Evaluation of *mupA* EVIGENE Assay for Determination of High-Level Mupirocin Resistance in *Staphylococcus aureus*[▽]

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Mupirocin is widely used to decolonize patients carrying *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA). The aim of this study was to determine the presence of high-level mupirocin resistance by a new commercially available *mupA* genotypic diagnostic product, *mupA* EVIGENE assay (AdvanDx).

Mupirocin is a naturally occurring antibiotic produced by *Pseudomonas fluorescens*. Mupirocin exerts its antimicrobial effect by inhibiting protein synthesis through irreversible and specific binding to bacterial isoleucyl-tRNA synthetase encoded by a chromosomal gene, *ileS* (8). Mupirocin is widely used, as it is established as the best topical antimicrobial available for both methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) (9) and is an important agent in preventing transmission of health care-associated staphylococcal infection, surgical-site infection (12), and bloodstream infection in patients undergoing hemodialysis (2, 14).

Unfortunately, bacterial resistance to mupirocin has been seen for both MSSA and MRSA. Mupirocin resistance occurs as two phenotypes: “high level” and “low level.” Low-level resistance is due to a genetic mutation, producing MICs in the range of 8 to 256 µg/ml, whereas high-level resistance (with an MIC of >256 µg/ml) is caused by presence of the plasmid gene *mupA* (or *ileS-2*) that encodes an additional isoleucyl-tRNA synthetase that is not inhibited by mupirocin (5, 7). Since application of mupirocin ointment into the external nares results in concentrations of ≥20,000 µg/ml, only high-level resistance is potentially significant in this setting (15).

High-level mupirocin resistance occurs in a variety of community-associated MRSA (CA-MRSA) and health care-associated MRSA (HA-MRSA) strains (13). Currently, isolates of the USA300 (ST8) lineage are the most prevalent CA-MRSA strain in United States, and they have been confirmed to carry the conjugative plasmid (pUSA03) expressing the *mupA* high-level mupirocin resistance gene (6). This indicates the need for susceptibility testing or detection of the *mupA* gene to guide therapeutic and prophylactic use of mupirocin.

Differentiation between high- and low-level resistances requires methods such as broth microdilution for determination of MIC, Etest (which is not cleared by the U.S. Food and Drug Administration [FDA]), or detection of the *mupA* gene asso-

ciated with high-level resistance (in-house PCR methods) (5, 15). Genotypic tests offer the advantage of detecting the genetic marker itself rather than phenotypic characteristics. Furthermore, genotypic tests can be performed directly using the clinical isolate without the need for incubation and culturing overnight. The aim of this study was to compare a new genotypic test for the detection of high-level mupirocin resistance, *mupA* EVIGENE assay (AdvanDx), with PCR for the *mupA* gene followed by bidirectional sequence analysis, the Clinical and Laboratory Standards Institute (CLSI)-recommended phenotypic mupirocin resistance testing (single-broth dilution test [256 µg/ml] and 200-µg disk diffusion test [3, 4]), and the mupirocin Etest.

The *mupA* EVIGENE assay is a sandwich hybridization assay that provides rapid (3.5-h) detection of the *mupA* gene from clinical isolates of *S. aureus* compared to the 18 to 24 needed for phenotypic tests. The EVIGENE plate allows testing in large batches (94 isolates) or testing of one sample at a time. The hands-on time is approximately 10 min for one sample and 30 min for 50 samples. The only equipment needed is a heating block and heated shaker (55°C, 400 rpm), and because the signal can be read by eye, this assay should be easy to implement for most laboratories.

The mupirocin gene has been associated with 46 different plasmid profiles (13). To make the collection of tested isolates as broad and diverse as possible, a total of 156 *S. aureus* isolates were collected and tested at one U.S. site and one European Union site (Denmark). In addition, a study was performed at the Centers for Disease Control and Prevention (CDC), Atlanta, GA, with 105 unrelated *S. aureus* isolates with known mupirocin MICs in the range of 8 to >1,024 µg/ml.

All 156 isolates were analyzed for the presence of *mupA* by using a PCR assay (11). As a control for the presence of PCR-inhibitory components in the template preparation, a second PCR was employed (1). Compared with PCR, the *mupA* EVIGENE assay exhibited 100% sensitivity (i.e., results by the two methods were the same for 16 out of 16 isolates tested) and 100% specificity (140 out of 140 isolates) (Table 1).

According to the CLSI guidelines, isolates with high-level mupirocin resistance should result in no zone of diffusion around a 200-µg mupirocin disk. A comparison of the *mupA*

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TABLE 1. *mupA* EVIGENE performance data versus genotypic testing (*mupA* PCR) data

Study site	<i>mupA</i> EVIGENE assay result compared to <i>mupA</i> PCR result ^a	
	No. testing positive/total no. tested	No. testing negative/total no. tested
Statens Serum Institute (Denmark)	12/12	39/39
University of Maryland	4/4	101/101
	Sensitivity 100% (16/16)	Specificity 100% (140/140)

^a No. testing positive or negative, *mupA* EVIGENE assay result. Total no. tested, number of samples that tested positive or negative by *mupA* PCR.

EVIGENE assay result to that of the disk diffusion assay showed a sensitivity of 93.8% (i.e., results by the two methods were the same for 15 out of 16 isolates), as one isolate which tested positive by the *mupA* EVIGENE assay was found to have a zone of inhibition of 10 mm. The specificity was 100% (140 out of 140) (Table 2). A study reported in the current CLSI guidelines demonstrated that the majority of *mupA*-negative isolates had 200- μ g mupirocin disk diffusion inhibition zone diameters of >18 mm. This agreed with results of our study, as all isolates (140 out of 140) that tested negative in the *mupA* EVIGENE assay demonstrated mupirocin 200- μ g inhibition zone diameters of >18 mm. This result suggests that the breakpoint for high-level mupirocin should be zone diameters of <18 mm rather than no zone around a 200- μ g mupirocin disk.

Performance data for the *mupA* EVIGENE assay compared to another recommended CLSI phenotypic test, single broth dilution (256 μ g/ml mupirocin), exhibited 100% sensitivity (with results for 16 out of 16 isolates in agreement) and 98.6% specificity (138 out of 140) (Table 2). The two tests performed equally for the majority of isolates ($n = 154$), except for two isolates that tested negative in the *mupA* EVIGENE assay but showed growth in the broth medium with 256 μ g/ml mupirocin. The same two isolates were confirmed negative with 200- μ g mupirocin disk diffusion, yielding zone diameters of 21 and 32 mm, and were thus classified as lacking high-level resistance according to the CLSI guidelines.

Furthermore, the 156 clinical isolates were evaluated for susceptibility using a mupirocin Etest strip (AB Biodisk).

The MIC data showed that all 16 *mupA* EVIGENE assay-positive isolates had MICs of >1,024 μ g/ml, and all 140 *mupA* EVIGENE assay-negative isolates had MICs of <0.064 to 64 μ g/ml (Table 2).

In a challenge study with 105 *S. aureus* isolates (54 *mupA*-positive and 51 *mupA*-negative strains with mupirocin concentrations from 2 to 1,024 μ g/ml confirmed by the CDC) (10), we observed that the *mupA* EVIGENE assay was negative for strains having low-level mupirocin resistance (8 to 64 μ g/ml) and positive for strains having high-level mupirocin resistance (1,024 μ g/ml) (Table 3). Sequencing of the 54 *S. aureus* clinical isolates with high-level mupirocin resistance showed that all 54 exhibited 100% to 90% sequence agreement with the *mupA* gene sequence. Of these, 50 clinical isolates had a DNA sequence in the amplified part of the *mupA* gene that was identical to that of the positive control AIS2006 079 (USA300), while one isolate had a single nucleotide substitution from G to A, leading to an amino acid change from serine to asparagine, and three isolates had a single nucleotide substitution from G to A, leading to an amino acid change from alanine to threonine. Strain AIS2006 079 from the CDC was used as a *mupA*-positive control, as the study was started before it was possible to get the American Type Culture Collection *mupA*-positive reference strain (BAA1708).

mupA EVIGENE is an easy to-use assay, requiring approximately 10 min of hands-on time, with a 3.5-h turnaround time with sample preparation. The *mupA* EVIGENE assay is a commercial alternative (Conformité Européenne [CE] labeled) to other molecular methods, such as in-house PCR methods, which for most routine microbiology laboratories are technically demanding. In this study, we compared our results to those obtained by standard PCR, which required identification by gel electrophoresis. Thus, for one sample, hands-on time was longer (40 min), but the turnaround time with sample preparation was shorter (2 h) for PCR than the EVIGENE assay when precast gels were used for identification.

The *mupA* EVIGENE assay is not yet approved by the FDA for detecting *mupA* in *S. aureus*, but the assay results show good discrimination between positive and negative test results in this two-site study and are supported by a challenge study with *S. aureus* isolates having mupirocin MICs from 8 to >1,024 μ g/ml. Thus, the use of the *mupA* EVIGENE assay is supported for the detection of high-level mupirocin resistance in clinical *S. aureus* isolates. The assay allows high-level mupi-

TABLE 2. *mupA* EVIGENE performance data versus phenotypic testing data^a

Study site	<i>mupA</i> EVIGENE assay result compared to phenotypic test result (no. testing positive/total no. tested)						
	Disk diffusion zone diameter			Mupirocin broth dilution (256 μ g/ml)		MIC range (μ g/ml)	
	No zone	<18 mm	>18 mm	Growth	No growth	>1,024	<0.064–64
Statens Serum Institute (Denmark)	11/12	12/12	39/39	12/12	38/39	12/12	39/39
University of Maryland	4/4	4/4	101/101	4/4	100/101	4/4	101/101
	Sensitivity 93.8% (15/16)	Sensitivity 100% (16/16)	Specificity 100% (140/140)	Sensitivity 100% (16/16)	Specificity 98.6% (138/140)	Sensitivity 100% (16/16)	Specificity 100% (140/140)

^a No. testing positive, *mupA* EVIGENE assay result. Total no. tested, number of samples with indicated result of 200- μ g mupirocin disk diffusion assay, single-dilution (256 μ g/ml) mupirocin broth assay, or mupirocin Etest.

TABLE 3. Study results for a panel of *S. aureus* isolates with mupirocin MICs from 8 to >1,024 µg/ml^a

No. of isolates	MIC range (µg/ml)	Result by:			
		<i>mupA</i> EVIGENE		<i>mupA</i> PCR	Sequencing
		Visual	OD range		
51	8–64	Negative	0.101–0.495	Negative	NA
54	>1,024	Positive	1.506–2.810	Positive	Positive

^a NA, not applicable.

rocin resistance to be detected 18 to 24 h sooner than by conventional methods without the potential errors inherent in the determination of inhibition zone sizes in disk diffusion testing and the possibility of false turbidity in broth dilution assays.

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