Probes for the study of mupirocin resistance in staphylococci

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Summary. Probes constructed from a 4·05-kb *EcoRI* digest fragment of a mupirocin resistance plasmid and a 751-bp internal part of this fragment hybridised with DNA from all of 36 independent high-level mupirocin-resistant staphylococci tested from seven centres; most were *Staphylococcus aureus*. In most instances the probes detected an *EcoRI* digest fragment of approximately 4 kb. Probes did not hybridise to DNA from low-level resistant strains, nor from strains sensitive to mupirocin.

Introduction

Mupirocin (SmithKline Beecham) is a relatively new topical antibiotic with a novel antibacterial activity; it inhibits the isoleucyl tRNA synthetase of staphylococci and streptococci, a mechanism not possessed by other commercially available antibiotics. High-level resistance to mupirocin (MIC > 2000 mg/L) was first described in 1987.^{1,2} It has been shown recently that strains exhibiting high-level resistance possess two isoleucyl tRNA synthetases, one of which is not inhibited by mupirocin.³ Resistance determinants were found to be carried on a diversity of plasmids as judged by *EcoRI* and *HindIII* restriction fragment patterns; only some plasmids were easily transferred and some plasmids could be demonstrated only by pulsed-field gel electrophoresis.⁴ In 1991, Dyke *et al.*⁵ cloned a mupirocin-resistance gene in *Escherichia coli* as a 4·05-kb *EcoRI* digestion fragment from a staphylococcal plasmid in strain 5 of Rahman *et al.*⁴ Here, the use of this fragment and of a smaller internal piece of it as probes for mupirocin resistance is reported.

Materials and methods

Bacterial isolates

The list of resistant staphylococci studied is shown in the table. These comprised chiefly strains isolated from patients attending the inpatient wards or outpatient clinics of the St John's Dermatology Centre and were judged to be independent on epidemiological data as well as antibiogram and plasmid profile (data not shown). Three sets of strains are described: (a) strains exhibiting high-level resistance (MIC >

2000 mg/L) and recognised initially by their ability to grow up to a disk containing mupirocin 200 µg; (b) low-level resistant strains (MIC 5–50 mg/L) that may grow close to a 5-µg disk but not a 200-µg disk; (c) mixed strains that exhibited a small number of resistant colonies in the zone around a 200-µg disk. In addition, 12 independent wholly sensitive *Staphylococcus aureus* strains were examined (epidemiological details not shown). Three strains of coagulase-negative staphylococci (nos. 19–21) reported previously⁴ have been studied in more detail and are reported separately.⁶

Probe construction

Antibiotic resistance patterns and plasmid profiles of the original strains were determined as described previously and, where possible, the mupirocin resistance plasmid was transferred to a standard strain by filter mating.⁴ DNA was prepared from staphylococci as described previously and from *E. coli* by the SDS method.⁷ Restriction enzymes were purchased from Gibco BRL (Paisley) and were used according to the manufacturer's instructions. Restriction fragment length patterns were prepared with transconjugant plasmid DNA from caesium chloride–ethidium bromide density gradients or from a plasmid preparation of the original strain as described previously.⁴ Standards were *E. coli* strain V517 for intact plasmids or phage λ DNA cut by *HindIII* (Gibco BRL) for fragments.

Probes were constructed from the 4·05-kb *EcoRI* fragment described by Dyke *et al.*⁵ cloned in *E. coli* and a 751-bp *XhoI/BglII* piece from within the 1·6-kb *NcoI* fragment of the gene.

Transfer of *EcoRI* digest fragments on to Flash Nylon membrane (Stratagene, Cambridge) was by standard methods.⁷ Probe DNA was recovered from agarose gels with the GeneClean II kit (Stratagene, Luton,

Table. Characteristics of resistant strains studied

Strain no.	Source	Year of isolation	Species	Resistance* patterns	Plasmid profile†	Mupirocin MIC (mg/L)	Resistance transferred to standard recipient
1.	STH	1987	<i>S. aureus</i>	Pc, Tc, Em	30, 4.2, 3.4, 3.1, 2.7, 2.2	> 2000	+
2.	Glasgow	1987	<i>S. aureus</i>	Pc, Tc, Em	30, 4.2	> 2000	+
3.	Bristol	1987	<i>S. aureus</i>	Pc	50, 30, 2.9, 2.3	> 2000	+
4.	STH	1988	<i>S. aureus</i>	Pc	35, 20	> 2000	+
5.	STH	1988	<i>S. aureus</i>	Pc	35, 25	> 2000	+
6.	Warr	1988	<i>S. aureus</i>	Pc, Tc, Em, Cl, Nm Gm, Mt	50, 35, 3.5	> 2000	+
7.	Warr	1988	<i>S. aureus</i>	Pc, Tc, Em, Cl, Nm Gm, Mt	35, 3.5	> 2000	+
8.	STH	1989	<i>S. aureus</i>	Pc, Tc, Em, Nm	30, 2.8	> 2000	+
9.	STH	1989	<i>S. aureus</i>	Sensitive	35	> 2000	—
10.	STH	1989	<i>S. aureus</i>	Sensitive	35	> 2000	—
11.	Nigeria	1965	<i>S. aureus</i>	Pc	20, 15, 6.5	> 2000	—
12.	Nigeria	1965	<i>S. aureus</i>	Pc	20, 15, 6.5	> 2000	—
13.	Nigeria	1965	<i>S. aureus</i>	Pc	25, 20	> 2000	+
14.	STH	1987	<i>S. epidermidis</i>	Pc, Tc, Em	30, 25, 5.9, 5.0, 2.9	> 2000	+
15.	STH	1987	<i>S. epidermidis</i>	Pc, Tc, Em	35, 4.2, 2.9	> 2000	—
16.	STH	1987	<i>S. epidermidis</i>	Pc, Tc, Em	30, 6.5, 4.0, 2.6	> 2000	+
17.	STH	1987	<i>S. epidermidis</i>	Pc, Tc	> 56, 4.3	> 2000	—
18.	Nigeria	1965	<i>S. epidermidis</i>	Sensitive	25, 3.2, 2.5	> 2000	+
22.	STH	1990	<i>S. aureus</i>	Pc, Em	2.8	> 2000	+
23.	STH	1990	<i>S. aureus</i>	Pc	47	> 2000	—
24.	STH	1990	<i>S. aureus</i>	Pc	47, 26	> 2000	—
25.	STH	1990	<i>S. aureus</i>	Pc, Tc	44, 17, 7, 4.8, and 2 less than 1.5 kb	> 2000	+
26.	STH	1990	<i>S. aureus</i>	Pc	> 56, 21	> 2000	—
27.	STH	1990	<i>S. aureus</i>	Tc	56, 6.3	> 2000	—
28.	STH	1990	<i>S. aureus</i>	Pc, Tc	56, 4.4, 1.8	> 2000	+
29.	STH	1990	<i>S. aureus</i>	Pc, Tc	35, 6.3, 4.4 and 2 smaller than 1.5 kb	> 2000	+
30.	STH	1990	<i>S. aureus</i>	Pc, Tc, Em	37, 4.0, 3.1, 2.8	> 2000	+
31.	Warr	1988	<i>S. aureus</i>	Pc, Tc, Em, Cl, Nm, Gm	> 56, 56	> 2000	—
32a.	STH	1990	<i>S. aureus</i>	Em	56, 22, 3.1, 2.5	5	—
32b.			<i>S. aureus</i>	Sensitive	None seen	> 2000	+
33a.	S.end	1990	<i>S. aureus</i>	Pc, Tc, Em, Gm, Mt	20.4	< 5	—
33b.			<i>S. aureus</i>	Pc	56	> 2000	—
34a.	STH	1990	<i>S. aureus</i>	Pc	24.6, 6.2	5	—
34b.			<i>S. aureus</i>	Pc	56	> 2000	—
35a.	STH	1988	<i>S. aureus</i>	Pc, Tc, Em	19.5, 4.6, 2.5	10	—
35b.			<i>S. aureus</i>	Pc	56	> 2000	—
36a.	W.X.	1988	<i>S. aureus</i>	Pc, Tc, Em	6.5, < 2.2	10	—
36b.			<i>S. aureus</i>	Pc	56	> 2000	—
37.	STH	1990	<i>S. aureus</i>	Sensitive	> 56, 19.5, 3.3, 2.6	10	—
38.	STH	1990	<i>S. aureus</i>	Pc	> 56	< 5	—
39.	STH	1990	<i>S. aureus</i>	Pc, Tc, Em	> 56, 15.5, 4.3, 3.5, 2.6	10–40	—

STH, St John's Dermatology Centre, St Thomas' Hospital; Warr, Warrington; W.X., Whipps Cross, London; S.end, Southend, Essex.

Nos 1–18 have been described previously and the *EcoRI* digests of the mupirocin plasmids have been determined.

Nos 19–21 have now been studied in greater detail and are included in a separate report.

Nos 28–30 possessed a very similar mupirocin plasmid.

No. 39 represents eight isolates tested from eight different inpatients over a period of 10 months in an apparent episode of cross-infection. Mixed strains are indicated by a and b (nos 32–36).

* Resistances examined were penicillin (Pc), tetracycline (Tc), erythromycin (Em), clindamycin (Cl), neomycin (Nm), gentamicin (Gm) and methicillin (Mt) in addition to mupirocin.

† Measured as intact plasmids in agarose gels.

Beds) and was labelled with the "Flash" Prime-It Random Labelling kit (Stratagene) according to the manufacturer's instructions. Biotinylated DNA was detected on membranes by the Flash Detection System (Stratagene) according to the manufacturer's instructions. Biotinylated λ DNA cut by *HindIII* (Gibco BRL) was used as a standard.

Results

Most of the original strains were epidemiologically independent as judged by antibiotic resistance patterns

and plasmid profiles (table) although some yielded mupirocin resistance plasmids with similar structures. Many of these mupirocin resistance plasmids were transferable to standard recipient strains and six new profiles were added (data not shown). The strains numbered 32–36 (Group C) were originally recorded as having low-level resistance but colonies grew within the inhibition zones to yield resistant variants. When these were subcultured they proved to have a different antibiotic resistance pattern from the low-level strains and four of the five strains (nos 33–36) carried plasmids with the same *EcoRI* digest pattern.

Figure. Hybridisation of 4.05-kb probe to *Eco*RI digests of transferred plasmids or whole cell digests. Lane 1, λ phage digests with *Hind*III; 2, strain 27; 3, strain 23; 4, strain 24; 5, strain 5; 6, strain 8; 7, strain 31. Strain numbers refer to the table.

In most instances, both probes hybridised to a *c.* 4-kb *Eco*RI restriction fragment. The exceptions were strain 1 which hybridised to a fragment of *c.* 18 kb (it has proved possible to separate the former large fragment into two, one of 18 kb and the other of *c.* 25 kb) and strain 8 which hybridised to a fragment of *c.* 4.8 kb (figure). Other DNA fragments did not hybridise to the probes. Neither probe hybridised to DNA from the low-level resistant strains nor from the fully sensitive strains.

Discussion

The addition of a further 10 apparently distinct high-level resistant strains to our previous 21, which yielded a further six distinct *Eco*RI restriction fragment patterns from the eight plasmids transferred or seen as single plasmids (nos. 32–36), confirms our previous observations⁴ on the diversity of plasmids which carry this resistance determinant.

The results with the 4.05-kb and 751-bp probes were identical. The probes revealed a single fragment in all high-level resistant strains corresponding in most cases to the 4.05-kb *Eco*RI fragment described from strain 5 in this series by Dyke *et al.*⁵ DNA from low-level resistant strains, which can be assumed to be similar to those obtained originally by serial culture on agar containing mupirocin,⁸ did not hybridise to the probe,

indicating a different mechanism of resistance. This has recently been confirmed by the report of two isoleucyl tRNA synthetases in mupirocin-resistant *S. aureus*.³

Five strains (nos. 32–36), which were apparently epidemiologically independent, were initially described as low-level resistant strains but were later observed to have a few colonies growing around the mupirocin disk during testing. On subculture, these proved to be high-level resistant strains with a different resistance pattern to the low-level “parent” strains. Four of these (nos. 33–36) possessed an indistinguishable resistance plasmid. There is no immediate explanation for this; laboratory cross-contamination cannot be excluded but the plasmid structure was not one that had been encountered previously. However, it is worth noting that co-existent high- and low-level resistant strains have been encountered previously.⁴

The almost constant size of the *Eco*RI fragment which hybridised with the probe suggests that the same genetic element is involved in all resistant strains; this is also true of the coagulase-negative staphylococci reported in detail by Connolly *et al.*⁶ If this is true, it may prove possible to modify the antibiotic in such a way as to inhibit this particular gene and thus eliminate or reduce the prevalence of resistance.

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