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Detection and Characterization of Mupirocin Resistance in *Staphylococcus aureus*

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Fourteen mupirocin-resistant *Staphylococcus aureus* strains were isolated over 18 months; 12 exhibited low-level resistance, while two showed high-level resistance. Highly mupirocin-resistant strains contained a large plasmid which transferred mupirocin resistance to other *S. aureus* strains and to *Staphylococcus epidermidis*. This plasmid and pAM899-1, a self-transferable gentamicin resistance plasmid, have molecular and biologic similarities.

Mupirocin is a topical antibiotic with excellent antistaphylococcal and antistreptococcal activity (3). It has been used to eradicate staphylococcal carriage in health care workers and patients (2, 4, 10, 11, 18, 20, 24). With increased use of mupirocin, especially with chronic use, resistance in *Staphylococcus aureus* has emerged (1, 5, 7, 11, 12, 16, 17, 20, 22, 25). Of most concern is high-level mupirocin resistance, reported initially from England (5, 16, 17, 22) and now from the United States (11) and associated with clinical failure (11, 16, 22).

In our long-term-care facility, we used mupirocin ointment in nares and wounds from June 1990 to December 1991 in an attempt to eradicate methicillin-resistant *S. aureus* from chronically colonized residents (11). This paper describes the emergence of mupirocin resistance in this setting, characterizes the mupirocin-resistant strains, and shows evidence for plasmid-mediated transfer of this resistance. This plasmid is further characterized by comparison with pAM899-1, a well-described conjugative plasmid.

Fourteen mupirocin-resistant *S. aureus* strains were isolated from clinical specimens collected between June 1990 and December 1991 (11). Antibiotic susceptibilities were determined by Kirby-Bauer disc diffusion (14). Methicillin resistance was determined by growth after 24 h at 35°C on plates containing Mueller-Hinton agar (Difco, Inc., Detroit, Mich.) containing 6 μg of oxacillin (Sigma, St. Louis, Mo.) per ml and 4% sodium chloride (23). Fusidic acid susceptibility was determined by using growth on brain heart infusion agar (Difco) containing 25 μg of fusidic acid per ml. Mupirocin MICs were determined by a microdilution method (9); a macrodilution assay with Mueller-Hinton broth was used to determine MICs of >100 μg of mupirocin per ml.

Transferability of mupirocin resistance was examined by filter matings as previously described by Forbes and Schaberg (8). Mupirocin-susceptible *S. aureus* 879R4 and RN450 served as recipients. Filter mating techniques were also employed in host range transfer experiments. With strain LZ-1 (*S. aureus*, Mup^r) as the donor, attempts to transfer high-level mupirocin resistance to SE-131 (*Staphylococcus epidermidis*) and to JH2-2 (*Enterococcus faecalis*) were made by using appropriate selective antibiotic-containing media. Additional mating experiments attempted to transfer high-level mupirocin resistance from LZ-1/879R4 (first-generation transconjugant, Mup^r) to *S. aureus* SA-136 containing the gentamicin resistance plasmid, pAM899-1, to determine compatibility between these two self-transferable elements. Selective antibiotic media allowed the examination of the transfer of gentamicin resistance to the mupirocin-resistant strain in the same mating.

DNA was isolated from *S. aureus* strains by the method described by Macrina et al. (13) and modified by Forbes and Schaberg (8). DNA was electrophoresed through 0.7% agarose (Bethesda Research Laboratories) in Tris-borate-EDTA buffer at 65 V for 5 h in a horizontal submarine electrophoresis chamber.

During the 18 months that mupirocin ointment was used to decrease methicillin-resistant *S. aureus* colonization, 14 mupirocin-resistant strains were isolated (Table 1). These included 13 strains cultured from the nares or wounds of 11 different patients and 1 strain found in the environment. Strains LZ-1 and LZ-6, isolated from the same patient 1 month apart, were different phage types, and strains LZ-2 and LZ-5, isolated from another patient 1 week apart, belonged to two different phage groups. There were four methicillin-susceptible *S. aureus* isolates, all of which had low-level resistance to mupirocin (MICs, 3.1 to 62.5 μg/ml). MICs for 8 of the 10 methicillin-resistant *S. aureus* strains ranged from 6.2 to 50.0 μg/ml, while 2 strains, LZ-1 and LZ-6, had high-level mupirocin resistance (MICs, >5,000 μg/ml).

Each mupirocin-resistant organism was used as a possible donor in filter matings with mupirocin-susceptible *S. aureus* recipient strains. Mupirocin MICs for only 2 of 14 isolates (LZ-1 and LZ-6), both methicillin-resistant *S. aureus*, were >5,000 μg/ml, and only these 2 transferred mupirocin resistance to *S. aureus* recipients (the lowest level of detection in our system was 10^{-10} transconjugants per recipient cell). Mupirocin resistance was transferred by both isolates into phage-free strains 879R4 and RN450 (Table 2). Subsequent experiments demonstrated the transfer of high-level mupirocin resistance in secondary and tertiary filter matings. No other antibiotic resistance marker was transferred with high-level mupirocin resistance.

We performed agarose gel electrophoresis on lysostaphin-

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TABLE 1. Phenotypic markers of 14 mupirocin-resistant *S. aureus* clinical isolates

Strain[a]	Date isolated (mo/yr)	Phage type	Methicillin resistance[b]	Phenotype[c]
LZ-1	7/91	83A	R	Cm^r Pen^r Cf^r Cl^r Em^r
LZ-2	2/91	84/75/83A	R	Cm^r Pen^r Cf^r Cl^r Em^r Gm^r
LZ-3	6/91	Nontypeable	R	Cm^r Pen^r Cf^r Cl^r Em^r Gm^r
LZ-4	3/91	53/83A/85	S	Cm^i Rif^r Pen^r Cl^r Em^r Gm^r
LZ-5	2/91	29	R	Cm^i Pen^r Cf^r Cl^r Em^r Gm^r
LZ-6	6/91	47/54/72/83A	R	Cm^r Pen^r Cf^r Cl^r Em^r
LZ-7	8/90	83A	R	Cm^r Pen^r Cl^r Em^r Gm^r
LZ-8	6/91	Nontypeable	R	Cm^r Pen^r Cf^r Cl^r Em^r Gm^r
LZ-9	11/90	Nontypeable	S	Pen^r Em^r
LZ-10	11/90	Nontypeable	R	Cm^r Pen^r Cf^r Cl^r Em^r Gm^r
LZ-11	12/90	71	S	Pen^r Gm^r
LZ-12	9/90	47/77/83A/95	R	Cm^r Pen^r Rif^r Cl^r Em^r Str^r Gm^r
LZ-13	8/90	Nontypeable	R	Cm^i Pen^r Tc^r Em^i
ENV-1	11/91	Nontypeable	S	Cm^i Fus^r Cl^i Nb^r

[a] Strains LZ-1 and LZ-6 were isolated from one patient at two different time points; strains LZ-2 and LZ-5 were isolated from another patient at two different time points. All other strains were each isolated from a different patient, except strain ENV-1, which was isolated from the environment.

[b] R, resistant; S, susceptible.

[c] Cf, cephalothin; Cl, clindamycin; Cm, chloramphenicol; Em, erythromycin; Fus, fusidic acid; Gm, gentamicin; Nb, novobiocin; Pen, penicillin; Rif, rifampin; Str, streptomycin; Tc, tetracycline; i, intermediate.

prepared minipreps of selected donor, recipient, and transconjugant strains (Fig. 1). Plasmid DNA was found in the high-level mupirocin-resistant donors (LZ-1 and LZ-6) as well as in the high-level mupirocin-resistant progeny resulting from the mating of LZ-1 and LZ-6 with RN450 (the mupirocin-susceptible, plasmid-free recipient). Identically sized plasmid DNA was found in recipients from secondary matings. The size of this plasmid can be approximated by comparing it with pAM899-1, a 49-kb self-transferable plasmid encoding for gentamicin resistance.

In additional filter mating experiments, high-level mupirocin resistance was transferred from LZ-1 to SE-131 at a frequency of 1.6×10^{-7} (Table 2). However, attempts to transfer this marker to a mupirocin-susceptible enterococcal strain, JH2-2, produced no transconjugants. Further matings were used to study the transfer of high-level mupirocin resistance into cells containing pAM899-1 and the reciprocal transfer of these markers by using the same cell lines. No transconjugants that simultaneously expressed high-level mupirocin resistance and gentamicin resistance could be detected.

Under the selective pressure of mupirocin use, 14 mupirocin-resistant *S. aureus* strains were isolated at a long-term care facility. Several different mupirocin-resistant strains were scattered geographically throughout the facility, were isolated chronologically over 18 months, and were phenotypically diverse, as determined by phage typing. We found no evidence of patient-to-patient transmission.

Low-level mupirocin resistance was found more commonly. Only the two highly mupirocin-resistant strains (MIC, >5,000 μg/ml) transferred mupirocin resistance on filter membranes. LZ-1 and LZ-6 each transferred high-level mupirocin resistance to several staphylococcal recipients,

TABLE 2. Frequency of transfer of mupirocin resistance

Donor strain	Recipient strain[a]	Transfer frequency[b]
LZ-1	RN450RF	9.9×10^{-8}
LZ-6	RN450RF	1.3×10^{-9}
LZ-1	879R4RF	2.2×10^{-8}
LZ-6	879R4RF	4.3×10^{-8}
LZ-1/879R4RF	879R4STR	3.6×10^{-6}
LZ-6/879R4RF	879R4STR	5.0×10^{-6}
(LZ-1/879R4RF)/879R4STR	879R4RF	6.4×10^{-5}
LZ-1	SE-131STR	1.6×10^{-7}
LZ-1	JH2-2RF	$<1.0 \times 10^{-10}$

[a] RF, chromosomal marker for rifampin and fusidic acid resistance; STR, chromosomal marker for streptomycin resistance.

[b] Expressed as the number of transconjugants per recipient cell.

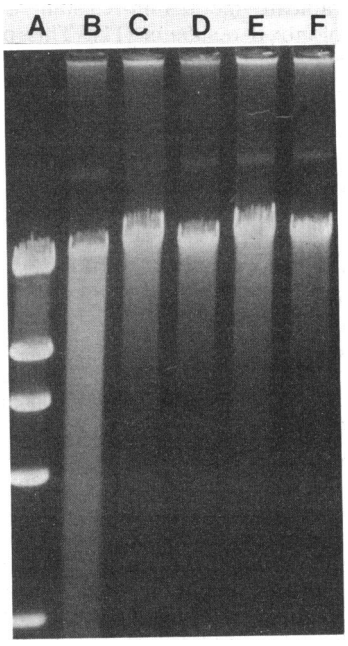

FIG. 1. Agarose gel electrophoresis of crude plasmid DNA from selected donor, recipient, and transconjugant strains. Lane A, bacteriophage lambda DNA (from top to bottom, bands represent 23.1, 9.4, 6.6, 4.4, and 2.3 kb); lane B, *S. aureus* donor LZ-1 (Mup^r); lane C, *S. aureus* recipient RN450RF (Mup^s) ("RF" is a chromosomal marker for rifampin and fusidic acid resistance); lanes D and E, *S. aureus* transconjugant LZ-1/RN450RF (Mup^r); lane F, *S. aureus* SA-136 (pAM899-1 Gm^r Rif^r Fus^r).

and this resistance was serially transferred in secondary and tertiary matings. Agarose gel electrophoresis demonstrated identically sized extrachromosomal DNA in LZ-1, LZ-6, and selected highly mupirocin-resistant progeny from primary and secondary matings. These results and the large size of the transferred extrachromosomal DNA implicate conjugation involving a self-transferable plasmid encoding high-level mupirocin resistance.

Mupirocin resistance has been noted previously, especially in settings of chronic use (5, 11, 16, 17, 20, 22). High-level mupirocin resistance has been transferred to suitable *S. aureus* recipients and found to be plasmid mediated (5, 15).

We transferred high-level mupirocin resistance to a strain of *S. epidermidis*; attempts at transfer to a strain of *E. faecalis* failed. High-level transferable mupirocin resistance has been described for coagulase-negative staphylococci isolated from clinical specimens (15); however, no descriptions of this marker in other gram-positive organisms have been reported. These findings suggest a narrow host range for this particular plasmid, similar to that of the gentamicin resistance plasmid pAM899-1 but in contrast to the broad host range of the enterococcal plasmid pAMB-1.

We examined the compatibility of pAM899-1 and our mupirocin plasmid by filter mating SA-136 and LZ-1/879R4. No recipient cells that simultaneously expressed gentamicin and high-level mupirocin resistance could be found, showing an inability of the plasmids to replicate within the same host cell. These plasmids likely belong to the same incompatibility group and therefore share regions of molecular homology. These characteristics may implicate a common evolutionary background for these self-transferable elements.

Rahman et al. also found that high-level mupirocin resistance was present on large plasmids of various sizes, some but not all of which were self-transferable (15). On the basis of restriction enzyme analysis and DNA hybridization studies, as well as the variation of transfer frequencies among strains and the existence of plasmid-free strains with nontransferable high-level resistance, some authors have suggested that high-level mupirocin resistance genes reside on transposons (5, 17, 21). We found high-level resistance only on self-transferable plasmid DNA.

We demonstrated the conjugative transfer of plasmid DNA encoding high-level mupirocin resistance. The transfer properties, presumed molecular weight, incompatibility group, and narrow host range of this plasmid are similar to those of pAM899-1. pAM899-1 can mobilize other nonconjugative coresident plasmids (8), and other self-transferable gentamicin plasmids carrying additional resistance markers have been described elsewhere (19). These phenomena have not been demonstrated with self-transferable mupirocin plasmids, although resistance to triclosan, curable by incubation at 42°C and transferable with high-level mupirocin resistance, has been reported (6). The heterogeneity of high-level resistance, the speculation that these genes may reside on transposons, and the similarity to pAM899-1 increase the concerns that the long-term application of mupirocin may encourage the dissemination of other resistance genes. The development of self-transferable high-level mupirocin resistance is further evidence in support of the judicious use of this topical antibiotic, especially in closed settings such as long-term-care facilities.

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