

Phenotypic and Genotypic Mupirocin Resistance among Staphylococci Causing Prosthetic Joint Infection

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Mupirocin MICs and *mupA* presence were determined in 108 staphylococci causing prosthetic joint infection. Zero of 35 isolates (0%) of methicillin-susceptible *Staphylococcus aureus*, 4/15 (27%) methicillin-resistant *S. aureus* isolates, 3/16 (19%) methicillin-susceptible coagulase-negative staphylococci, and 11/42 (26%) methicillin-resistant coagulase-negative staphylococci were mupirocin resistant. *mupA* was detected in all five high-level mupirocin-resistant staphylococci and one mupirocin-susceptible staphylococcus.

Most *Staphylococcus aureus* infections appear to originate from endogenous nasal flora (1, 10, 24). Decolonization of nasal carriers prior to orthopedic surgery may reduce the incidence of surgical site infection caused by *S. aureus* and the subsequent development of prosthetic joint infection (PJI) (9, 26). Topical mupirocin is an effective *S. aureus* nasal decolonization agent (14, 21); however, mupirocin resistance may be associated with decolonization failure (4, 7, 25). Low-level resistance to mupirocin is associated with mutations in endogenous bacterial isoleucyl-tRNA synthetase; high-level mupirocin resistance is due to acquisition of *mupA* which encodes an exogenous isoleucyl-tRNA synthetase not inhibited by mupirocin (6, 8). Coagulase-negative staphylococci (CNS) may act as a reservoir for *mupA*, which may be transferred to *S. aureus*; transfer of *mupA* from CNS to *S. aureus* has been demonstrated in vitro (17, 23).

To determine the frequency of phenotypic and genotypic resistance to mupirocin, we collected staphylococci from patients who had infected knee or hip prostheses and were hospitalized at the Mayo Clinic, Rochester, Minn., between January 1999 and December 2002. One staphylococcal isolate per patient from the site of the infection was studied. PJI was defined by the presence of at least one of the following criteria: (i) growth of the same microorganism in two or more synovial fluid or intraoperative tissue cultures, (ii) synovial fluid or intraoperative tissue purulence, (iii) acute inflammation on histopathologic examination of intraoperative tissue, and (iv) a sinus tract communicating with the prosthesis (22, 28).

Mupirocin MICs were determined by broth microdilution according to Clinical and Laboratory Standards Institute (formerly NCCLS) guidelines (15). Mupirocin resistance was classified as low level (MIC, 8 to 256 $\mu\text{g/ml}$) or high level (MIC, >256 $\mu\text{g/ml}$) (3). *S. aureus* ATCC 29213 was used for assay control.

The presence of *mupA* was determined by using PCR with previously described primers Mup 1 (5' CCC ATG GCT TAC CAG TTG A) and Mup 2 (5' CCA TGG AGC ACT ATC

CGA A) (8, 18). DNA was extracted from 10⁸ bacterial cells by using DNA Stat DNA-60 (Tel-Test, Friendswood, TX). Cycling parameters consisted of 95°C for 10 min followed by 40 cycles of 1 min at 94°C, 2 min at 46°C, and 3 min at 72°C, with a final extension of 3 min at 72°C.

To evaluate differences in categorical variables between groups, the two-tailed Fisher's exact test was used. A *P* value of <0.05 was considered statistically significant. All calculations were performed using the statistical software package JMP (version 5.1; SAS Institute Inc., Cary, NC). The study was approved by the Mayo Clinic Institutional Review Board.

A total of 108 staphylococcal isolates from 57 men and 51 women (median age, 70 years; range, 17 to 90 years) with infected knee (*n* = 61) or hip (*n* = 47) prostheses were studied. Fifty isolates (46%) were *S. aureus* isolates and 58 isolates (54%) were CNS, including *Staphylococcus epidermidis* (*n* = 45), *Staphylococcus lugdunensis* (*n* = 6), *Staphylococcus caprae/capitis* (*n* = 2), *Staphylococcus warneri* (*n* = 2), *Staphylococcus hominis* (*n* = 1), *Staphylococcus simulans* (*n* = 1), and *Staphylococcus saprophyticus* (*n* = 1). Coagulase-negative staphylococci were identified to the species level by using conventional biochemical testing. For isolates for which identification was unclear by use of conventional biochemical testing, 16S ribosomal RNA gene PCR and bidirectional sequence analysis were performed, as previously described (11). Data were analyzed by use of Sequencher 3.1 (Gene Codes Corporation, Ann Arbor, MI) and GenBank.

Table 1 shows the distribution of mupirocin resistance among staphylococcal isolates. Phenotypic mupirocin resistance was more common among CNS (14 of 58 isolates, 24%) than among *S. aureus* isolates (4 of 50 isolates, 8%) (*P* = 0.037). Among the 35 methicillin-susceptible *S. aureus* isolates, no mupirocin resistance was detected, whereas 4 of 15 (27%) methicillin-resistant *S. aureus* (MRSA) isolates were resistant to mupirocin (*P* = 0.006). Three MRSA isolates exhibited low-level mupirocin resistance (mupirocin MICs were 8, 32, and 32 $\mu\text{g/ml}$), and one exhibited high-level mupirocin resistance. Among CNS, 3 of 16 (19%) methicillin-susceptible isolates and 11 of 42 (26%) methicillin-resistant isolates exhibited mupirocin resistance (*P* = 0.736). Two methicillin-susceptible CNS exhibited low-level mupirocin resistance (mupirocin MICs were 32 and 64 $\mu\text{g/ml}$), and one exhibited high-level

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TABLE 1. Phenotypic and genotypic mupirocin resistance in staphylococci causing prosthetic joint infection

Parameter	Value for group			
	<i>Staphylococcus aureus</i> isolates		Coagulase-negative staphylococci	
	Methicillin susceptible (n = 35)	Methicillin resistant (n = 15)	Methicillin susceptible (n = 16)	Methicillin resistant (n = 42)
MIC ₉₀ (μg/ml)	0.25	32	32	64
MIC ₅₀ (μg/ml)	0.25	0.5	0.25	0.25
MIC range (μg/ml)	0.06–0.5	0.25–1,024	0.06–>1,024	0.06–>1,024
No. (%) of isolates of indicated category				
Susceptible (MIC, <8 μg/ml)	35 (100)	11 (73)	13 (81)	31 (74)
Low-level resistance (MIC, 8–256 μg/ml)	0	3 (20)	2 (13)	8 (19)
High-level resistance (MIC, >256 μg/ml)	0	1 (7)	1 (6)	3 (7)
Detection of <i>mupA</i> (n)	0	1	2	3

mupirocin resistance. In contrast, eight methicillin-resistant CNS exhibited low-level mupirocin resistance (mupirocin MICs were 32 μg/ml [six isolates] and 64 μg/ml [two isolates]), and three exhibited high-level mupirocin resistance.

mupA was detected in all isolates exhibiting high-level mupirocin resistance, as well as in one mupirocin-susceptible (mupirocin MIC, 2 μg/ml) *S. epidermidis* isolate. PCR amplification and product sequence analysis confirmed the presence of a *mupA*-specific sequence in this mupirocin-susceptible *S. epidermidis* isolate (GenBank accession number X75439) (8).

In our study, mupirocin resistance was more prevalent in CNS than in *S. aureus* (24% versus 8%), as was previously shown in studies with bloodstream, nosocomial pneumonia, skin and soft tissue infection, environmental, and carriage isolates (12, 13, 16, 20, 27). We also found that the mupirocin resistance rate among methicillin-resistant staphylococci causing PJI was higher than that of methicillin-susceptible staphylococci causing PJI, especially among *S. aureus* isolates (i.e., 27% in MRSA isolates versus 0% in methicillin-susceptible *S. aureus* isolates). This finding has been previously reported (2, 12, 20, 27); however, our study is the first to include a defined group of individuals with PJI. The presence of *mupA* in our study did not consistently correlate with high-level mupirocin resistance. *mupA* has been previously reported among *S. aureus* isolates exhibiting low-level mupirocin resistance (5, 18, 19) but not (as reported herein) in mupirocin-susceptible *S. epidermidis*. *mupA* may be present but not expressed, potentially as a result of mutations outside of the region sequenced herein or inadequate gene copy (18).

In conclusion, mupirocin resistance was found in 27% of MRSA isolates causing hip or knee PJI. This finding is important because mupirocin is used for decolonization of MRSA carriers, including the prevention of infection after implantation of prosthetic devices. Mupirocin susceptibility testing of *S. aureus* isolates to be treated with mupirocin is advised. New strategies for MRSA nasal decolonization are warranted.

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