Epidem. Inf. (1989), **102**, 261–270
Printed in Great Britain

Transmissible mupirocin resistance in *Staphylococcus aureus*

By MARY RAHMAN[1], W. C. NOBLE[1] and B. COOKSON[2]

[1] *Department of Microbiology, Institute of Dermatology*
[2] *Department of Medical Microbiology, St Thomas's Hospital, United Medical and Dental Schools, London*

(*Accepted 3 October 1988*)

SUMMARY

The spread of two strains of *Staphylococcus aureus* with high level resistance to mupirocin is described. The resistance proved to be easily transferred to other *S. aureus* strains by filter mating experiments and on the skin of mice. No plasmid band corresponding to the resistance could be demonstrated by agarose gel electrophoresis or by caesium chloride gradient centrifugation but cleavage of 'chromosomal' DNA from resistant recipients showed bright bands of DNA absent from sensitive controls.

INTRODUCTION

Initial clinical studies on a new topical antibiotic, mupirocin (Bactroban, Beecham Pharmaceuticals) were published in 1985 (Dobson *et al.* 1985). Mupirocin is a new class of antibiotic which blocks protein synthesis by inhibition of isoleucyl tRNA synthetase. Early studies showed that low level resistance (MIC 12–50 mg/l) could be selected by repeated subculture of staphylococci in the presence of the antibiotic (White *et al.* 1985). However, in 1987 we observed and reported (Rahman, Noble & Cookson, 1987) high level mupirocin resistance in *Staphylococcus aureus* isolated from patients in the dermatology wards at our hospital. Others have also reported high level resistance (Smith & Kennedy, 1988). This paper reports details of the outbreak of colonization/infection in our wards and the initial studies on transmissibility and nature of the resistance genes.

MATERIALS AND METHODS

There are two wards for dermatology patients (E and B) each consisting of 5 bays of 4–6 beds and 4 single bedded side rooms. Patients admitted to these wards are in the care of several different consultants but shared junior staff.

S. aureus isolates from clinical microbiology specimens were routinely tested for resistance to penicillin (10 i.u.), tetracycline (30 μg), erythromycin (15 μg), gentamicin (10 μg) fusidic acid (10 μg) and neomycin (30 μg) using a disk diffusion

Correspondence to: Professor W. C. Noble, Department of Microbiology, Institute of Dermatology, United Medical and Dental Schools, Lambeth Hospital, London SE11 4TH, UK.

method on Mueller–Hinton agar at 37 °C and to methicillin (5 μg) at 30 °C; all disks and media are from Oxoid. Isolates were not tested for resistance to mupirocin unless the request form stated that this antibiotic had been prescribed. When the first mupirocin resistant isolate was recovered, all patients in E ward were screened for carriage of mupirocin-resistant *S. aureus* in the nose, throat, perineum and any skin lesions using swabs moistened in peptone water. Similar studies were made at weekly intervals until we were convinced that all mupirocin resistant staphylococci had disappeared. Thereafter all *S. aureus* isolates from patients admitted to the wards have been tested for resistance to mupirocin by inclusion of a 5 μg disk in the routine testing.

The minimal inhibitory concentration of mupirocin was determined by incorporating the antibiotic in blood agar base (Oxoid) and spot-inoculating *c.* 10^4 cells in broth culture followed by overnight incubation at 37 °C. Phage-typing was carried out at the Institute laboratories using the International set of phages and standard techniques, and also by the Division of Hospital Infection, Central Public Health Laboratory. Plasmid profiling was carried out as described in Dowsett *et al.* (1984). Curing of resistance was attempted by growing staphylococci at 42 °C with repeated subculture (Asheshov, 1966). Resistance transfers on mouse skin and on filters were made as described in Naidoo & Noble (1987), mixed broth culture studies were made as described by Cookson & Phillips (1988) and attempts to induce phage made using mitomycin C. Centrifugation in caesium chloride/ethidium bromide was carried out using an MSE Europa centrifuge at 50000 rev./min. for 45–65 h. Endonuclease cleavage of density gradient derived DNA was carried out according to the suppliers instructions using *Eco* RI, *Hin*d III and *Bam* HI (Gibco Ltd).

RESULTS

When the index case (patient A) was noted on ward E, all patients were screened and a further six carriers in ward E were detected (Fig. 1). A single positive patient (N) was later revealed on ward B; records showed that two patients B and C had been transferred from ward E to B for Christmas though patient N was not present at this time. No further carriers were detected on ward B but several more became positive on ward E. Swabs from the nose and any skin lesions of staff failed to reveal any carriers of mupirocin-resistant staphylococci and environmental contamination was slight (data not shown).

Clinicians were requested to withdraw mupirocin therapy and heavily colonized patients were nursed in the side rooms. The value of Hibisol (ICI) hand disinfection between patients was emphasised for staff. Application of chlorhexidine-containing products to patients' skin was generally contra-indicated for fear of exacerbating skin lesions.

A study of prescriptions issued on the ward revealed that five patients (A, B, C, D, N) had been receiving mupirocin for several weeks or months, without our knowledge. In all five cases the antibiotic had been prescribed for use as required and in three patients (B, C, D) this had been intermittent. Other therapies included topical steroids, flucloxacillin and erythromycin.

Retrospective testing of *S. aureus* isolates available from the weeks immediately

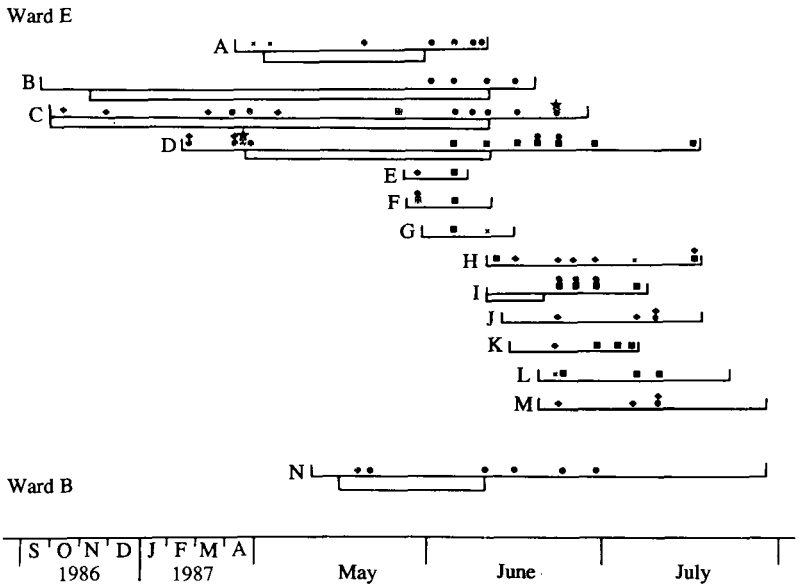

Fig. 1. Details of spreed of mupirocin resistant staphylococci in two hospital wards. Symbols: *Staphylococcus aureus* strains: ●, Pc^R Em^R Mu^R; ■, Pc^R Tc^R Em^R Mu^R; ☮, Pc^R Em^R Mu^S; ★, Pc^R Tc^R Em^R Mu^S; ⊕, Pc^R Em^R Mu not tested; ⊞, Pc^R Tc^R Em^R Mu not tested; ◆, other strains; ×, *S. aureus* not isolated. Block underlining indicates period of mupirocin therapy.

prior to the outbreak revealed only one further mupirocin-resistant isolate, a blood culture contaminant from patient B isolated at the same time as the 'index' case. Sixteen isolates of *S. aureus* which were resistant to penicillin and erythromycin or to penicillin, tetracycline and erythromycin were available from the period October 1986 to February 1987; all were mupirocin sensitive and were of different phage types to the epidemic strains.

The epidemic strains

Mupirocin resistance at MIC > 2000 mg/l was found in two strains of *S. aureus*. One strain was also resistant to penicillin and erythromycin and the other to penicillin, erythromycin and tetracycline. Although both were lysed by several phages of group III, the plasmid profiles were so different (Fig. 2) as to suggest that they were wholly independent. In some instances mupirocin sensitive isolates were available which showed the same remaining antibiotic resistance pattern, phage type and plasmid profile as the subsequent mupirocin resistant strains (see Fig. 1). Patient C had at one stage carried an epidemic methicillin-resistant *S. aureus* (EMRSA) (Cookson & Phillips, 1988). None of the five available isolates of EMRSA from this patient was mupirocin resistant but mupirocin resistance transfer was achieved to this strain (see below).

Prospective studies in the two wards for the 6 months following the end of the epidemic revealed that 989 staphylococcal isolates from 191 colonized patients were reported by the clinical laboratory. Only two isolates were mupirocin resistant and these came from patient B during a routine readmission.

https://doi.org/10.1017/S0950268800029939 Published online by Cambridge University Press

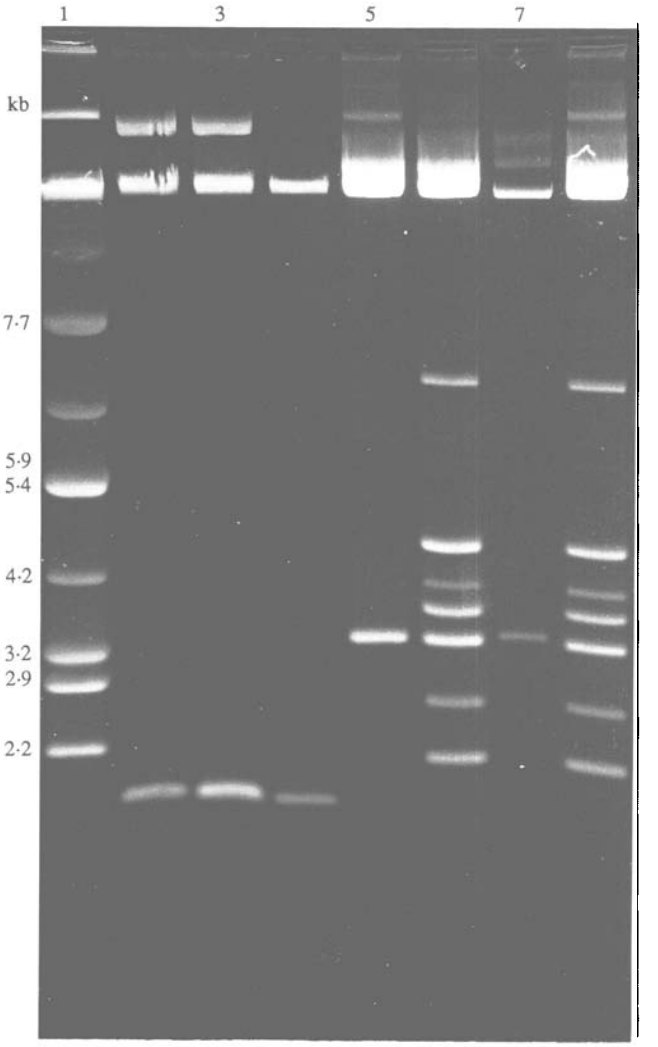

Fig. 2. Plasmid profiles of DNA from strains isolated from patients. Lane 1, *Escherichia coli* V517 standards. Lanes 2 and 3, EMRSA from patient C. Lane 4, EMRSA with gentamicin plasmid cured. Lanes 5 and 7, Pc^R Em^R Mu^R epidemic strain. Lanes 6 and 8, Pc^R Tc^R Em^R Mu^R epidemic strain.

Table 1A. *Characteristics of isolates used as donors in transfer experiments*

Isolate *S. aureus*	Resistance pattern*	Phage pattern	Plasmid profile (kb)
C	Pc Em Mu	6/47/54/77	3·1, 30†
B	Pc Em Mu	6/47/54/77	3·1, 30
F4A	Pc Tc Em Mu	6/47/54/77	2·2, 2·7, 3·1, 3·4, 4·2, 30
F4B	Pc Tc Em Mu	6/47/54/77	2·2, 2·7, 3·4, 4·2, 30
G	Pc Tc Em Mu	6/47/54/77	2·2, 2·7, 3·1, 3·4, 4·2, 30

* Resistances only shown, see text for full panel.

† Measured as intact plasmids on agar gels; large plasmids therefore subject to large error.

https://doi.org/10.1017/S0950268800029939 Published online by Cambridge University Press

Table 1 B. *Characteristics of isolates used as recipients in transient experiments*

Isolate	Resistance pattern	Phage type	Plasmid profile (kb)	Source
8325 Rif	Rif*	29/47/54/75/84/85	—	NCTC 8325
8325 Rif Fd	Rif Fd†	29/47/54/75/84/85	—	NCTC 8325
8325 Rif Fd GBW	Gm Rif Fd‡	29/47/54/75/84/85	pJ BW 30	NCTC 8325
C S/G Rif Fd	Pc Tc Em Me	NT, 85w	1·8	This study
C NAB Rif Fd	Pc Tc Em Gm Me	83A, 83A/85	1·8, 30	This study; contains the gentamicin and antiseptic resistance plasmid for EMRSA (NAB plasmid)
80CR5 Rif Fd	Rif Fd	29/47/84	—	Engel, see Naidoo & Noble (1987)
8325–4	Rif, Nov§	NT	—	Novick (1967)
WG 3358	Rif, Nov§	NT (Inh, 6, 75, 77)	—	Townsend *et al.* (1987)
BC 3HG	Rif, Nov§, Gm, Pc, Prop, Eth.	Not done	C.30	This study; this is WG 3358 containing the NAB plasmid (Cookson & Phillips, 1988)
W 57	Rif, Nov§	Not done	—	Winkler *et al.* (1965)
RN450	Rif, Nov§	Not done	—	Novick (1967)

Inh, Inhibition reactions at RTD100.
* Made chromosomally resistant to > 80 mg/l rifampicin.
† Made chromosomally resistant to > 30 mg/l fusidic acid.
‡ Contains the conjugative gentamicin plasmid from *S. hominis* BW (Naidoo & Noble, 1987).
§ Chromosomal resistance to > 8 mg/l of novobiocin.

Table 2. *Curing of mupirocin resistance and other antibiotic resistances*

Isolate	Total colonies studied	Colonies now sensitive to		
		Mupirocin	Erythromycin	Tetracycline
F4B	451	113	27	1
B	260	41	28	NA
G	87	8	8	0

NA, not applicable, original strain sensitive.

Table 3. *Frequency of transfer of mupirocin resistance in filter-mating experiments given as transferants per final number of recipients*

Recipient	Donor		
	C	G	F4A
8325 Rif	9×10^{-7}, 6×10^{-5}	7×10^{-7}, 7×10^{-5}	3×10^{-7}
8325 Rif Fd GBW	$1{\cdot}4 \times 10^{-4}$, 3×10^{-6}, 3×10^{-7}	7×10^{-5}, $2{\cdot}5 \times 10^{-6}$	N.D.
C MRSA R/G	6×10^{-5}, 3×10^{-5}	$1{\cdot}6 \times 10^{-5}$	N.D.
C MRSA S/G1	3×10^{-5}, 8×10^{-5}	4×10^{-5}	N.D.
RN 450	1×10^{-6}	N.D.	3×10^{-5}, 3×10^{-5}
WG 3358	5×10^{-7}	N.D.	1×10^{-4}
BC 3HG	N.D.	N.D.	1×10^{-4}, 3×10^{-3}, 6×10^{-3}
W 57	3×10^{-7}	N.D.	3×10^{-5}, 4×10^{-5}, 2×10^{-5}

N.D., not done.

Laboratory studies

The donor isolates used in laboratory studies are shown in Table 1A.

Initial experiments revealed that high level mupirocin resistance was readily lost from strains F4B, B and G under conditions of elevated temperature at which other resistances were also lost (Table 2). Electrophoresis of cured isolates showed that loss of erythromycin resistance was associated with loss of a plasmid band at about 2·7 kb and loss of tetracycline resistance with loss of a plasmid band at about 4·2 kb. However, no plasmid band could be associated with loss of mupirocin resistance. This was initially felt to be due partly to the faint OC and linear forms of other plasmids, especially in the Pc^R Tc^R Em^R isolates.

Accordingly, mupirocin resistance was transferred into a number of recipient strains (Table 1B) known to contain no plasmids or to contain a specific plasmid. The results for transfer frequencies in filter mating are shown in Table 3. No high level mupirocin resistance was detected when only the recipient strain was inoculated on to selective agar.

Transfer occurred at comparable rates even when 0·02 M sodium citrate was incorporated in the agar on which the filter was incubated (data not shown). Culture supernatants and mitomycin C induction lysates failed to transfer mupirocin resistance (data not shown).

In vivo transfer of mupirocin resistance occurred to recipient 8325 Rif from F4B (2×10^{-3}) and G (1×10^{-5}). These are mean rates for two animals in each instance.

https://doi.org/10.1017/S0950268800029939 Published online by Cambridge University Press

Table 4. *Serial transfer of mupirocin resistance*

	Donor	
Recipient	8325 Rif Mup (C)	8325 Rif Mup (G)
80CR5 Rif Fd	6×10^{-6}	2×10^{-6}
8325 pI 524	$1{\cdot}8 \times 10^{-6}$	1×10^{-6}

Selection of 8325 pI 524 was made on agar containing cadmium and mupirocin.

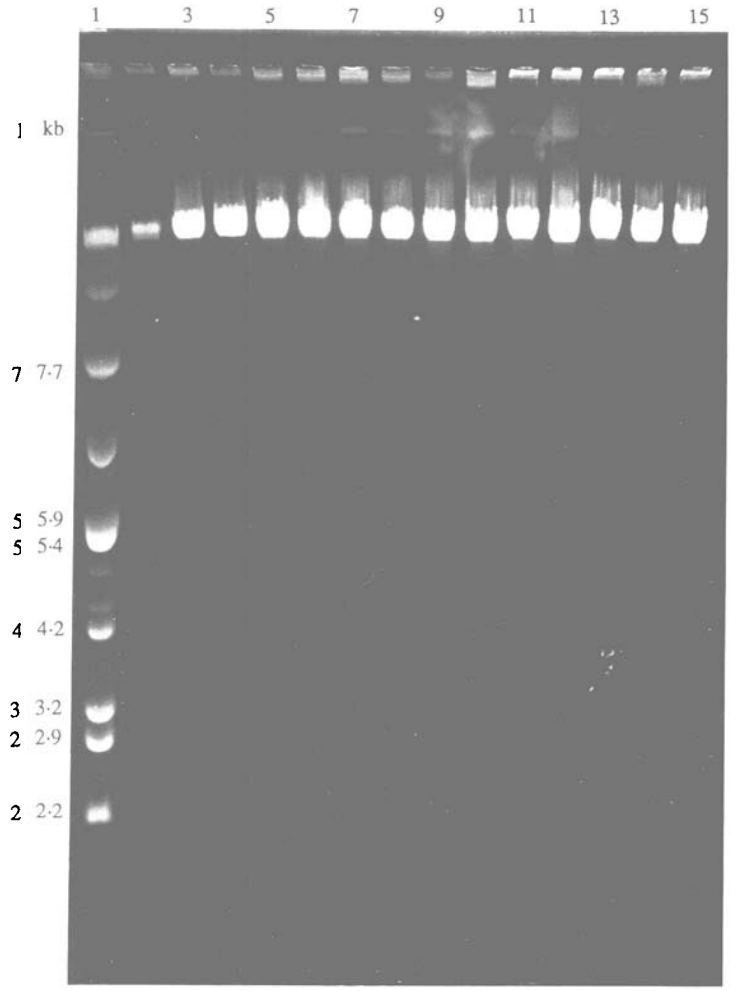

Fig. 3. Gel electrophoresis of DNA from mupirocin-resistant transferants. Lane 1, *Escherichia coli* V517 standards. Lane 2, *S. aureus* 80CR5 recipient Mu^S. Lanes 3 and 11–15, *S. aureus* 80CR5 Mu^R transferants from filter matings with epidemic strain from patient C. Lanes 4–10, from patient G.

Several representative colonies of each successful transfer were checked for the appropriate antibiotic sensitivity and plasmid profile in order to eliminate breakthrough of the donor, an acknowledged problem when rifampicin or fusidic acid are used as selective agents. Transferants were resistant to mupirocin at the same high level as the donors.

https://doi.org/10.1017/S0950268800029939 Published online by Cambridge University Press

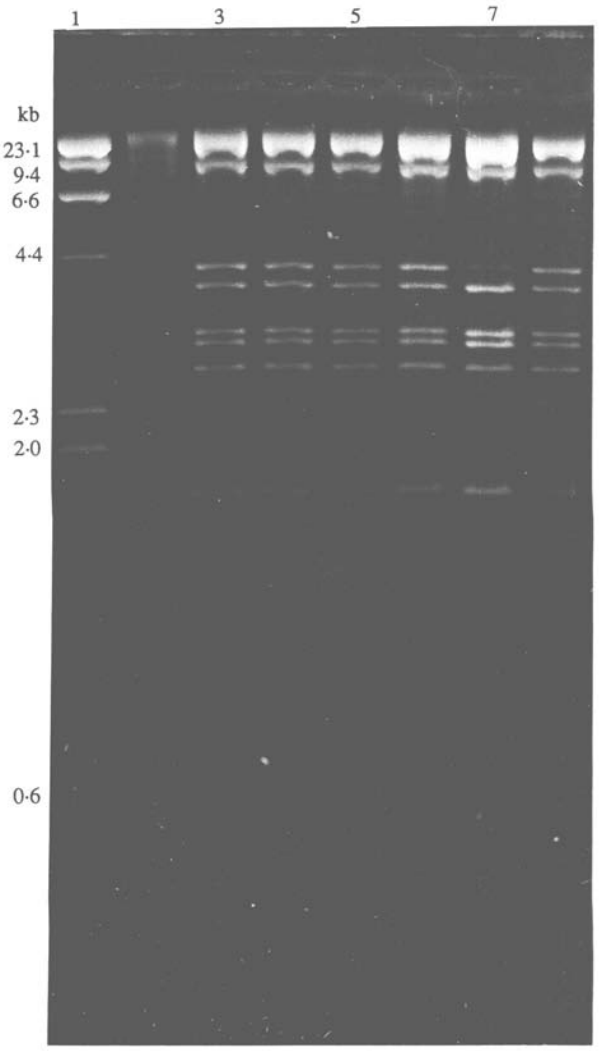

Fig. 4. *Eco* RI cleavage patterns of DNA from caesium chloride density gradients. Lane 1, *Hin*dIII cleaved standard from Gibco. Lane 2, *S. aureus* 8325 recipient Mu^S. Lanes 3–8, *S. aureus* 8325 Mu^R DNA from transferants from filter matings with epidemic strains from patients G, E, F, C, B, N.

Mupirocin resistance could also be serially transferred from 8325 to other 8325 derivatives and to 80CR5 as shown in Table 4. Transferants could also be cured of mupirocin resistance.

Electrophoresis of transferants showed that some possessed a sharp band at about 30 kb (Fig. 3) and it was initially assumed that this represented the 'Mupirocin-resistance plasmid'. Not all transferants possessed this band and a faint band could be seen in 8325 sensitive to mupirocin; when present, the sharp band was brighter if the isolates had first been grown on media containing mupirocin at 20 mg/l. Caesium chloride centrifugation of DNA prepared from eight transferants in 8325 revealed only one band, that corresponding to the chromosomal DNA in control preparations.

Endonuclease cleavage of this density gradient derived, apparently chromosomal, DNA showed that several bright bands could be seen in electrophoretic gels of DNA prepared from resistant transferants. These were superimposed on the faint bands of cleaved chromosomal DNA also seen in the sensitive controls (Fig. 4). Each enzyme gave different bands and cleavage by two enzymes gave the expected increase in number of bands and decrease in band size indicated by increased mobility in the gel. All transferants gave the same band pattern.

DISCUSSION

The epidemiology of this outbreak has not been fully investigated because we were alerted to the possibility of resistance only after five patients had been receiving intermittent mupirocin therapy for long periods. The presence of high level resistance in two distinct strains of *S. aureus* in one ward suggested to us that the resistance might be transferable.

Initial results indicated that mupirocin resistance might be located on a conjugative plasmid in the same manner as gentamicin. The rates of transfer were about the same as for gentamicin in our experience (Naidoo & Noble, 1978) and in that of others (McDonnell, Sweeney & Cohen, 1983; Forbes & Schaberg, 1983) and transfer occurred on mouse skin at lower densities of organisms than was the case on filters. It seemed improbable that selection of high level resistance amongst the recipients occurred in the absence of gene-transfer because (*a*) controls comprising only recipients failed to yield resistant variants, (*b*) acquisition of resistance occurred only in the presence of certain potential donors (data not shown).

If a plasmid band had been found consistently in all transferants we should have concluded that plasmid transfer, probably conjugative transfer, had occurred. However plasmid bands were not always seen and it is doubtful if the sharp band at *c.* 30 kb represents conventional closed circular plasmid DNA. Centrifugation in density gradients also failed to reveal CCC plasmid DNA though endonuclease cleavage of this DNA seems to reveal extra-chromosomal DNA associated with the transfer of high level mupirocin resistance. Conjugative plasmids bearing genes for gentamicin resistance are well established in *S. aureus* and the conjugative transfer of aminoglycoside resistance in the absence of plasmid DNA has been recorded (El Sohl *et al.* 1986). Conjugative transposons which might also explain the phenomenon have not yet been reported in *S. aureus* (Murphy, 1988) though well established in streptococci (Clewell, 1986). The results reported here seem more likely to reflect a new type of plasmid element, perhaps similar to the open circular plasmids described by Townsend *et al.* (1986), co-migrating with the chromosomal DNA in both agarose gels and caesium chloride density gradients, than to be a multicopy transposon which is enriched during preparative procedures designed to demonstrate plasmid DNA.

REFERENCES

Asheshov, E. H. (1966). Loss of antibiotic resistance in *Staphylococcus aureus* resulting from growth at high temperature. *Journal of General Microbiology* **42**, 403–410.

Clewell, D. B. (1986). Conjugative transposons and the dissemination of antibiotic resistance in streptococci. *Annual Review of Microbiology* **40**, 635–659.

COOKSON, B. D. & PHILLIPS, I. (1988). Epidemic methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy* **21**, Suppl. C. 57–65.

DOBSON, R. L., LEYDEN, J. J., NOBLE, W. C. & PRICE, J. D. (1985). *Bactroban* (*Mupirocin*). Proceedings of an International Symposium. *Excerpta Medica*.

DOWSETT, E. G., PETTS, D. N., BAKER, S. L., DESAXE, M. J., COE, A. E., NAIDOO, J. & NOBLE, W. C. (1984). Analysis of an outbreak of staphylococcal scalded skin syndrome: strategies for typing 'non-typable' strains. *Journal of Hospital Infection* **5**, 391–397.

EL SOHL, N., ALLIGNET, J., BISMUTH, R., BURET, B. & FOUACE, J. M. (1986). Conjugative transfer of staphylococcal antibiotic resistance markers in the absence of detectable plasmid DNA. *Antimicrobial Agents and Chemotherapy* **30**, 161–169.

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.

MCDONNELL, R. W., SWEENEY, H. M. & COHEN, S. (1983). Conjugational tranfer to gentamicin-resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrobial Agents and Chemotherapy* **23**, 151–160.

MURPHY, E. (1988). Transposable elements in *Staphylococcus*. In *Transposition*, pp. 59–89. Cambridge: Cambridge University Press.

NAIDOO, J. & NOBLE, W. C. (1978). Transfer of gentamicin resistance between strains of *Staphylococcus aureus* on skin. *Journal of General Microbiology* **107**, 391–393.

NAIDOO, J. & NOBLE, W. C. (1987). Skin as a source of transferable antibiotic resistance in coagulase-negative cocci. *Zentralblatt für Bakteriologie*, Supplementum **16**, 225–234.

NOVICK, R. P. (1967). Properties of a cryptic high frequency transducing phage in *Staphylococcus aureus*. *Virology* **33**, 155–166.

RAHMAN, M., NOBLE, W. C. & COOKSON, B. D. (1987). Mupirocin-resistant *Staphylococcus aureus*. *Lancet* ii, 387.

SMITH, G. E. & KENNEDY, C. T. C. (1988). *Staphylococcus aureus* resistant to mupirocin. *Journal of Antimicrobial Chemotherapy* **21**, 141–142.

TOWNSEND, D. E., ASHDOWN, N., BOLTON, J., DUCKWORTH, G., MOORHOUSE, E. C. & GRUBB, W. B. (1987). The International spread of methicillin-resistant *Staphylococcus aureus*. *Journal of Hospital Infection* **9**, 60–71.

TOWNSEND, D. E., BOLTON, S., ASHDOWN, N., ANNEAR, D. I. & GRUBB, W. B. (1986). Conjugative staphylococcal plasmids carrying hitch-hiking transposons similar to Tn 554: intra and inter-species dissemination of erythromycin resistance. *Australian Journal of Experimental Biology and Medical Science* **64**, 367–379.

WHITE, A. R., BEALE, A. S., BOON, R. J., GRIFFIN, K. E., MASTERS, P. J. & SUTHERLAND, R. (1985). Antibacterial activity of mupirocin. In Bactroban (mupirocin). Proceedings of an International Symposium. (ed. R. L. Dobson, J. J. Leyden, W. C. Noble & J. D. Price). *Excerpta Medica*, pp. 19–36.

WINKLER, K. C., DE WAARF, J., GROOTSEN, C., ZEGERS, B. J. M., TELLIER, N. F. & WERTREGT, C. D. (1965). Lysogenic conversion of staphylococci to loss of B toxin. *Journal of General Microbiology* **39**, 321–333.