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Sensitivity and Specificity of a Rapid rRNA Gene Probe Assay for Simultaneous Identification of *Staphylococcus aureus* and Detection of *mecA*

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rRNA gene sequences were used for identification and target adequacy controls in a DNA probe assay to identify isolates as *Staphylococcus* and, more specifically, as *S. aureus* within 1 hour. *mecA* status was simultaneously determined using a specific DNA probe. The target adequacy control guarded against false-negative *mecA* results.

Methicillin resistance in *Staphylococcus aureus* and coagulase–negative *Staphylococcus* (CoNS) are leading causes of nosocomial infections worldwide (13). These high levels of methicillin resistance have led to an overuse of vancomycin, resulting in vancomycin-intermediate and -resistant *Staphylococcus* (5, 19, 21, 22). Over the last several years, methicillin-resistant *Staphylococcus aureus* (MRSA) has been emerging in the community (community-acquired MRSA [CA-MRSA]). CA-MRSA is associated with strains that have some unique properties (such as virulence factors and susceptibility patterns) compared to the traditional hospital-acquired MRSA strains. Consequently, the need for rapid methods to identify and detect resistant *Staphylococcus* remains (3).

Common methods for susceptibility testing include broth microdilution, disk diffusion, and screening agars, which require timely incubations after isolate identification. Alternatively, the Oxoid PBP2′ latex agglutination (OLA) assay (Oxoid, United Kingdom) is a rapid test for differentiating MRSA from methicillin-sensitive *Staphylococcus aureus* (MSSA) on agar plates (2, 11, 14, 23, 24, 25, 26, 27). Unfortunately, reliable testing of CoNS with the OLA assay requires induction with oxacillin (Ox), and a heavier inoculum size and longer lysis are critical for accurate results (11, 14, 26).

Recently, molecular assays for methicillin resistance and *Staphylococcus* identification using *mecA* and *Staphylococcus*-specific genes have yielded fast and reliable results (4, 6, 9, 12, 13, 16, 20). Molecular assays have an advantage because they do not rely on time-consuming incubations or phenotypic expression (7). However, because of the technical expertise required, many formats are not practical for routine use in most clinical laboratories.

The objective of this study was to evaluate the performance of a Gen-Probe (San Diego, Calif.) prototype DNA probe hybridization protection assay (HPA). This assay, currently under development, can simultaneously identify a *Staphylococcus* isolate and the *mecA* gene for rapid detection of MRSA and methicillin-resistant CoNS (MRCoNS) (Fig. 1). This is the first report describing the use of DNA probe HPA for detecting single-stranded DNA. A total of 589 clinical isolates and 24 American Type Culture Collection (ATCC) isolates were tested using the oxacillin MIC, the DNA probe HPA, and the OLA assay. Specificities for 368 clinical isolates representing 288 *S. aureus* and 80 CoNS strains were tested on subculture plates. To test assay sensitivity and specificity in a clinical setting, 221 isolates representing 110 *S. aureus* and 111 CoNS strains were tested from primary culture plates. Each clinical isolate represented only one strain per patient. Ox MIC was the reference method, since it is used in the laboratory for routine susceptibility testing. PCR of the *mecA* gene was used to resolve any discrepant results.

Clinical isolates were collected from the UCLA Medical Center and Sunnybrook and Women's College Health Sciences Centre (SWCHSC). All frozen isolates were passed three times on blood agar plates prior to being tested. All clinical isolates collected from primary plates were collected from the UCLA Medical Center. Colonies from primary plates presumed to be *S. aureus* or CoNS were used for OLA and probe analysis. These colonies were subcultured for MIC testing, identification confirmation, and preparing glycerol stocks. Isolates were identified using conventional biochemical methods. Oxacillin susceptibilities were determined for all isolates according to CLSI (formerly NCCLS) methods (17). Isolates from UCLA were tested using a broth microdilution reference method with in-house panels (17). SWCHSC tested isolates by disk diffusion and broth microdilution (17). The OLA test was performed with modifications as described previously (11, 14). PCR amplification was performed at UCLA, SWCHSC, and Gen-Probe (10, 15).

Probe analysis was performed using HPA (1, 18), which employs probes with chemiluminescent acridinium ester labels complementary to a highly conserved bacterial rRNA gene

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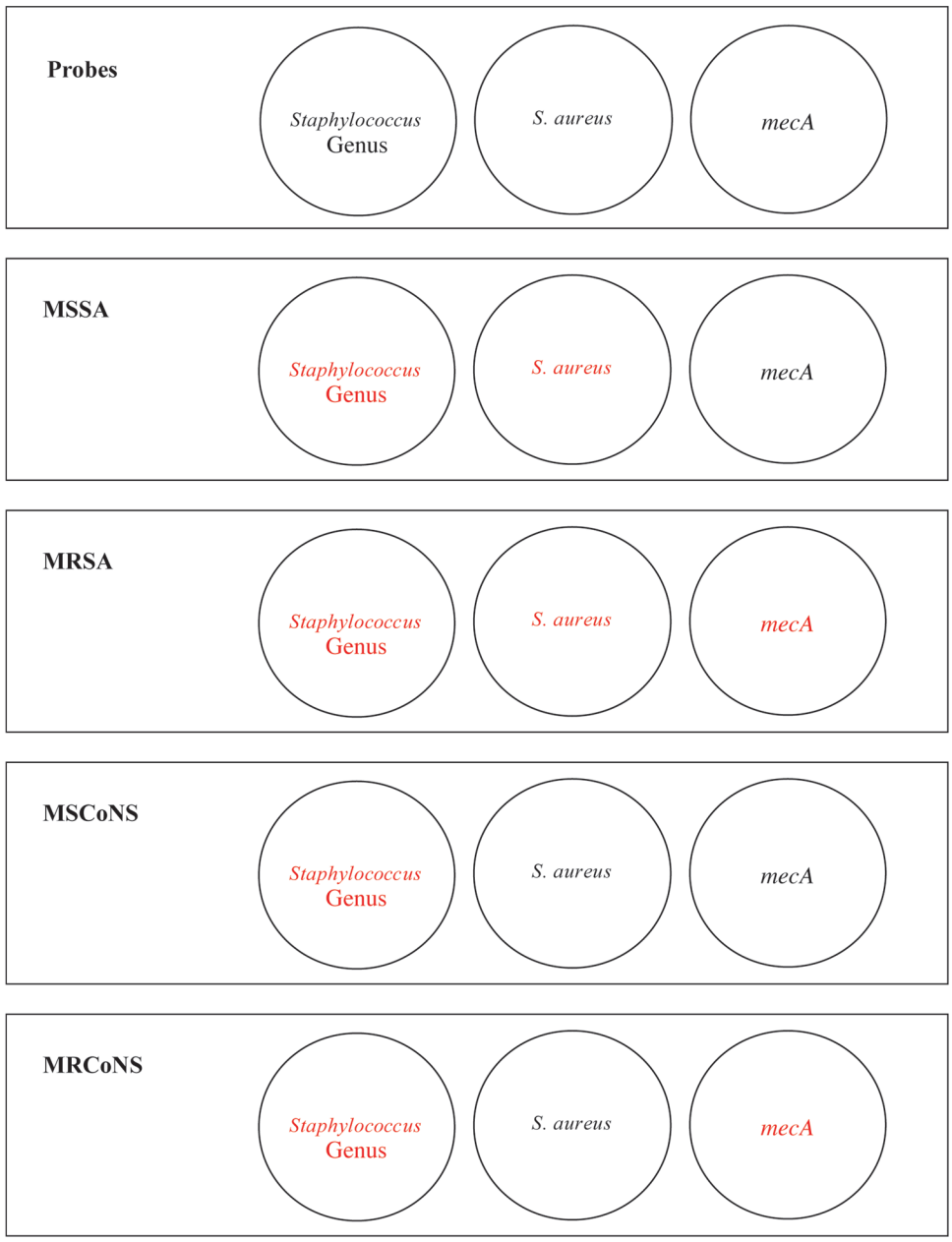

FIG. 1. Simultaneous identification of *Staphylococcus* and determination of *mecA* status. Positive probe results are indicated in red.

region or antibiotic resistance gene. These highly conserved bacterial rRNA gene probes have been extensively characterized for identification specificity (15). Gen-Probe Incorporated supplied all proprietary reagents. The assay was run in white 96-well microtiter plates (Whatman, Clifton, N.J.). Each microtiter plate contained three probe mixtures in individual wells to make up the reaction set (Fig. 1). A 1-μl loop of cells was lysed at 100°C in 150 μl of a succinate-buffered detergent reagent. After the addition of 100 μl of base solution and a 10-min 60°C incubation, the reaction mixture was cooled to room temperature and 100 μl of a hybridization buffer was added. Of this mixture, 50 μl was aliquoted to each of the three wells and a 50-μl oil overlay was added to prevent evaporation throughout the 60°C assay. HPA was automated using a BMG luminometer (Lumistar 500; BMG Technologies, Offenburg, Germany), and the results were available in under 60 min.

The probe results for detecting MRSA and MRCoNS from both primary and subculture plates are summarized in Tables 1 and 2. Of the 398 *S. aureus* clinical isolates, 196 were Ox resistant (MICs ≥ 4 μg/ml) and 202 were Ox susceptible (MICs ≤ 2 μg/ml) (Table 1). The *mecA* probe results demonstrated that 184 of the 196 Ox-resistant isolates produced positive probe results and that 199 of the 202 Ox-susceptible isolates produced negative probe results. The *mecA* probe results showed 184/196 MRSA and 199/202 MSSA. The *mecA* probe, OLA, and PCR were negative for 12/196 MRSA isolates formally defined as borderline resistant (MICs ≥ 4 to 8 μg/ml). Regarding the three OLA-, *mecA* probe-, and PCR-

TABLE 1. Results for 411 *S. aureus* isolates in Ox MIC, GP probe, and OLA assays

Organism	No. of isolates	Oxacillin MIC (μg/ml)	GP probe (no.)				OLA (no.)	
			Staphylococcus sp.	*S. aureus*	*mecA* positive	*mecA* negative	Positive	Negative
Clinical MRSA	14	≥4	14	14	6	8	6	8
	13	8	13	13	9	4	9	4
	14	16	14	14	14	0	14	0
	155	>16	155	155	155	0	154	1
Clinical MSSA	149	≤0.25	149	149	1	148	1	148
	32	0.5	32	32	0	32	0	32
	13	1	13	13	0	13	0	13
	8	2	8	8	2	6	2	6
ATCC MRSA[a]	1	8	1	1	1	0	1	0
	1	16	1	1	1	0	1	0
ATCC MSSA[a]	6	≤0.25	6	6	0	6	0	6
	4	0.5	4	4	0	4	0	4
	1	1	1	1	0	1	0	1

[a] ATCC MRSA tested included no. 33591 and 43866. ATCC MSSA tested included no. 6538, 12598, 27661, 12600, 29213, 25923, 27660, 13301, 25904, 29247, and 33589.

TABLE 2. Results for 202 CoNS clinical and ATCC isolates in Ox MIC, GP Probe, and OLA assays

Organism	No. of isolates	Oxacillin MIC (μg/ml)	GP probe (no.)				OLA (no.)	
			Staphylococcus sp.	*S. aureus*	*mecA* positive	*mecA* negative	Positive	Negative
S. capitis	10	≤0.25	10	0	0	10	0	10
	1	4	1	0	1	0	1	0
	1	>16	1	0	1	0	1	0
S. epidermidis[a]	36	≤0.25	36	0	4	32	0	36
	2	0.5	2	0	2	0	2	0
	5	1	5	0	5	0	5	0
	14	2	14	0	14	0	14	0
	14	4	14	0	14	0	14	0
	8	8	8	0	8	0	8	0
	8	16	8	0	8	0	8	0
	38	>16	38	0	38	0	38	0
S. intermedius[a]	1	≤0.25	1	0	0	1	0	1
	1	0.5	1	0	0	1	1	0
	1	8	1	0	1	0	1	0
	1	>16	1	0	1	0	1	0
S. auricularis	1	≤0.25	1	0	0	1	0	1
S. hyicus-hyicus[a]	1	≤0.25	1	0	0	1	0	1
S. lugdunensis	3	0.5	3	0	0	3	0	3
S. warneri[a]	5	≤0.25	5	0	1	4	2	3
	3	0.5	3	0	0	3	0	3
	1	16	1	0	0	1	1	0
	4	>16	4	0	4	0	4	0
S. haemolyticus[a]	6	≤0.25	6	0	0	6	0	6
	1	0.5	1	0	1	0	1	0
	1	4	1	0	1	0	1	0
	10	>16	10	0	10	0	10	0
S. saprophyticus[a]	2	≤0.25	2	0	0	2	0	2
	2	0.5	2	0	0	2	0	2
	4	1	4	0	0	4	0	4
S. cohnii	1	≤0.25	1	0	0	1	0	1
S. sciuri	1	≤0.25	1	0	1	0	0	1
S. simulans[a]	1	≤0.25	1	0	0	1	0	1
S. hominis[a]	5	≤0.25	5	0	0	5	0	5
	2	0.5	2	0	2	0	1	1
	7	>16	7	0	7	0	7	0

[a] ATCC isolates tested included *S. epidermidis* (12228, 29887, 14990, and 35983), *S. intermedius* (29663), *S. hyicus-hyicus* (11249), *S. warneri* (27836), *S. haemolyticus* (29970), *S. saprophyticus* (15305), *S. simulans* (11631), and *S. hominis* (29885).

positive MSSA samples, the initial OLA results for two of the three were negative, but upon using the modified protocol, both samples converted to OLA positive. Converted OLA-positive isolates may reflect low expression levels of the *mecA* protein due to regulatory mutations.

Of the 191 CoNS clinical isolates, 129 were Ox resistant (MICs $\geq$ 0.5 μg/ml) and 62 were Ox susceptible (MICs $\leq$ 0.25 μg/ml) (Table 2). Of the 129 MRCoNS isolates, 116 were positive by *mecA* probe, PCR, and OLA. Eleven of the 13 discrepant MRCoNS isolates (MIC = 0.5 to 1.0 μg/ml) were *S. saprophyticus*, *S. lugdunensis*, or *S. warneri* (Table 2). Mechanisms of resistance other than those mediated by *mecA* may be responsible for these results. Other investigators using similar molecular methods with CoNS have observed this phenomenon (8, 11). Based on these MIC cutoffs, CLSI suggests further testing for clarification of resistance status using molecularly based techniques (17). The other two discrepant MRCoNS isolates were OLA positive and PCR negative and were identified as *S. intermedius* (MIC $\leq$ 0.5 μg/ml) and *S. warneri* (MIC = 16 μg/ml).

The two OLA-positive MRCoNS isolates that disagreed with the *mecA* probe and PCR results might reflect mutations. However, with three different DNA probes widely dispersed along the *mecA* gene, three different mutations yielding a false-negative result are unlikely. A possible explanation is that the material tested by the *mecA* probe and PCR was different from the colonies used for MIC and OLA. This hypothesis is supported by OLA-negative results when glycerol retention samples of these colonies were grown on plates containing an oxacillin disk. These isolates were originally identified only as CoNS using a coagulase test, so a correlation at the species level could not be performed between the first and second rounds of test isolates.

Of 62 MSCoNS isolates, 56 were negative by *mecA* probe, PCR, and OLA. PCR confirmed the six *mec*A probe-positive MSCoNS isolates, with one displaying a positive OLA result identified as *S. warneri*. Of the five that disagreed with OLA, four were *S. epidermidis* and one was *S. sciuri*. The five OLA-negative isolates may represent an unexpressed *mecA* gene due to mutations in the regulatory regions. The single OLA-positive isolate may be an example of down regulation as opposed to nonexpression of the *mecA* gene.

Testing of 24 ATCC *Staphylococcus* isolates showed 100% agreement for *S. aureus* with the *mecA* probe and PCR. Of the 11 ATCC CoNS isolates, 1 of 3 MRCoNS isolates was *mecA* probe and PCR negative and was identified as *S. saprophyticus*. OLA results for the ATCC isolates agreed with MIC, *mecA* probe, and PCR except for one false positive for a methicillin-sensitive *S. warneri* isolate and the false negative for *S. saprophyticus* (Table 2). The OLA manufacturer's insert states that *S. warneri* may give a false positive, and in such cases, the Ox MIC should be used for determining resistance.

Rapid identification of *Staphylococcus*, more specifically *S. aureus*, along with the methicillin resistance status has been the focus of many recent studies (4, 6, 9, 12, 13, 20). Many direct and amplified formats have problems with false negatives due to lack of an internal control to verify that sufficient target was present and accessible. The DNA probe system described here addresses this problem by including an internal control consisting of rRNA gene-targeted *Staphylococcus* identification probes (Fig. 1). If there is sufficient DNA target to give a valid identification signal, there should be sufficient target for determination of the *mecA* status, thus eliminating false-negative results.

This study demonstrates that the identification of an isolate as a member of the *Staphylococcus* genus, and more specifically as *S. aureus*, coupled with the determination of *mecA* status in a 1-hour timeframe is feasible. The DNA probe identification results for *S. aureus* displayed 100% sensitivity and specificity. The *mecA* DNA probe results for *S. aureus* demonstrated 93.9% sensitivity and 98.6% specificity, and CoNS had 89.4% sensitivity and 91.4% specificity compared to MICs. When PCR was used as the means to resolve the *mecA* discrepant results, the *mecA* probe test demonstrated 100% sensitivity and specificity with all *S. aureus* and CoNS isolates tested.

We have shown that this *Staphylococcus-S. aureus-mecA* probe system is an accurate, easy, and rapid assay. The DNA probe sample-handling methodology requires less than 5 min of hands-on time, is amendable to batch processing, and is performed in under 15 min. The probe hybridization and detection steps are fully automated and can be completed in 45 min using the automated microtiter instrument. The data are automatically exported into a spreadsheet at the end of the run, which makes analysis simple. Coupled with effective communication to the physician, this 1-hour DNA probe assay could improve management and infection control practices for methicillin-resistant *Staphylococcus* infections.

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