visualized the plasmid associated with gentamicin resistance by agarose gel electrophoresis of DNA in transferants of the phage propagating strains (unpublished observations). The results indicate a large plasmid, greater than 20×10^6 D, a value similar to that reported by deSaxe and Porthouse (1979).

Recently Jaffe *et al.* (1980) reported transfer on skin of gentamicin resistance from an *S. epidermidis* strain to an *S. aureus* though at lower frequency; as in the studies reported here transfer was achieved with epidemiologically relevant strains.

Our results show that transfer of gentamicin resistance can occur at a higher rate on skin than in the test tube; the conditions which govern the transfer have not yet been determined. Nevertheless the observations may account for the frequency with which skin patients feature in accounts of the appearance of gentamicin resistant staphylococci in hospitals (Warren & Roberts, 1976; Bint *et al.* 1977; Wyatt *et al.* 1977).

The support of the Wellcome Trust is gratefully acknowledged. Mrs Mary Rahman is thanked for technical assistance.

REFERENCES

BINT, A. J., GEORGE, R. H., HEALING, D. E., WISE, R. & DAVIES, M. (1977). An outbreak of infection caused by a gentamicin resistant *Staphylococcus aureus*. *Journal of Clinical Pathology* **30**, 165–7.

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**, 738–47.

NAIDOO, JAY & NOBLE, W. C. (1978*a*). Transfer of gentamicin resistance between strains of *Staphylococcus aureus* on skin. *Journal of General Microbiology* **107**, 391–3.

NAIDOO, JAY & NOBLE, W. C. (1978*b*). Acquisition of antibiotic resistance by *Staphylococcus aureus* in skin patients. *Journal of Clinical Pathology* **31**, 1187–92.

NOBLE, W. C. (1966). Virulence and the biochemical characteristics of *Staphylococci*. *Journal of Pathology and Bacteriology* **91**, 181–93.

NOVICK, R. P. (1963). Analysis by transduction of mutations affecting penicillinase formation. *Journal of General Microbiology* **33**, 121–36.

DESAXE, MAUREEN & PORTHOUSE, AUDREY (1979). Gentamicin resistance in *Staphylococcus aureus*. *Lancet*, ii, 370–1.

WARREN, R. E. & ROBERTS, S. O. B. (1976). Gentamicin-resistant staphylococci. *Lancet*, i, 543–4.

WYATT, T. D., FERGUSON, W. P., WILSON, T. S. & MCCORMACH E. (1977). Gentamicin resistant *Staphylococcus aureus* associated with the use of topical gentamicin. *Journal of Antimicrobial Chemotherapy* **3**, 213–17.