Mupirocin resistance in coagulase-negative staphylococci

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Summary. High-level mupirocin resistance in coagulase-negative staphylococci isolated from patients undergoing peritoneal dialysis was investigated by transfer of the resistance determinants, usually in the form of a plasmid, to *Staphylococcus aureus* strains, cleavage of the plasmid by restriction endonuclease and hybridisation with a probe comprising a 4.05 kb *EcoRI* fragment of a plasmid from a *S. aureus* strain. In most instances the mupirocin-resistant staphylococci isolated from each patient were different according to the species, antibiogram and plasmid profile data. The mupirocin resistance determinant was carried on various plasmids as judged by *EcoRI* restriction fragment length polymorphisms. All hybridised at about 4 kb with the *S. aureus* probe.

Introduction

Coagulase-negative staphylococci (CNS) are well established members of the normal flora of human skin¹ but are also evident in infection associated with indwelling catheters or prostheses. Control of such infection with the topical antibiotic Mupirocin (Smith-Kline Beecham) has been investigated.^{2–5} Although the rate of resistance to mupirocin in CNS has been reported to be *c.* 3%⁶ there are few reports of specific studies. We have been particularly interested in infection in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) and have collected staphylococci from the skin, nose and exit site of these patients.^{7,8} In a continuing study of high-level resistance (MIC > 2000 mg/l) to mupirocin, we have examined staphylococci from CAPD patients collected during 1987. A number of isolates proved to be resistant although the patients were not known to have received this antibiotic. This paper reports studies on these resistant staphylococci.

Materials and methods

Staphylococci and cultural methods

Bacterial strains and plasmids are listed in table I. Species were identified by conventional tests and the API STAPH and ATB32 STAPH kits (API bio-Mérieux, Basingstoke, Hants). Data on single isolates from patients A, B and V have been partially described in a previous paper.⁹

Strains were routinely cultured on Blood Agar Base (BAB; Oxoid). Sensitivity testing was performed on Mueller-Hinton Agar (Oxoid) with Oxoid sensitivity

test disks as follows: Penicillin (Pc, 10 IU), tetracycline (Tc, 30 µg), erythromycin (Em, 15 µg), gentamicin (Gm, 10 µg), neomycin (Nm, 30 µg), mupirocin (Mup, 5 µg, 200 µg), chloramphenicol (Cm, 30 µg). Minimum inhibitory concentrations (MICs) were measured by incorporating antibiotic in BAB.

Resistance transfer

Resistance transfer was performed on filters and on the skin of hairless-obese mice as described previously.¹⁰ Plasmid DNA was prepared from the staphylococci by the method of Wilson *et al.*¹¹ and from *Escherichia coli* by alkaline lysis.¹² DNA was purified by caesium chloride-ethidium bromide density gradient centrifugation by standard methods¹³ and plasmid profiles were analysed by conventional or pulsed-field gel electrophoresis.

Plasmid manipulation

Plasmid DNA was digested with restriction endonuclease from Gibco BRL (Paisley) according to the manufacturer's instructions and analysed by conventional or pulsed-field gel electrophoresis in agarose in TBE buffer as described previously. Standards were *E. coli* strain V517 for intact plasmids¹³ and λ phage cut by *HindIII* (Gibco BRL) for plasmid fragments.

Plasmids to be probed were digested with *EcoRI*, electrophoresed and transferred to a nylon membrane, either from Amersham (Slough, Berks) or Stratagene (Cambridge), according to the manufacturers' instructions.

Probe construction

Probe DNA was a 4.05-kb *EcoRI* fragment from an *S. aureus* plasmid¹⁴ cloned in *E. coli* JM 107 and was a

Table I. Characteristics of coagulase-negative staphylococci

Code no.	Source*	Species	Antibiogram†	Plasmid profile‡	Number of determinations§
A14E	Nose	<i>S. epidermidis</i>	Pc, Tc, Em	None detected	9+1
B×6	Exit	<i>S. epidermidis</i>	Pc, Tc, Em	56, 45, 6, 4·6, 2·6	4
B×F	Nose	<i>S. epidermidis</i>	Pc, Tc, Em	55, 45, 6, 4·6, 2·6	4
B×8	Exit	<i>S. epidermidis</i>	Pc	45	2
B×13	Nose	<i>S. epidermidis</i>	Pc	45	6+2
B×19	Exit	<i>S. epidermidis</i>	Pc	45	4+1
By Bi	Nose	<i>S. epidermidis</i>	Pc, Tc	40, 11, 6·3, 4·7	2
By Bii	Nose	<i>S. capitis</i>	Pc, Tc	57, 7, 2·5	2
By Hi	Nose	<i>S. epidermidis</i>	Pc, Tc	57, 9, 7·5, 4·4	2
By Hii	Nose	<i>S. capitis</i>	Pc, Tc	57, 7, 2·5	2
Cs	Nose	<i>S. epidermidis</i>	Pc, Em	45, 2·7	4
D2	Nose	<i>S. haemolyticus</i>	Pc, Tc	4·7	2+2
E	Exit	<i>S. epidermidis</i>	Pc	45	6+2
VB	Exit	<i>S. epidermidis</i>	Pc	45	2+1
VC	Exit	<i>S. epidermidis</i>	Pc	45	2+1
VD	Exit	<i>S. hominis</i>	Tc	57, 7·4, 3, 2·5	2
VE	Exit	<i>S. hominis</i>	Tc	57, 7·4, 3, 2·5	1+1
V3E	Exit	<i>S. epidermidis</i>	Pc	45	2
V3F	Exit	<i>S. hominis</i>	Tc	57, 7·4, 3, 2·5	2
V3J	Exit	<i>S. epidermidis</i>	Pc	45	2+1

* Nose swab or CAPD exit site swab.

† Only resistances recorded, see text for antibiotics tested; all were resistant to mupirocin MIC > 2000 mg/L.

‡ Size in kb of intact plasmids seen by conventional gel electrophoresis.

§ Number of determinations by conventional gel electrophoresis plus number by pulse-field gel electrophoresis.

Table II. *Eco*RI restriction fragment patterns from mupirocin-resistant transconjugants

Strain no.	Species	Mean restriction fragment sizes
A14E (2)*	<i>S. epidermidis</i>	> 25, 13, 10·5, 4, 2·5
Cs (4)	<i>S. epidermidis</i>	11, 6, 5·4, 4, 2·6, 2·4, 2·3
D2 (4)†	<i>S. haemolyticus</i>	11, 10, 5·9, 5·4, 4, 2·2, 1·8
E (3)	<i>S. epidermidis</i>	11, 9·4, 5·4, 4, 2·4, 1·8
B×8 (2)		
B×13 (2)		
B×19 (2)		
B×6 (2)	<i>S. epidermidis</i>	11·2, 6, 5·6, 5·3, 4, 2·7, 2·5, 2·3
B×F (2)		
By B1 (2)	<i>S. epidermidis</i>	15, 8, 5·4, 5·3, 4·1, 2·5, 2·2, 1·7, 1·3
By H (i) (3)	<i>S. epidermidis</i>	15·5, 12, 11, 8, 5·7, 4·7, 4·2, 2·2, 1·4, 1·0
By B (ii) (3)	<i>S. capitis</i>	15, 11, 10, 7, 4·5, 4·3, 4·2, 3·0, 2·0, 1·4
By H (ii) (3)†		
VB (2)	<i>S. epidermidis</i>	16, 8·9, 5·7, 5·4, 4·3, 3·5, 2·5, 2·3, 1·4, 1·0
VC (2)		
V3E (3)		
V3J (2)		
VD (2)	<i>S. hominis</i>	15, 11, 10, 7, 6, 4·5, 4·3, 4·1, 2·0, 1·0
VE (2)		
V3F (2)		

* Transconjugant from isolate no. and (number of restrictions performed).

† Restriction digest of whole cell DNA showed bright bands.

gift from Dr. K. G. H. Dyke. Probe DNA was prepared by *Eco*RI digestion of recombinant DNA and the 4·05-kb fragment was recovered from the gel with Gene-clean II (Stratagene). The probe fragment was labelled with bio-11-dUTP/dTTP (Flash System, Stratagene), hybridised to blocked DNA at 43°C and visualised according to the manufacturer's instructions.

Results

A total of 403 isolates of CNS from 48 patients was screened and 20 isolates were found to be resistant to mupirocin > 2000 mg/L. These 20 isolates were of four species, although most¹⁴ were *S. epidermidis*, and were from seven patients designated A, Bx, By, Cs, D, E and V. Patients Bx, By and V each carried two or

more different species or apparently different strains of *S. epidermidis* as judged by antibiogram and plasmid profile (table I). With two exceptions, strains D2 and A14E, a large plasmid was seen in conventional gel electrophoresis plasmid profiles. Plasmid DNA was detected by pulsed-field gel electrophoresis in all isolates tested except D2, where the 4.7-kb plasmid had most probably run off the gel.

The large plasmids could be transferred to *S. aureus* strains by filter mating or on mouse skin at rates between 1×10^{-4} and 1×10^{-11} except in the case of strain D2 with which no transfer was observed. Transconjugants, including that of strain A14E, were shown to possess a single large plasmid by both conventional and density gradient centrifugation except for the ByH(1) transconjugant which showed a single "chromosomal" band. Strain D2 revealed bright "plasmid-like" bands on restriction endonuclease digestion of the original strain. All transconjugants plus the original D2 strain hybridised with the 4.05-kb probe from *S. aureus* at about 4 kb when the *EcoRI* restriction patterns were tested.

Four *S. aureus* isolates were available from patient F and 19 additional isolates from patient By, but none was resistant to mupirocin.

Discussion

It appears from table I that the seven patients yielding high-level mupirocin-resistant CNS carried distinct strains except, perhaps, for patients E and Bx, both of whom had an *S. epidermidis* strain resistant only to penicillin in addition to mupirocin and possessing only a single large plasmid. This might be regarded as rather tenuous evidence of cross-infection, but the restriction patterns shown in table II confirm that these single plasmids were indistinguishable and, in all probability, these represent a single strain. More surprising is the fact that the mupirocin resistance plasmids in the two *S. epidermidis* isolates from patient By differed and were also different from that in the *S. capitis* strain of that patient. Equally, the mupirocin

resistance plasmids in the *S. epidermidis* and *S. hominis* strains of patient V differed, though there was very close similarity between the plasmids of *S. capitis* and *S. hominis* isolates from these two patients.

In all but one instance the plasmids were transferable, although in some cases at low frequencies, to *S. aureus* strains. Some rates were sufficiently high (1×10^{-4}), to warrant the description of conjugative plasmid, these rates being typical of those for other mupirocin resistance plasmids, gentamicin resistance plasmids and others.^{10,15}

One plasmid (strain D2) was detectable only by pulsed-field electrophoresis and, in this respect, resembled the original mupirocin resistance plasmid described in *S. aureus*⁹ although the structure of the plasmid determined by *EcoRI* restriction patterns was quite different.

The probe used was a 4.05-kb *EcoRI* fragment from a plasmid in *S. aureus* shown to contain the entire mupirocin resistance gene which resembles, in some respects, the isoleucyl tRNA synthetase gene in *E. coli*.¹⁴ In all instances, the probe hybridised with an *EcoRI* restriction fragment of the plasmids at *c.* 4 kb and it is probable that the resistance determinants were all the same. A great variety of structures has also been seen in mupirocin resistance plasmids in clinical strains of *S. aureus*⁹ and, unless the determinant is a transposon, it is difficult to account for its presumed promiscuity.

As with other antibiotic resistance determinants it may be that the normal, CNS flora of the skin acts as a reservoir of mupirocin resistance genes which are available to *S. aureus*. The mupirocin-resistance rate in staphylococci not exposed to therapeutic mupirocin is not known for a general population, although we have reported previously mupirocin resistance in both *S. aureus* and *S. epidermidis* strains collected many years prior to the therapeutic use of the antibiotic.⁹ This finding is important because of the resistance in potential opportunist pathogens such as *S. epidermidis*, which may cause half of the peritonitis seen in CAPD patients, and because the resistance determinants are carried on transmissible replicons which may spread to other pathogenic bacteria.

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