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Mupirocin resistance in staphylococci: development and transfer of isoleucyl-tRNA synthetase-mediated resistance *in vitro*

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D.G. THOMAS, J.M. WILSON, M.J. DAY AND A.D. RUSSELL. 1999. Mupirocin resistance could be transferred from highly resistant clinical isolates of *Staphylococcus aureus* to highly sensitive recipients of *Staph. aureus*, *Staph. epidermidis* and *Staph. haemolyticus*. Transconjugants of the latter two organisms could transfer this resistance into mupirocin-sensitive *Staph. aureus*. Moderately resistant strains did not transfer this resistance to sensitive recipients, nor did strains with high-level mupirocin resistance developed by serial transfer or habituation. The inhibitory effects of mupirocin on crude isoleucyl-tRNA synthetases (IRS) isolated from mupirocin-sensitive and -resistant strains of *Staph. aureus* have been determined. Drug concentrations needed to produce 50% inhibition, I_{50} values, were very low against IRS from a highly sensitive strain, somewhat higher against IRS from moderately resistant strains, much higher against enzyme from strains trained *in vitro* to high-level resistance, and considerably higher still against IRS extracted from clinical isolates possessing high-level mupirocin resistance and from the transconjugates of such strains resulting from crosses with mupirocin-sensitive strains. It is concluded that high-level resistance in clinical isolates is plasmid-mediated involving a second, mupirocin-resistant IRS whereas in moderately resistant strains, and in strains trained *in vitro* to high-level resistance, chromosomal mutations are likely to be responsible for decreasing IRS sensitivity.

INTRODUCTION

Mupirocin (pseudomonic acid A) is a narrow-spectrum antibiotic originally isolated from *Pseudomonas fluorescens* (Sutherland *et al*. 1985; Hill *et al*. 1988). It is active predominantly against staphylococci and more permeable Gram-negative species such as *Hemophilus* and *Neisseria*. Enterobacteriaceae are intrinsically resistant due to a permeability barrier (Al-Masaudi *et al*. 1988). In staphylococci, mupirocin-resistant isolates which show a high level of resistance to this antibiotic have occasionally been clinically isolated. Moderately resistant strains of *Staph. aureus* have also been isolated (Rahman *et al*. 1987, 1989, 1990, 1993). Mupirocin is used topically as an ointment against methicillin-resistant *Staph. aureus* (MRSA), usually for periods of not less than 10 d.

The mechanism of antibacterial action of mupirocin involves specific inhibition of bacterial isoleucyl tRNA synthetase (IRS; Hughes and Mellows 1978a,b, 1980; Capobianco *et al*. 1989). The mupirocin structure resembles the isoleucyl adenylate complex and thus, the target for its activity is the first part of the aminoacylation reaction in which isoleucyl adenylate is formed.

In this paper, the development and transferability of mupirocin resistance is described.

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MATERIALS AND METHODS

Chemicals and antibiotics

Mupirocin was a gift from SmithKline Beecham, Brockham Park, Betchworth, Surrey. Chemicals were purchased from Sigma.

Bacterial strains and culture

The strains used are listed in Table 1 together with their resistance phenotypes, which were used as markers in the transfer experiments. The strains were routinely cultured on nutrient agar (Oxoid) and their identity confirmed by conventional tests and the API STAPH (BioMerieux). Diagnostic sensitivity test (DST) agar (Oxoid) was used instead of nutrient agar when culturing coagulase-negative staphylococci, due to their poor growth on nutrient agar.

Determination of minimum inhibitory concentrations (MIC)

A 2 ml volume of the appropriate concentration of antibiotic was added to 18 ml molten agar to give a series of doubling concentrations from 0·13 to 512 μg ml^{-1}. The plates were poured and overdried before use. Overnight cultures of test bacteria were diluted 1 : 100, and 10 μl were spotted onto the plates using the Denley multipoint inoculator (Denley, Billingshurst, UK) and incubated at 37 °C for 48 h. The MIC was taken as being the lowest concentration of antibiotic that prevented growth.

Stepwise development of mupirocin resistance

Cultures containing a series of increasing concentrations of mupirocin in 10 ml volumes of nutrient broth (Oxoid) were inoculated with 100 μl of an overnight culture of moderately mupirocin-resistant *Staph. aureus*. The cultures were incubated at 37 °C until there was a clear differential of growth between two dilutions. The culture with the highest concentration of antibiotic which permitted growth was used to inoculate a further series of media containing increasing concentrations of mupirocin. This procedure was repeated for a maximum of 10 sub-cultures. Mupirocin resistance thus produced was found to be stable. The resistant variants selected were tested for cross-resistance to other antibiotics.

Antibiotic disc susceptibility testing

A sterile swab dipped into an overnight culture of test organism was used to inoculate evenly DST agar. When the inoculum had dried, the antibiotic sensitivity discs (Oxoid) were placed on the inoculated plates (no more than six to a plate) and incubated overnight at 37 °C. *Staphylococcus aureus* NCTC 6571 acted as control organism.

Table 1 Staphylococcal strains† fused in transfer experiments or isoleucyl-tRNA synthetase studies

Organism	Resistance phenotype‡	Organism	Resistance phenotype
L2*	Mup Gm Ery Te Met Pen Amp Km	F89*	Mup
L8*	Mup Gm Ery Te Met Pen Amp Km	C7*	Mup
Kelesh*	Mup Ery Te Pen	C9**	Mup
Eagles*	Mup Ery Te Pen	C20**	Mup
Sau 2	Sm Fd (Mup^s)	11561 (R)	Rif
KS*	Transconjugant of Kelesh × Sau 2	RN450 (R)	Rif
ES*	Transconjugant of Eagles × Sau 2	RN 2677	Rif Nov
Clarke (T0)**	Mup Gm Ery Te Met	6571 (R)	Rif
Clarke (T10)*	Mup Gm Ery Te Met	UHW 1	Gm Met Pen Amp Cef
K227 (T0)**	Mup Gm Ery Te	UHW 2	Gm Rif Med Cip Cef
K227 (T8)*	Mup Gm Ery Te	UHW 4	Gm Met Amp Cef
G1217 (T0)**	Mup Ery	UHW 7	Gm Met Pen Amp Cip Cef
G1217 (T10)*	Mup Ery	UHW 8	Gm Met Amp Cef

† All strains are *Staphylococcus aureus* except for UHW 1, UHW 2, UHW 4 (*Staph. epidermidis*) and UHW 7, UHW 8 (*Staph. haemolyticus*).

Amp, ampicillin; Cip, ciprofloxacin; Ery, erythromycin; Fd, fusidic acid; Gm, gentamicin; Km, kanamycin; Met, methicillin; Mup, mupirocin; Nov, novobiocin; Pen, benzylpenicillin; Rif, rifampicin; Sm, streptomycin; Te, tetracycline.

* High-level mupirocin resistance (MIC >512 μg ml^{-1}).

** Moderate-level mupirocin resistance (MIC 8–32 μg ml^{-1}).

Mating procedure

Donor and recipient cells were grown overnight at 37 °C in 10 ml nutrient broth; 1 ml of the donor cell culture and 3 ml of the recipient cell culture were mixed, and the mixture filtered (Millipore filter, pore size 0·4 μm). The filter was placed, with the bacterial cells facing upwards, onto a nutrient agar plate (DST agar was used for transfers involving coagulase-negative staphylococci as recipients). After incubation overnight at 37 °C, the filter was removed and vortexed in 1 ml nutrient broth. The suspended mating mixtures were serially diluted and dilutions spread onto plates containing appropriate selective antibiotics at a concentration of 5 μg ml^{-1}. Donor and recipient controls were always plated separately. Colonies were counted after incubation for 48 h at 37 °C. The transfer frequencies were expressed as the number of transconjugants per recipient cell. The direction of transfer was determined by replica plating and utilizing secondary markers not used for selection in the original cross. All crosses were repeated three times unless stated otherwise, and all the results are presented as mean values.

Extraction of crude IRS and determination of activity

A 10 ml aliquot of an overnight broth culture of the test organism was inoculated into 500 ml nutrient broth in a 2 litre flask and incubated at 37 °C in a shaking water-bath at 200–220 rev min^{-1}. Cell growth was monitored spectrophotometrically at an absorbance of 660 nm. When the culture was at the top of the exponential phase, the cells were harvested.

The culture was harvested in the RC5C centrifuge (Dupont, Stevenage, UK) at 5500 rev min^{-1} and 4 °C for 10 min. The cell pellet was washed with phosphate-buffered saline (PBS) and centrifuged twice before determining the weight of the pellet. The cells were resuspended in lysis/sonication buffer (20 mmol l^{-1} Tris, 2 mmol l^{-1} dithiothreitol (DTT), 5 mmol l^{-1} $MgCl_2$, 0·5 mmol l^{-1} EDTA, pH 8·2) to a final density of 0·4 g cells ml^{-1} of buffer. Lysostaphin and DNAse 1 were added to final concentrations of 150 μg ml^{-1} and 15 μg ml^{-1}, respectively, and incubated overnight at 4 °C. The suspension was sonicated for five cycles of 20 s on and 30 s off at 18–20 microns amplitude. The broken cell suspension was centrifuged at 19 000 rev min^{-1} for 20 min. The supernatant fluid was removed and glycerol added to a final concentration of 30% (v/v). The sample was stored at −20 °C until required for use. The dilute crude IRS in enzyme buffer (Tris 0·138 g in 9 ml H_2O, pH 8·2) was placed on ice; 2 ml 100 mmol l^{-1} DTT solution were then added and the solution made up to 100 ml with water. From this, 1 : 2·5, 1 : 5, 1 : 10, 1 : 20 and 1 : 50 dilutions were prepared. A 50 μl volume of each dilution was added to 100 μl reagent mixture of the following composition: buffer 5 ml, ATP 2·5 ml (30 g l^{-1}), tRNA 2·5 ml (20 g l^{-1}), ^{14}C isoleucine 1·0 ml, water 9 ml. This was incubated for 10 min; 2 ml of 7% trichloracetic acid (TCA) were then added and the samples left on ice for 30 min to allow the precipitate to form.

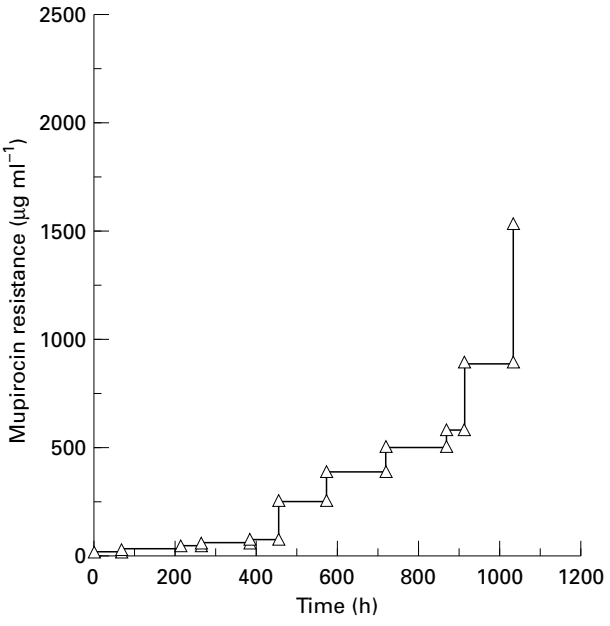

Fig. 1 Stepwise development of resistance to mupirocin in *Staphylococcus aureus* Clarke (MIC of mupirocin against parent strain, 16 μg ml^{-1})

Determination for I_{50} for crude IRS sample

Aliquots (10 μl) of the appropriate dilution of mupirocin or water were placed in reaction vials and equilibrated at 37 °C. Diluted IRS (50 μl) was added and incubated for 5 min, then 100 μl of warmed reagent mixture were added. After incubation for 15 min, 2 ml 7% TCA were added and the samples left on ice for 30 min to allow the precipitate to form.

Filtration and counting

The reaction mixture from the vial was deposited onto a Millipore filtration manifold. The vial was rinsed with 2 ml TCA and the washings deposited on the same filter. The filter was washed with 2 × 10 ml aliquots of TCA and 2 × 10 ml aliquots of ethanol. The filters were then placed in a scintillation vial in 6 ml Optisafe scintillation fluid. The vials were then counted for 2 min. The percentage inhibition of IRS activity at each concentration was calculated as follows:

$$\% \text{ inhibition} = (C - S) \times 100/C$$

where C is the control count and S is the sample count for the concentration of enzyme. The percentage inhibition was

Table 2 Mupirocin transfer frequencies from highly resistant strains of *Staphylococcus aureus* to sensitive strains of *Staph. aureus*

	Donor (Mup^r)					
Recipient (Mup^r)	L2	L8	Kelesh	Eagles	F89	C7
Sau2	$4{\cdot}6 \times 10^{-6}$	$2{\cdot}1 \times 1{\cdot}0^{-6}$	$9{\cdot}7 \times 10^{-6}$	$4{\cdot}4 \times 10^{-6}$	$<1{\cdot}9 \times 10^{-6}$	$3{\cdot}2 \times 10^{-6}$
11561 (R)	$<3{\cdot}9 \times 10^{-9}$	$<2{\cdot}2 \times 10^{-9}$	$<7{\cdot}7 \times 10^{-10}$	$<6{\cdot}7 \times 10^{-10}$	$<2{\cdot}6 \times 10^{-9}$	$<8{\cdot}1 \times 10^{-10}$
RN450 (R)	$<1{\cdot}1 \times 10^{-10}$	$<1{\cdot}7 \times 10^{-9}$	$<5{\cdot}8 \times 10^{-10}$	$<4{\cdot}5 \times 10^{-10}$	$<2{\cdot}6 \times 10^{-9}$	$<6{\cdot}4 \times 10^{-10}$
6571 (R)	$<4{\cdot}3 \times 10^{-10}$	$<4{\cdot}8 \times 10^{-10}$	$<6{\cdot}5 \times 10^{-10}$	$<4{\cdot}9 \times 10^{-10}$	$<1{\cdot}8 \times 10^{-9}$	$<2{\cdot}1 \times 10^{-9}$
RN2677	$4{\cdot}4 \times 10^{-6}$	$1{\cdot}2 \times 10^{-6}$	$4{\cdot}0 \times 10^{-6}$	$1{\cdot}6 \times 10^{-6}$	$<1{\cdot}2 \times 10^{-9}$	$1{\cdot}0 \times 10^{-6}$

Figures are transfer frequencies.
(R), trained to rifampicin resistance.

then plotted against mupirocin concentrations for each crude IRS sample, and the I_{50} calculated as the concentration (μg l^{-1}) of mupirocin that reduced the activity of IRS by 50%. IRS determinations were carried out in duplicate.

RESULTS

Training to mupirocin resistance

Three mupirocin-sensitive strains of *Staph. aureus* (NCTC 6571, 11561 and RN2677) did not readily gain mupirocin resistance, a frequency of $<1 \times 10^{-7}$ cells spontaneously mutating to give a resistance (MIC) of $>5\,\mu$g ml^{-1}. Conversely, three strains of *Staph. aureus* with moderate resistance to the antibiotic (MICs 8–32 μg ml^{-1}, i.e. Clarke, G1217 and K227) developed high resistance (MICs >512 μg ml^{-1}) when trained to grow on media containing the drug. An example is provided in Fig. 1. These new strains were termed *Staph. aureus* Clarke (T10), G1217 (T10) and K227 (T8), where the figure in brackets indicates the number of transfers for each strain to become highly mupirocin-resistant.

Moderately resistant strains and highly resistant trained strains possessed stable mupirocin resistance. By comparison, the highly resistant *Staph. aureus* strains L2 and L8 became sensitive to mupirocin (MICs $<0{\cdot}25\,\mu$g ml^{-1}) when exposed to unfavourable conditions of incubation at 40 °C or growth in non-selective media.

Table 3 Mupirocin transfer frequencies from highly resistant strains of *Staphylococcus aureus* to mupirocin-sensitive coagulase-negative staphylococci

	Donor	
Recipient and strain	Kelesh	Eagles
Staph. epidermidis UHW 1	$<2{\cdot}9 \times 10^{-9}$	$<2{\cdot}4 \times 10^{-6}$
Staph. epidermidis UHW 2	$7{\cdot}4 \times 10^{-7}$	$1{\cdot}0 \times 10^{-6}$
Staph. epidermidis UHW 4	$<3{\cdot}2 \times 10^{-9}$	$<1{\cdot}9 \times 10^{-9}$
Staph. haemolyticus UHW 7	$2{\cdot}5 \times 10^{-8}$	$6{\cdot}8 \times 10^{-7}$
Staph. haemolyticus UHW 8	$4{\cdot}2 \times 10^{-8}$	$7{\cdot}9 \times 10^{-7}$

Transferability of mupirocin resistance between *Staph. aureus* strains

The initial series of transfer experiments detected transfer of high-level mupirocin resistance to mupirocin-sensitive *Staph. aureus* strains. Transconjugants were detected on plates containing mupirocin and a selective antibiotic for recipient, i.e. streptomycin for Sau 2, rifampicin for 11561 (R), RN450 (R) and 6571 (R), and novobiocin for RN2677. Neither donor nor recipient cultures alone grew on these media.

Mupirocin resistance was transferred at a frequency of $9{\cdot}7 \times 10^{-6}$ from highly resistant *Staph. aureus* strains L2, L8, Kelesh, Eagles and C7, but no transfer was demonstrated from strain F89. Resistance was transferred into two (Sau 2 and RN2677) of five recipient strains used (Table 2). Confirmation that mupirocin resistance had been transferred was obtained by testing the susceptibility of the possible transconjugants against secondary markers, in each case 50 colonies for each cross being tested. The donor strains (L2, L8, Kelesh and Eagles) were all resistant to erythromycin but the transconjugants from crosses with either Sau2 or RN 2677 were sensitive. All the transconjugants from crosses involving Sau2 were resistant to rifampicin. *Staphylococcus aureus* strain C7 is resistant to mupirocin only, but the transconjugants involving this strain with Sau 7 or RN2677 were resistant to at least three antibiotics. It can thus be concluded that mupirocin resistance was transferred from the donors to the recipients.

Mupirocin resistance was not transferred above detectable levels to mupirocin-sensitive strains from the moderately resistant *Staph. aureus* strains, or from the strains trained to high-level resistance.

Table 4 Transfer frequencies of mupirocin resistance from mupirocin-resistant coagulase-negative staphylococci (transconjugants) to mupirocin-sensitive strains of *Straphylococcus aureus*

	Donor*					
Recipient†	K2	E2	K7	E7	K8	E8
Sau2	$6{\cdot}4 \times 10^{-6}$	$3{\cdot}4 \times 10^{-6}$	$4{\cdot}2 \times 10^{-6}$	$2{\cdot}4 \times 10^{-6}$	$4{\cdot}6 \times 10^{-6}$	$2{\cdot}2 \times 10^{-6}$
RN2677	$5{\cdot}1 \times 10^{-6}$	$2{\cdot}2 \times 10^{-6}$	$4{\cdot}6 \times 10^{-6}$	$5{\cdot}8 \times 10^{-6}$	$3{\cdot}4 \times 10^{-6}$	$5{\cdot}6 \times 10^{-6}$

† Recipient, Mup^s *Staph. aureus* strains.
* Donor, Mup^r coagulase-negative staphylococci (transconjugants).

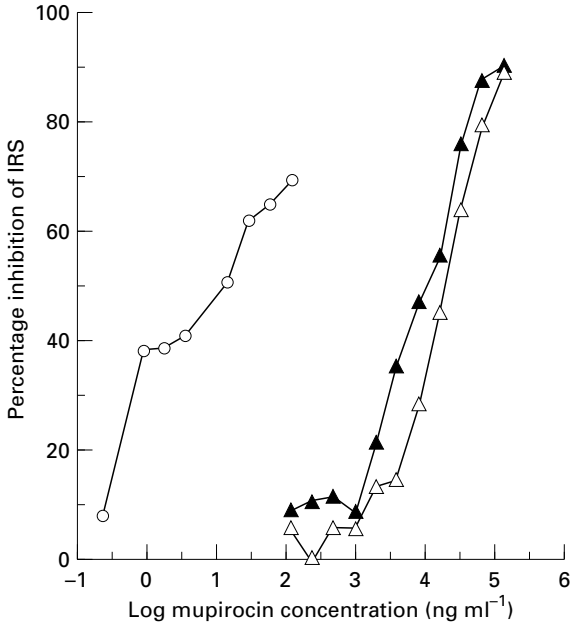

Fig. 2 Inhibition by mupirocin of isoleucyl-tRNA synthetase (IRS) extracted from some *Staphylococcus aureus* strains. (○) Sau2; (△) Kelesh; (▲) KS (transconjugant)

Table 5 I_{50} concentrations of mupirocin against IRS and sensitivity of *Staphylococcus aureus* strains to the antibiotic

Staph. aureus	I_{50} (ng ml^{-1})	MIC (μg ml^{-1})
Eagles	12 600	>512
Kelesh	14 100	>512
KS*	10 000	>512
ES†	11 200	>512
Sau2	12·6	0·5
K227 (T0)	15·8	16
K227 (T8)	891	>512
Clarke (T0)	17·8	16
Clarke (T10)	891	>512
G1217	5·6	8
G1217 (T10)	224	>512

* Transconjugants from *Staph. aureus* Kelesh × *Staph. aureus* Sau 2.
† Transconjugants from *Staph. aureus* Eagles × *Staph. aureus* Sau 2.

Interspecies transfer of mupirocin resistance

Donors (*Staph. aureus* strains Kelesh and Eagles) which showed a high level of mupirocin resistance (MICs >512 μg ml^{-1}) were used to study interspecies transferability. Transconjugants were selected on DST agar with mupirocin and gentamicin (an antibiotic to which the non-*Staph. aureus* strains were resistant; Table 1). The results (Table 3) demonstrate that mupirocin resistance was transferred into three (*Staph. epidermidis* UHW2, and *Staph. haemolyticus* UHW7 and UHW8) of the five recipient strains employed. The transconjugants were confirmed by their secondary markers. Strain Kelesh was a less efficient donor than Eagles. Transconjugants (K2 and E2) from the crosses of *Staph. aureus* Kelesh and Eagles with UHW 2 were resistant to rifampicin and sensitive to erythromycin, and those (K7, E7, K8 and E8) from the crosses with UHW7 and UHW8 were resistant to ampicillin and sensitive to erythromycin. Using the API STAPH system, transconjugants were confirmed as *Staph. epidermidis* or *Staph. haemolyticus*, thus demonstrating conclusively that mupirocin resistance had been transferred.

These mupirocin-resistant transconjugants of *Staph. epidermidis* and *Staph. haemoloyticus* transferred mupirocin resistance to mupirocin-sensitive *Staph. aureus* (Table 4).

Inhibition of IRS by mupirocin

The degrees of inhibition of IRS by different concentrations of mupirocin are shown in Fig. 2. In the donors, Kelesh and Eagles, and their respective transconjugants, the I_{50} values of mupirocin are high (Table 5) whereas in the recipient, Sau

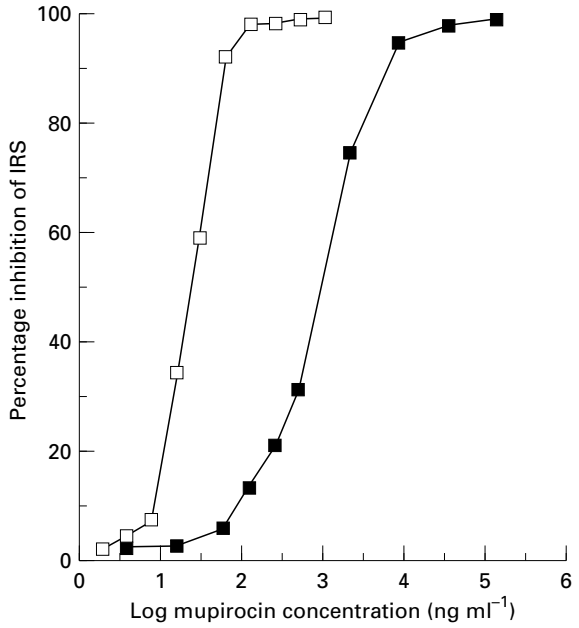

Fig. 3 Inhibition by mupirocin of isoleucyl-tRNA synthetase (IRS) extracted from a moderately-resistant strain (K227) and its trained highly-resistant strain, K227 (T8). (□) K227; (■) K227 (T8)

2, used in the transconjugant experiments, the I_{50} value of mupirocin is only 1/1000 of that value.

Some moderately resistant strains, and strains trained to high-level resistance, were also investigated. I_{50} values of mupirocin against IRS from the parent strains ranged from 5·6 to 17·8 μg ml^{-1} and from 224 to 891 ng ml^{-1} against IRS from the trained strains (see Table 5; Fig. 3 exemplifies the results obtained with K227 and K227 (T8)). I_{50} values against these highly resistant strains are thus considerably below those described for clinical isolates and their transconjugants.

DISCUSSION

Highly sensitive strains of *Staph. aureus* (MICs $<1\,\mu$g l^{-1}) could not be trained to mupirocin resistance, although moderately resistant strains (mupirocin MIC 8–16 μg ml^{-1}) could be trained to give a stable, high-level resistance (MIC $>512\,\mu$g ml^{-1}). This trained resistance could not, however, be transferred to mupirocin-sensitive recipients, which suggests that such resistance is chromosomally mediated rather than located on a plasmid. In this context, it is interesting to note the findings of Antonio *et al.* (1995) who concluded that moderate mupirocin resistance resulted from a point mutation on the *Staph. aureus* chromosome.

Studies with two strains (L2 and L8) of *Staph. aureus* showing high-level resistance to mupirocin demonstrated that growth at 40 °C in a non-selective medium produced a loss of resistance (MICs $<0{\cdot}25\,\mu$g ml^{-1}), implying that high-level resistance is located on a plasmid. Noble *et al.* (1988) and Rahman *et al.* (1987, 1989, 1990, 1993) have amply demonstrated the association of plasmids with high-level resistance.

Al-Masaudi *et al.* (1991) and Kloos and Lamb (1991) showed that gene transfer between staphylococcal populations is possible at room temperature. In the experiments described in the present paper, it was found that mupirocin resistance is transferred from some (but not all) highly mupirocin-resistant strains of *Staph. aureus* to some highly sensitive strains of this organism (Table 2) and of other staphylococcal species (Table 3). Furthermore, mupirocin resistance from such transconjugants of *Staph. epidermidis* and *Staph. haemolyticus* could then be transferred into sensitive *Staph. aureus* recipients, interestingly at a higher transfer frequency (Table 4). Non-transferable high-level mupirocin resistance, e.g. from *Staph. aureus* F89 (Table 2) may be due to a narrow host-range plasmid or to the incorporation of the gene responsible into the chromosome to give permanent high-level resistance. It is also possible that F89 has become trained to high-level resistance in the environment, although this is considered to be unlikely due to the favourable conditions needed to generate a stable high resistance.

The exchange of genetic material between *Staph. aureus* and *Staph. epidermidis* is probably associated with the high degree of DNA homology between the species involved (Kloos 1980). Such a genetic exchange could provide a reservoir of resistance genes for *Staph. aureus*, as both species occupy the same niche on the human skin (Mellows 1985).

Early studies with cell-free systems from *Escherichia coli* demonstrated that the target site of mupirocin is isoleucyl-tRNA synthetase (IRS), the enzyme which charges the appropriate tRNA with isoleucine (Farmer *et al.* 1992).

The I_{50} values of mupirocin were low against crude IRS enzyme isolated from mupirocin-sensitive strains, rather higher against mupirocin moderately resistant strains, much higher against strains trained to a high mupirocin resistance, and considerably higher still against highly resistant clinical isolates (Table 5). Farmer *et al.* (1992) reported that a general increase in IRS I_{50} values corresponded to a general increase in MIC values.

The transferable mupirocin resistance gene is located on a large plasmid (Rahman *et al.* 1987, 1989, 1990, 1993) and is responsible for producing a different IRS enzyme from that present on the *Staph. aureus* chromosome (Gilbart *et al.* 1993; Cookson 1998). This second, plasmid-specified enzyme may compete more strongly for the active site in the acylation process than the chromosomal IRS, and will thus require a higher mupirocin concentration to reduce its activity. The IRS from transconjugants had similar I_{50} values to those from the donor strains, which suggests strongly that such an IRS enzyme is responsible for mupirocin resistance and that high-level mupirocin resistance is located on a plasmid.

Table 6 Characteristics of mupirocin resistance in *Staphylococcus aureus* strains

Response to mupirocin	MIC (mg l^{-1})	Characteristics	Growth affected by mupirocin: Below MIC	Growth affected by mupirocin: Above MIC	I_{50} (ng ml^{-1})†	I_{50} (ng ml^{-1})*
Sensitive	<4	Can become moderately resistant	+++	+++	10	0·7–3·0
Moderate resistance	8–256	Chromosomal base change; can become highly resistant	+++	+++	6–17·8	19–43
High resistance	>512	Plasmid-mediated: resistance transferable and curable	++	++	224–891 (trained) 10 000–14 100 (transferable)	7000–10 000

+++, Large effect of mupirocin; ++, moderate effect; +, small effect; −, no effect.
† This study.
* Data of Gilbart *et al.* (1993).

In the trained strains, resistance to the drug is likely to be due to chromosomal mutations being selected at increasing mupirocin concentrations, as also suggested by Cookson (1998). Such mutations are one-point mutations in the gene (Antonio *et al*. 1995), the IRS produced having a lower affinity for the binding process than the plasmid-encoded IRS.

Characteristics of mupirocin resistance in *Staph. aureus* are summarized in Table 6, which examines the types of response to mupirocin, MIC values, how sub- and supra-MIC levels affect growth, and the I_{50} values. High-level and moderate-level mupirocin resistance poses a significant clinical problem.

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