Diversity of staphylococci exhibiting high-level resistance to mupirocin

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Summary. Plasmids mediating high-level resistance to mupirocin (MIC > 1000 mg/L) in staphylococci from various sources were studied by restriction endonuclease cleavage. Several patterns were obtained but six plasmids isolated from various *Staphylococcus aureus* and *S. epidermidis* strains were indistinguishable. The diversity and spread of these plasmids is illustrated.

Introduction

Mupirocin (Beecham, UK) is a topical antibiotic which acts by inhibiting isoleucyl-tRNA synthetase, a mechanism not employed by any other therapeutically available antibiotic.¹ It is of value in the treatment of staphylococcal and streptococcal infection, in the suppression of nasal staphylococci, and in the management of eczematous and other lesions infected with *Staphylococcus aureus*.

In 1987 we reported high-level resistance to mupirocin in strains of *S. aureus* isolated from patients in dermatology wards.^{2,3} We also drew attention to the difference in endonuclease cleavage patterns of genetic elements which mediated the conjugative transfer of mupirocin resistance.⁴ We now report on the diversity of strains we have encountered.

Materials and methods

Staphylococcal strains

Strains of *S. aureus* resistant to > 1000 mg of mupirocin/L were isolated in St Thomas' Hospital, London or sent to us from elsewhere. Details are given in table I. Additionally, we have screened 357 isolates of coagulase-negative staphylococci from patients receiving continuous ambulatory peritoneal dialysis (CAPD), 290 strains of *S. aureus* collected in 1964–1965 from random samples of a rural population in the Netherlands, 32 strains

collected from a Nigerian population in 1965–1966 and 360 strains of various staphylococcal species isolated from pigs during a study of porcine exudative epidermitis during 1987–1989.

Coagulase-negative strains were identified by the API Staph system (API Products, Basingstoke).

Examination of plasmid properties

Conjugative transfer of plasmids to *S. aureus* strains NCTC 8325, B111 or RN 2677 made resistant to rifampicin 80 mg/L, fusidic acid 30 mg/L or novobiocin 5 mg/L, was performed on filters as described by Rahman *et al.*³ Plasmids or restriction-endonuclease fragments were separated on agarose gels with *Escherichia coli* V517 or phage λ cut by *Hind*III (Gibco BRL) as standards. Endonuclease cleavage with *Eco*RI and *Hind*III (Gibco BRL) was done according to the maker's instructions.

Plasmids that co-migrated with the chromosomal DNA in both caesium chloride-ethidium bromide density gradients and in conventional agarose gels^{2,3} were demonstrated by pulsed field gel electrophoresis (PFGE) in the Pharmacia hexagonal array with agarose 1%, at 170 V with a 5-s pulse time for c. 20 h. Plasmids not separable in caesium chloride density gradients were demonstrated as described elsewhere³ by cleaving whole-cell DNA from a *S. aureus* NCTC 8325 or B111 recipient or by recovery of plasmid DNA by elution from PFGE gels.

Results

Coagulase-positive staphylococci

The two original epidemic strains from St Thomas' Hospital^{2,3} possessed an identical plasmid that mediated mupirocin resistance, here typified by strain J2385. All other strains from St Thomas'

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Table I. Sources and characteristics of mupirocin-resistant *S. aureus*

Source and year of isolation	Domestic code	Resistance pattern*	Plasmid profile†	Transferability of mupirocin resistance	Plasmid DNA detected in	
					CsCl gradients	PFGE gel
1 STH 1987	J2385	Pc Tc Em	30, 4·2, 3·4, 3·1, 2·7, 2·2	++	—	+
2 Glasgow 1987	J2625	Pc Tc Em	30, 4·2	++	—	+
3 Bristol 1987	J2627	Pc	50, 30, 2·9, 2·3	±	+	+
4 STH 1988	J2872	Pc	35, 20	±	+	NT
5 STH 1988	J2870	Pc	35, 25	++	+	+
6 Warr 1988	J2878	Pc Tc Nm Em Cl Gm Mt	50, 35, 3·5	++	—	+
7 Warr 1988	J2880	Pc Tc Nm Em Cl Gm Mt	35, 3·5	+	—	+
8 STH 1989	J2925	Pc Tc Nm Em	30, 2·8	++	+	+
9 STH 1989	J2956	Sensitive	35	—	+	+
10 STH 1989	J3090	Sensitive	35	—	+	NT
11 Nigeria 1965	DUT 471	Pc	20, 15, 6·5	—	NK	NT
12 Nigeria 1965	DUT 473	Pc	20, 15, 6·5	—	NK	NT
13 Nigeria 1965	DUT 476	Pc	25, 20	+	+	NT

NT, not tested; NK, not known; STH, St Thomas' Hospital (*S. aureus* J2385 is the original strain); Warr, Warrington.

* Pc, penicillin; Tc, tetracycline; Nm, neomycin; Em, erythromycin; Cl, clindamycin; Gm, gentamicin; Mt, methicillin. Only resistances are shown. All strains were resistant to mupirocin (MIC > 1000 mg/L).

† Measured as intact plasmids on agarose gels (kb).

Hospital were epidemiologically distinct. Strains from Warrington also formed part of an epidemic of infection; no other strain was associated with spread of infection or colonisation.

Some mupirocin resistance plasmids were readily transferable to *S. aureus* strain NCTC 8325 or B111 and are assumed to be conjugative plasmids (table I). In other instances, resistance did not transfer to *S. aureus* NCTC 8325 or B111 despite several attempts, but in a few cases transferred at a low frequency of $<10^{-8}$ to the restriction-deficient strain RN 2677.

Plasmids that co-migrated with chromosomal DNA, but which could be separated by PFGE, were isolated from the original St Thomas' Hospital *S. aureus* strain J2385 and the Glasgow *S. aureus* strain J2625. In all other cases an apparently closed circular DNA plasmid was demonstrated in conventional agarose gels and by ethidium bromide-caesium chloride density gradients.

No mupirocin-resistant strain was found among *S. aureus* isolated during 1964–1965 or among the staphylococci from pigs. Four strains resistant to mupirocin were found in the strains from Nigeria, one of which proved to be coagulase-negative (see below).

Coagulase-negative staphylococci

High-level resistance in coagulase-negative

staphylococci was recorded from three sources (table II). Four isolates encountered in routine clinical specimens during or shortly after the *S. aureus* outbreak at St Thomas' Hospital were all *S. epidermidis*: three were also resistant to penicillin, tetracycline and erythromycin but had different plasmid profiles, and the remaining strain was resistant to penicillin and tetracycline as well as mupirocin. In two instances (*S. epidermidis* J2516 and J2435) mupirocin resistance was transferred to *S. aureus* NCTC 8325, and produced a conventional plasmid band on caesium chloride gradients.

Coagulase-negative staphylococci resistant to high levels of mupirocin were isolated from nine of 18 patients who were undergoing CAPD; of 357 isolates from these 18 patients, 26 were resistant. The true number of unrelated strains is not known. Five patients yielded resistant *S. epidermidis* strains, two *S. capitis*, one *S. hominis* and one *S. haemolyticus*. All strains transferred mupirocin resistance to *S. aureus* NCTC 8325 or B111 but conventional plasmids could not be demonstrated in these transferants by electrophoresis. However, with one exception, separate plasmid bands were seen by PFGE. Restriction patterns obtained for transferants from three of these isolates were different.

One Nigerian strain (*S. epidermidis* DUT 451) differed from the three *S. aureus* strains in being sensitive to penicillin. Mupirocin resistance was transferable to *S. aureus* NCTC 8325.

Table II. Sources and characteristics of mupirocin-resistant coagulase-negative staphylococci

Source and year of isolation	Identity	Domestic code	Resistance pattern	Plasmid profile	Transferability	Plasmid DNA detected on CsCl gradient
14 STH 1987	<i>S. epidermidis</i>	J2516	Pc Tc Em	30, 25, 5.9, 5.0, 2.9	±	+
15 STH 1987	<i>S. epidermidis</i>	J2518	Pc Tc Em	35, 4.2, 2.9	—	NK
16 STH 1987	<i>S. epidermidis</i>	J2435	Pc Tc Em	30, 6.5, 4.0, 2.6	±	+
17 STH 1987	<i>S. epidermidis</i>	J2869	Pc Tc	None seen	—	+
18 Nigeria 1965	<i>S. epidermidis</i>	DUT 451	Sensitive	25, 3.2, 2.5	+	+
19 GH 1987	<i>S. hominis</i>	VIA	Sensitive	None seen	+	—
20 GH 1987	<i>S. epidermidis</i>	A14C	Pc Tc Em	None seen	+	—
21 GH 1987	<i>S. capitis</i>	BY8H	Sensitive	5.8, 5.3, 2.6, 1.7	+	—

GH, Guy's Hospital. Other abbreviations as for table I.

Restriction patterns

Restriction patterns of most of the plasmids transferred to standard strains were different (table III). However, the plasmids from *S. aureus* strains J2872, J3090 and J2880, and those from *S. epidermidis* strains DUT 451, J2516 and J2435 had indistinguishable *EcoRI* and *HindIII* digest patterns. There were marked similarities between the patterns from this group of six indistinguishable plasmids (table III, column 7) and those from *S. aureus* strains J2878 and J2956 (table III, columns

6 and 9) which differed in the size of some bands; this was confirmed by electrophoresis of the cleaved DNA in adjacent lanes of a gel.

Discussion

A diversity of plasmids was encountered in this study. After double digestion with *EcoRI* and *HindIII* several common bands were visualised, but these were not universal and could be related to other plasmid functions. However, since the plas-

Table III. *EcoRI* restriction fragments of some mupirocin resistance plasmids

Fragment sizes (kb) for plasmid from strain number*												
	1	2	3	5	6	7	8	9	13	19	20	21
	>15	14	15	5.9	15	13	7.9	12	13	9.3	11.5	c. 25
	13.7	13	9.3	5.2	13	6.6	5.8	8.4	8.4	7.2	7.4	15
	11	10.6	7.7	4.1	5.9	5.7	4.9	5.5	4.0	6.2	6.4	13
	4.0	6.8	6.5	2.5	4.1	4.2	3.4	3.9	2.4	5.4	5.0	8.7
	3.5	5.4	3.9	2.4	2.3	2.2	2.6	2.4	2.2	4.0	4.7	3.4
	2.8	5.0	(2.9)	1.8	2.1	2.1	1.8	2.1		3.7	3.9	2.9
	2.7	4.5	2.4	1.4	1.7	1.7		1.7		3.0	3.1	2.7
	2.4	4.2	2.2							2.9		2.5
	1.7	3.8	1.4							2.6		1.8
		3.3										
		2.6										
		2.3										
		2.1										
		1.8										
		(1.7)										
		1.5										
		1.2										
		1.0										
Number of patterns analysed	6	4	3	2	3	6	3	3	1	1	1	1
Total size (kb)	57	87	52	23	29	36	26	36	30	44	42	75

Figures in parenthesis indicate a probable doublet.

* See table I for identity of strains.

mids showed diversity when first recognised in 1965, diversity in recent strains is to be anticipated. In contrast, the similarity of some plasmids, e.g., in *S. aureus* strains J2872, J2880, J3090, and *S. epidermidis* strains DUT 451, J2516 and J2435, as shown by *EcoRI* and *HindIII* digests, shows their wide spread. The existence of resistance plasmids 20 years before commercial introduction of the antibiotic should not be surprising; mupirocin is a naturally-occurring product of *Pseudomonas fluorescens* and exposure of staphylococci under natural circumstances can be assumed.

Low-level mupirocin resistance, with minimal inhibitory concentrations ≤ 50 –100 mg/L, can be achieved by training staphylococci *in vitro*⁵ and such low-level resistance occurs naturally *in vivo* in strains not described here. The relationship, if any, between low and high resistance must remain speculative.

Several of the plasmids described here cannot be demonstrated on caesium chloride density gradients or by conventional gel electrophoresis. This might indicate an open-circular or perhaps linear plasmid co-migrating with the chromosomal DNA, since plasmid DNA could be demonstrated by PFGE. These plasmids do not migrate at the same rate as conventional plasmids in PFGE but are proportionately faster than their size would suggest (data not shown). The genes, if any, that mediate this phenomenon are unknown but appear to be independent of the *tra*⁺ genes presumed to be present on the conjugative plasmids, since not all plasmids that appear to be conjugative show the phenomenon.

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