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# Molecular Evaluation of Mupirocin Resistances Among *Staphylococcus aureus* Collected in United States (USA) Hospitals During 2008-2010

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SENTRY ANTIMICROBIAL SURVEILLANCE

## ABSTRACT

**Background**: Recent reports indicate an escalation in high-level resistance (HLR, MIC, ≥512 µg/mL) to mupirocin among *S. aureus* (SA) following the increased use of this agent to control the carriage of MRSA on skin and in nasal passages, as well as for treatment of cutaneous infections. Low-level resistance (LLR; MIC, 8-256 µg/mL) arises by mutation of the mupirocin target, isoleucyl-tRNA synthetase, whereas HLR is due to presence of the plasmid encoded *mupA*, an exogenous isoleucyl-tRNA synthetase. We analyzed mupirocin resistance (R) rates among SA collected from USA hospitals in a 3 year period and evaluated the R mechanisms against this agent in isolates showing HLR.

**Methods**: 16,762 SA isolates collected from bloodstream, respiratory and skin and skin structure infections in 77 hospitals located in 41 USA states during 2008-2010 were susceptibility (S) tested by reference broth microdilution methods against mupirocin and comparator agents. All 2009 and 2010 strains displaying HLR to mupirocin were tested for *mupA* by PCR and results were analyzed by melting curve analysis. *nucA* was tested as an internal control.

**Results**: Overall, 4.8% of the SA strains were R to mupirocin (2.5% HLR). S rates were stable over the years (94.9, 96.5 and 95.3% in 2008, 2009 and 2010, respectively). R was more common among MRSA when compared to methicillin-susceptible (MS) SA and rates among these groups showed very small variations over the years. The LLR rate was higher in 2008, whereas HLR were more elevated in 2010, but no definitive trends were observed. Among 261 HLR SA tested for *mupA*, only two (0.8%; one MSSA and one MRSA) were negative for the presence of this gene.

**Conclusions**: Our results demonstrated a stable rate of mupirocin R in a large recent multi-year USA collection of SA strains. *mupA* was detected in the vast majority (99.2%) of the isolates displaying HLR. Elevated mupirocin-R rates noted in prior studies could be due to limited sample sizes or evaluation of isolates from a unique institutional clone.

| Organisms group (no. tested) | No. (%): Susceptible (≤4 µg/mL) | Low Level R (8-256 µg/mL) | High Level R (≥512 µg/mL) |
|---|---|---|---|
| 2008 | | | |
| All *S. aureus* (5609) | 5326 (94.9) | 149 (2.7) | 134 (2.4) |
| MSSA (2411) | 2366 (98.1) | 16 (0.7) | 29 (1.2) |
| MRSA (3198) | 2960 (92.4) | 133 (4.3) | 105 (3.3) |
| 2009 | | | |
| All *S. aureus* (4989) | 4815 (96.5) | 80 (1.6) | 94 (1.8) |
| MSSA (2441) | 2404 (98.5) | 11 (0.5) | 26 (1.1) |
| MRSA (2548) | 2409 (94.5) | 71 (2.8) | 68 (2.7) |
| 2010 | | | |
| All *S. aureus* (6164) | 5875 (95.3) | 119 (2.0) | 167 (2.7) |
| MSSA (3028) | 2962 (97.8) | 21 (0.7) | 43 (1.5) |
| MRSA (3136) | 2913 (92.9) | 98 (3.1) | 124 (4.0) |

## INTRODUCTION

Mupirocin is approved by the United States (USA) Food and Drug Administration (FDA) for eradication of *Staphylococcus aureus* nasal carriage in adult patients and healthcare personnel to control the dissemination of methicillin-resistant *S. aureus* (MRSA). In other countries mupirocin is also used to treat superficial skin and skin-structure infections (SSSI) and this use has lead to the emergence of resistance among community-acquired isolates, where mupirocin was made available without prescription. Several studies showed that high-level mupirocin resistance (HLR) in *S. aureus* is associated with decolonization failure, thus, resistance to this compound should be closely monitored.

Among *S. aureus*, HLR (MIC, ≥512 µg/mL) is caused by the acquisition of a plasmid-mediated gene named *mupA*, which encodes a novel isoleucyl RNA synthetase. *mupA*-carrying plasmids typically harbor resistance determinants to other antimicrobial agents including macrolides, gentamicin, tetracycline, and trimethoprim, suggesting that mupirocin use could select for increased drug resistance among *S. aureus*. Isolates with low-level mupirocin resistance (LLR) usually have acquired base changes in the native isoleucyl RNA synthetase gene, *ileS* or a single copy of *mupA* in the chromosome.

In this study, we analyzed mupirocin resistance rates among *S. aureus* collected from USA hospitals in a 3 year period and evaluated the resistance mechanisms against this agent in isolates showing HLR from 2009-2010.

## MATERIALS AND METHODS

Bacterial strains. A total of 16,762 *S. aureus* strains collected during 2008-2010 (5,609, 4,989 and 6,164 for 2008, 2009 and 2010, respectively) in 77 USA hospitals, located in the nine Census Regions (41 states) were analyzed as part of the SENTRY Antimicrobial Surveillance Program. These isolates were obtained from bloodstream, respiratory tract infections, and skin and skin structure infections, according to defined protocols. Only one isolate per patient from documented infections were included. Species identification was confirmed by standard biochemical tests, the Vitek 2 System (bioMerieux, Hazelwood, Missouri, USA) or 16S rRNA sequencing, when necessary.

Antimicrobial susceptibility testing. Isolates were susceptibility tested by a reference broth microdilution procedure as described by the Clinical and Laboratory Standards Institute (CLSI; M07-A8 document) using validated broth microdilution panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Categorical interpretations for all antimicrobials were those found in M100-S21 (2011) and quality control (QC) was performed using, *S. aureus* ATCC 29213. All QC results were within specified ranges as published in CLSI documents.

Detection of *mupA*. All strains displaying mupirocin MIC at ≥512 µg/mL from 2009 and 2010 were tested for the presence of *mupA* and *nucA* (confirm identification and extraction control) in separate reactions. Oligonucleotides previously described for *mupA* (5'-GGG CCT TAA TTT CGG ATA GTG CTC-3' and 5'-TAA TCT GGC TGC GGA AGT GAA ATC-3') and *nucA* (5'-TAT GGT CCT GAA GCA AGT G-3' and 5'-GCC ACG TCC ATA TTT ATC AG-3') were used in an amplification reactions containing SYT09 (Invitrogen; California, USA). Detection of amplicons was performed by melting curve assays using the iCycler iQ™5 System (Bio-Rad, California, USA).

## RESULTS

- Overall, 95.6% of the *S. aureus* isolates tested were mupirocin-susceptible according to the CLSI breakpoints (≤4 µg/mL, susceptible).
- Variations by year were noted, but no clear trend could be detected comparing 2008 and 2010 results (*P*=0.2938; odds ratio [OR; 95% confidence interval (CI)], 0.91 [0.77-1.08]; Table 1). Using CLSI breakpoints, 94.9, 96.5 and 95.3% of the strains were susceptible in 2008, 2009 and 2010, respectively.
- LLR to mupirocin (MIC, 8-256 µg/mL) showed a slight variation among the three years analyzed: 2.7, 1.6 and 2.0% for 2008, 2009 and 2010, respectively (Table 1); however these differences were not statistically meaningful (*P*=0.0041; odds ratio [OR; 95% confidence interval (CI)], 0.70 [0.55-0.90]).
- Strains showing HLR (MIC, ≥512 µg/mL) were detected in comparable rates for the three years: 2.4, 2.0 and 2.7% for 2008, 2009 and 2010, respectively (*P*=0.0041; odds ratio [OR; 95% confidence interval (CI)], 0.70 [0.55-0.90]; Table 1).
- Resistance rates were higher among MRSA isolates compared to MSSA: 7.6, 5.5 and 7.1% resistant (CLSI criteria) for MRSA and 1.9, 1.6 and 2.2% resistant for MSSA in 2008, 2009 and 2010, respectively (Table 1).
- All 261 strains showing HLR (MIC, ≥512 µg/mL) from 2009 and 2010 were screened for the presence of *mupA* and 259 strains were positive for this gene. All strains were positive for *nucA* (*S. aureus* confirmatory ID).
- Isolates showing mupirocin MIC values ≥512 µg/mL and negative for *mupA* were one MSSA and one MRSA from Tulsa, OK and New Orleans, LA, respectively, both collected in 2010.

**Table 1.** Mupirocin MIC distributions for *S. aureus* in the USA SENTRY Program determined by the CLSI reference broth microdilution method.

| Organism (no. tested) | Cumulative no. % inhibited at MIC (µg/mL)[a]: ≤0.25 | 0.5 | 1[b] | 2 | 4[c] | 8 | 16 | 32 | 64 | 128 | 256 | ≥512 | No. at resistance categories (%): Susceptible (≤4 µg/ml) | LLR[d] (8-256 µg/ml) | HLR[d] (≥512 µg/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2008 | | | | | | | | | | | | | | | |
| All (5609) | 3673 (65.5) | 53 (66.4) | 17 (66.7) | 3 (66.8) | 1580 (94.9) | 15 (95.2) | 108 (97.1) | 15 (97.4) | 0 (97.4) | 0 (97.4) | 11 (97.6) | 134 (100.0) | 5326 (94.9) | 149 (2.7) | 134 (2.4) |
| MRSA (3198) | 2028 (63.4) | 33 (64.5) | 13 (64.8) | 1 (64.9) | 885 (92.6) | 11 (92.9) | 99 (96.0) | 14 (96.4) | 0 (96.4) | 0 (96.4) | 9 (96.7) | 105 (100.0) | 2960 (92.4) | 133 (4.3) | 105 (3.3) |
| MSSA (2411) | 1645 (68.2) | 20 (69.1) | 4 (69.2) | 2 (69.3) | 695 (98.1) | 4 (98.3) | 9 (98.7) | 1 (98.7) | 0 (98.7) | 0 (98.7) | 2 (98.8) | 29 (100.0) | 2366 (98.1) | 16 (0.7) | 29 (1.2) |
| 2009 | | | | | | | | | | | | | | | |
| All (4989) | - | - | - | - | 4815 (96.5) | 2 (96.5) | 55 (97.6) | 19 (98.0) | 2 (98.0) | 0 (98.0) | 2 (98.1) | 94 | 4815 (96.5) | 80 (1.6) | 94 (1.8) |
| MRSA (2548) | - | - | - | - | 2423 (94.9) | 2 (94.7) | 51 (96.7) | 15 (97.2) | 0 (97.2) | 0 (97.2) | 1 (97.3) | 68 (100.0) | 2409 (94.5) | 71 (2.8) | 68 (2.7) |
| MSSA (2441) | - | - | - | - | 2404 (98.5) | 4 (98.6) | 4 (98.8) | 2 (98.9) | 2 (98.9) | 0 (98.9) | 1 (98.9) | 26 (100.0) | 2404 (98.5) | 11 (0.5) | 26 (1.1) |
| 2010 | | | | | | | | | | | | | | | |
| All (6161) | - | - | - | - | 5877 (95.4) | 31 (95.9) | 63 (96.9) | 8 (97.0) | 1 (97.0) | 3 (97.1) | 11 (97.3) | 167 (100.0) | 5875 (95.3) | 119 (2.0) | 167 (2.7) |
| MRSA (3135) | - | - | - | - | 2915 (93.0) | 26 (93.8) | 53 (95.5) | 6 (95.7) | 1 (95.7) | 2 (95.8) | 8 (96.0) | 124 (100.0) | 2913 (92.9) | 98 (3.1) | 124 (4.0) |
| MSSA (3026) | - | - | - | - | 2962 (97.9) | 5 (98.0) | 10 (98.4) | 2 (98.4) | 0 (98.4) | 1 (98.5) | 3 (98.6) | 43 (100.0) | 2962 (97.8) | 21 (0.7) | 43 (1.5) |

a. A total of 3961 strains collected in 2008 were tested with a dilution range of 0.25-512 µg/mL, whereas 1648 were tested with a dilution range of 4-512 µg/mL also used for 2009 and 2010 isolates.
b. EUCAST susceptible breakpoint (≤1 µg/mL).
c. CLSI susceptible breakpoint (≤4 µg/mL).
d. LLR= low level resistance; HLR= high level resistance.

## CONCLUSIONS

- In this study analyzing a large collection of *S. aureus* strains collected throughout the USA, mupirocin resistance rates were stable and showed no increasing trend during three years monitored, contradicting some recent reports of escalating resistance rates against this compound in the USA.
- Among HLR strains, *mupA* is still the main resistance mechanism, but strains showing elevated MIC results that did not carry this gene (only in 2010) were detected and will be further characterized.

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