Mupirocin Resistance in *Staphylococcus aureus* Causing Recurrent Skin and Soft Tissue Infections in Children[▽]

J. Chase McNeil, Kristina G. Hulten, Sheldon L. Kaplan, and Edward O. Mason*

Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas

Received 17 November 2010/Returned for modification 11 December 2010/Accepted 21 January 2011

***Staphylococcus aureus* resistance to mupirocin is often caused by acquisition of a novel isoleucyl-tRNA synthetase encoded on the plasmid gene *mupA*. We tested *S. aureus* isolates from children at Texas Children's Hospital with recurrent skin and soft tissue infections for mupirocin resistance and *mupA*. Of 136 isolates, 20 were resistant to mupirocin (14.7%). Fifteen isolates (11%) carried *mupA*, and the gene was more common in methicillin-susceptible *S. aureus* (21.4%) than methicillin-resistant *S. aureus* (8.3%; $P = 0.03$). Seven of 20 mupirocin-resistant isolates displayed clindamycin resistance.**

Mupirocin inhibits isoleucyl-tRNA synthetase, an enzyme necessary for bacterial protein synthesis. Mupirocin resistance exists as high-level resistance (HMR), with a MIC that is ≥ 512 $\mu\text{g/ml}$, or low-level resistance (LMR), with a MIC between 8 and 256 $\mu\text{g/ml}$ (7). HMR is mediated by *mupA*, a plasmid-associated gene, which encodes a novel isoleucyl-tRNA synthetase (8, 9). LMR is facilitated by a mutation in the native gene *ileS-1* (1).

Mupirocin resistance in adults ranges from 2 to 28% (14, 16). Few data are available on mupirocin resistance in children. We determined the prevalence of mupirocin resistance among *S. aureus* isolates causing recurrent skin and soft tissue infections (SSTI) at Texas Children's Hospital (TCH).

Isolates were chosen from a *Staphylococcus aureus* database approved by the Baylor College of Medicine Institutional Review Board (12). We reviewed medical records for patients who had at least three SSTI separated by at least 6 months between 2001 and 2009; patients with immunocompromising or chronic skin diseases were excluded. The initial and last isolates were analyzed. Isolates were classified as community acquired (CA), community-onset health care associated (CO-HCA), or nosocomial (11).

Oxacillin susceptibility was determined following Clinical and Laboratory Standards Institute guidelines (3). Mupirocin MICs were determined by Etest (Biomérieux, Sweden). Mupirocin agar dilution was used for a *mupA*⁺ strain with an LMR phenotype to identify resistant subpopulations.

A *mupA* probe was obtained using *S. aureus* control strain FPR3757, published *mupA* primers (4), and the DIG-High Prime labeling kit (Roche, Mannheim, Germany) according to the manufacturer's instructions and then used for a dot blot hybridization assay. Lysed bacterial suspensions were transferred to a Hybond-N+ membrane (GE Healthcare, Piscataway, NJ) by using a dot blot apparatus (Schleicher & Schuell, Keene, NH).

Pulsed-field gel electrophoresis (PFGE) was performed on

SmaI digestions of *mupA*-positive and/or resistant isolates and their pairs (11). For two isolates with a discrepancy between their MICs and the presence or absence of *mupA*, the *mupA* and *ileS-1* genes were sequenced (Seqwright, Inc.).

A total of 136 isolates representing 68 patients were available for molecular testing and were included in the final analysis. A total of 108 (79.5%) isolates were methicillin-resistant *S. aureus* (MRSA) and 28 (20.5%) were methicillin-susceptible *S. aureus* (MSSA). Twenty (14.7%) mupirocin-resistant isolates were identified from 16 patients; 16 of the isolates were HMR (Table 1).

The mupirocin MIC₅₀ was 0.064 $\mu\text{g/ml}$ (range, 0.064 to $>1,024$ $\mu\text{g/ml}$), which did not change over time. Resistance occurred more commonly among recurrent (19.1%) than initial (10.3%) isolates ($P = 0.06$). The rate of mupirocin resistance was higher among MSSA (21.4%) than MRSA (13%) isolates.

Eleven of 16 (68.8%) patients with mupirocin-resistant isolates were nonwhite, which was similar to the frequency for susceptible isolates (35/52, or 67.3%; $P = 0.46$). The median age of patients with resistant isolates was 61.5 months versus 29.5 months among susceptible isolates ($P = 0.07$).

Fifteen isolates carried *mupA*, occurring more commonly among recurrent (14.7%) than initial (7.3%) isolates (Table 1). *mupA* carriage was greater among MSSA (21.4%) than MRSA (8.3%) isolates ($P = 0.03$). One *mupA*-positive isolate displayed an LMR phenotype; sequencing revealed a single adenine residue deletion that caused a premature stop codon. Mupirocin agar dilution from 33 colonies from this isolate revealed that 31/33 clones had a mupirocin MIC of <2 $\mu\text{g/ml}$; one isolate had a MIC of 4 $\mu\text{g/ml}$, and another had a MIC of 16 $\mu\text{g/ml}$. The subclones were found to have identical PFGE patterns. Two *mupA*-negative isolates were HMR. Sequencing of both isolates identified no mutations in the *ileS-1* gene.

From review of medical records, mupirocin was used in 16/68 patients prior to acquisition of the isolate. Five of these 16 patients with resistant isolates had previous mupirocin exposure.

A total of 28/136 organisms exhibited clindamycin resistance (20.4%). Seven of 20 mupirocin-resistant organisms were clindamycin resistant (35%; $P = 0.05$); 5/15 *mupA*-positive isolates were also clindamycin resistant.

* Corresponding author. Mailing address: Texas Children's Hospital, Feigin Center, Suite 1150, 1102 Bates St., Houston, TX 77030. Phone: (832) 824-4330. Fax: (832) 825-4347. E-mail: emason@bcm.edu.

[▽] Published ahead of print on 31 January 2011.

TABLE 1. Prevalence of mupirocin resistance and *mupA*

Isolate group	Total no. of isolates with indicated level of mupirocin resistance/total no. of isolates (%)			Total no. of <i>mupA</i> -positive isolates/total no. analyzed (%)
	Resistant	HMR	LMR	
Overall	20/136 (14.7)	16/136 (11.7)	4/136 (2.9)	15/136 (11)
Initial isolates	7/68 (10.3)	5/68 (7.4)	2/68 (2.9)	5/68 (7.3)
Recurrent isolates	13/68 (19.1)	11/68 (16.1)	2/68 (2.9) ^a	10/68 (14.7) ^b
MRSA	14/108 (13)	11/108 (10.1)	3/108 (2.7)	9/108 (8.3)
MSSA	6/28 (21.4)	5/28 (17.8)	1/28 (3.5) ^c	6/28 (21.4) ^d

^a $P = 0.06$ for comparison of initial versus recurrent isolates for mupirocin resistance.

^b $P = 0.09$ for comparison of initial versus recurrent isolates for *mupA* positivity.

^c $P = 0.1$ for comparison of MRSA versus MSSA for mupirocin resistance.

^d $P = 0.03$ for comparison of MRSA versus MSSA for *mupA* positivity.

Isolates were derived from CA ($n = 78$), CO-HCA ($n = 56$), and nosocomial ($n = 2$) infections; 13/20 mupirocin-resistant isolates and 12/15 *mupA*-positive isolates were CA. By PFGE, 18 mupirocin-resistant organisms were USA300 (Table 2).

This is one of two studies examining mupirocin resistance in children in a community setting. We found mupirocin resistance in 14.7% of isolates, which is higher than the rate of 1.8% determined in the other community-based study of children (10).

mupA was carried significantly more frequently by MSSA than MRSA, in contrast to previous studies (2). USA300 has been associated with carriage of multidrug resistance plasmids, such as pUSA03, which confers resistance to mupirocin and clindamycin (5). Studies of *S. aureus* USA300 isolates from

TABLE 2. Comparison of paired patient isolates

Patient no. ^a	Initial or recurrence isolate ^b	Diagnosis	Methicillin resistance phenotype	Mupirocin resistance phenotype	Mupirocin MIC (μ g/ml)	<i>mupA</i>	PFGE type
1	Initial	Pustule	MRSA	LMR	16	Negative	USA300.0114
	Recurrence	Abscess	MRSA	S	0.064	Negative	USA300.0114
2	Initial	Paronychia	MRSA	S	0.064	Negative	USA300.0114
	Recurrence	Pustule	MRSA	HMR	>1,024	Positive	Non-USA300
3	Initial	Pustule	MRSA	LMR	128	Negative	USA300.0114
	Recurrence#	Abscess	MRSA	S	0.5	Negative	USA300.0114
4	Initial	Abscess	MRSA	S	0.094	Negative	USA300.0114
	Recurrence	Abscess	MRSA	LMR	128	Negative	USA300.0114
5	Initial	Abscess	MRSA	HMR	512	Negative	USA300.0114
	Recurrence	Abscess	MRSA	S	0.094	Negative	USA300.0114
6	Initial	Abscess	MRSA	HMR	>1,024	Positive	USA300.0114
	Recurrence#	Abscess	MSSA	S	0.25	Negative	USA300 var 1 ^c
7	Initial#	Abscess	MRSA	HMR	>1,024	Positive	USA300.0114
	Recurrence#	Abscess	MRSA	HMR	>1,024	Positive	USA300 var 2
8	Initial	Abscess	MSSA	HMR	>1,024	Positive	USA300 var 3
	Recurrence	Abscess	MSSA	HMR	>1,024	Positive	Non-USA300
9	Initial	Abscess	MSSA	S	0.064	Negative	USA300 var 4
	Recurrence	Abscess	MRSA	HMR	>1,024	Positive	USA300.0114
10	Initial	Pustule	MRSA	S	0.19	Negative	USA300.0114
	Recurrence#	Abscess	MSSA	HMR	>1,024	Positive	USA300 var 1
11	Initial#	Abscess	MRSA	S	0.125	Negative	USA300.0114
	Recurrence	Abscess	MRSA	HMR	>1,024	Positive	USA300.0114
12	Initial	Abscess	MRSA	S	0.094	Negative	USA300.0114
	Recurrence	Abscess	MRSA	HMR	>1,024	Positive	USA300.0114
13	Initial	Lymphadenitis, cellulitis	MSSA	LMR	192	Positive	USA300 var 1
	Recurrence	Abscess	MSSA	HMR	>1,024	Positive	USA300 var 1
14	Initial	Abscess	MRSA	HMR	512	Positive	USA300.0114
	Recurrence	Abscess	MRSA	HMR	512	Negative	USA300.0114
15	Initial	Abscess	MSSA	S	0.064	Negative	USA300 var 1
	Recurrence#	Abscess	MSSA	HMR	>1,024	Positive	USA300 var 1
16	Initial#	Abscess	MRSA	S	0.125	Negative	USA300 var 5
	Recurrence	Abscess	MRSA	HMR	>1,024	Positive	USA300 var 5

^a Patients include all those with at least one mupirocin-resistant and/or *mupA*-positive isolate along with its pair.

^b #, the isolate was obtained after mupirocin exposure.

^c var denotes a USA300 variant.

HIV-positive men found that all isolates harboring pUSA03 were mupirocin resistant (5).

Eighteen of 20 mupirocin-resistant isolates were USA300 by PFGE. Of the *mupA*-positive isolates, one-third were clindamycin resistant, consistent with acquisition of multidrug resistance determinants by USA300. Studies at our institution have shown higher rates of clindamycin resistance among MSSA than among MRSA (12). This may explain the higher prevalence of *mupA* among MSSA. Furthermore, both clindamycin- and mupirocin-resistant *S. aureus* isolates have disseminated in our community; four of five clindamycin-resistant, *mupA*-positive isolates were isolated from community-acquired infections.

One isolate with an LMR phenotype possessed *mupA*. Such a discrepancy has been described when mutations occur within *mupA* or when the plasmid-mediated gene becomes incorporated into the chromosome (6, 13). Our sequencing data revealed a single nucleotide deletion that caused a frameshift and a nonfunctional peptide. Population analysis further revealed that this isolate contained an LMR subpopulation. The LMR clone may have obtained further mutations within *ileS-1*, partially restoring the resistance phenotype, or it may have lacked the single nucleotide deletion in *mupA*. As noted by others (15), two isolates were found to have an HMR phenotype but lacked *mupA*. No mutations were noted within the native *ileS-1* gene, and it is possible that these isolates acquired HMR by another unknown mechanism.

Our retrospective design limits a complete determination of mupirocin use in our community. Furthermore, the true incidence of mupirocin resistance may be underrepresented. We only evaluated SSTI, and from these data it is difficult to state the impact that mupirocin resistance may have on invasive disease.

The rate of mupirocin resistance among pediatric *S. aureus* isolates in the Houston area is approximately 15% and is particularly a problem for infections by community-acquired MSSA and clindamycin-resistant isolates. Continued surveillance of mupirocin resistance is warranted.

This study was supported in part by Pfizer.

REFERENCES

1. Antonio, M., N. McFerran, and M. J. Pallen. 2002. Mutations affecting the Rossman fold of isoleucyl-tRNA synthetase are correlated with low-level mupirocin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **46**:438–442.
2. Chaves, F., J. Garcia-Martinez, S. de Miguel, and J. R. Otero. 2004. Molecular characterization of resistance to mupirocin in methicillin-susceptible and -resistant isolates of *Staphylococcus aureus* from nasal samples. *J. Clin. Microbiol.* **42**:822–824.
3. Clinical and Laboratory Standards Institute. 2010. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. CLSI document M100-S20-U. CLSI, Wayne, PA.
4. Diep, B. A., et al. 2008. Emergence of multidrug-resistant, community-associated, methicillin-resistant *Staphylococcus aureus* clone USA300 in men who have sex with men. *Ann. Intern. Med.* **148**:249–257.
5. Diep, B. A., et al. 2006. Complete genome sequence of USA300, an epidemic clone of community-acquired *Staphylococcus aureus*. *Lancet* **367**:731–739.
6. Driscoll, D. G., C. L. Young, and U. A. Ochsner. 2007. Transient loss of high-level mupirocin resistance in *Staphylococcus aureus* due to *mupA* polymorphism. *Antimicrob. Agents Chemother.* **51**:2247–2248.
7. Farmer, T. H., J. Gilbert, and S. W. Elson. 1992. Biochemical basis of mupirocin resistance in strains of *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **30**:587–596.
8. Gilbert, J., C. R. Perry, and B. Slocumbe. 1993. High-level mupirocin resistance in *Staphylococcus aureus*: evidence for two distinct isoleucyl-tRNA synthetases. *Antimicrob. Agents Chemother.* **37**:32–38.
9. Hodgson, J. E., et al. 1994. Molecular characterization of the gene encoding high-level mupirocin resistance in *Staphylococcus aureus* J2870. *Antimicrob. Agents Chemother.* **38**:1205–1208.
10. Hogue, J. S., P. Buttke, L. E. Braun, and M. P. Fairchok. 2010. Mupirocin resistance related to increasing mupirocin use in the clinical isolates of methicillin-resistant *Staphylococcus aureus* in a pediatric population. *J. Clin. Microbiol.* **48**:2599–2600.
11. Hulten, K. G., et al. 2006. Three-year surveillance of community onset health care-associated *Staphylococcus aureus* infections in children. *Pediatr. Infect. Dis. J.* **25**:349–353.
12. Kaplan, S. L., et al. 2005. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin. Infect. Dis.* **40**:1785–1791.
13. Ramsey, M. A., S. F. Bradley, C. A. Kauffman, and T. M. Morton. 1996. Identification of chromosomal location of *mupA* gene encoding low-level mupirocin resistance in staphylococcal isolates. *Antimicrob. Agents Chemother.* **40**:2820–2823.
14. Simor, A. E., et al. 2007. Mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* strains in Canadian hospitals. *Antimicrob. Agents Chemother.* **51**:3880–3886.
15. Swenson, J. M., et al. 2010. Multicenter study to determine disk diffusion and broth microdilution criteria for prediction of high- and low-level mupirocin resistance in *Staphylococcus aureus*. *J. Clin. Microbiol.* **48**:2469–2475.
16. Upton, A., S. Lang, and H. Heffernan. 2003. Mupirocin and *Staphylococcus aureus*: a recent paradigm of emerging antibiotic resistance. *J. Antimicrob. Chemother.* **51**:613–617.