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Detection of Oxacillin-Susceptible *mecA*-Positive *Staphylococcus aureus* Isolates by Use of Chromogenic Medium MRSA ID

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Reports of oxacillin-susceptible *mecA*-positive *Staphylococcus aureus* strains are on the rise. Because of their susceptibility to oxacillin and cefoxitin, it is very difficult to detect them by using routine phenotypic methods. We describe two such isolates that were detected by chromogenic medium and confirmed by characterization of the *mecA* gene element.

By definition, methicillin-resistant *Staphylococcus aureus* (MRSA) strains have an oxacillin MIC of ≥4 μg/ml or harbor the *mecA* gene, which encodes the low-affinity penicillin-binding protein (PBP) designated PBP2a (1, 2). Among the MRSA isolates, only a few express homogeneous oxacillin resistance (i.e., ≥1 in 10^2 express high-level resistance) while the majority show heterogeneous drug resistance (heteroresistance) (3). Phenotypically oxacillin-susceptible and *mecA*-positive *S. aureus* clinical isolates are being increasingly reported (4–9, 16). Dependence on growth conditions like temperature and osmolarity of the medium for phenotypic expression of resistance further complicates susceptibility testing of MRSA by standard microbiological methods (3). On the basis of the Clinical and Laboratory Standards Institute (CLSI) guidelines, a method based on agar containing 6 μg/ml of oxacillin was developed to screen *S. aureus* isolates (2). Though it can detect true MRSA effectively, it is likely to miss *mecA*-positive *S. aureus* having an oxacillin MIC of <2 μg/ml. Such isolates have been considered to be extremely heteroresistant (<1 in 10^8 of the population is highly resistant to methicillin), but there are also reports documenting the existence of nonheterogeneous phenotypically oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA) (6). The use of β-lactams to treat such isolates may cause an increase in the MIC of oxacillin well above the established breakpoint for resistance (oxacillin MIC, ≥4 μg/ml), ultimately leading to failure of therapy (10).

From August to December 2011, 30 consecutive *S. aureus* isolates recovered from various clinical samples (blood, tissue, pus) from hospitalized patients in a university hospital in southern India were tested. Bacteria were identified as *S. aureus* by Gram stain, catalase, DNase, mannitol fermentation, and coagulase positivity or by Vitek2 ID-GP card (bioMérieux, Marcy l' Etoile, France). Methicillin susceptibility was determined by oxacillin and cefoxitin disc diffusion (1) and the absence of green colonies on a chromogenic medium MRSA ID agar (bioMérieux, Marcy l' Etoile, France). Isolates giving discordant results (green colonies only on MRSA ID agar) were further investigated by using oxacillin screening agar (1) and the Vitek 2 AST-GP67 automated system (bioMérieux, Marcy l' Etoile, France). The results were reconfirmed by MRSA latex agglutination (Slidex MRSA Detection; bioMérieux) and *mecA* PCR (11). Susceptibility testing of oxacillin-susceptible *mecA*-positive isolates for 14 antimicrobial agents was done by the Vitek 2 AST-GP67 card and disc diffusion method, and the MICs of oxacillin and cefoxitin was determined by the Etest method according to CLSI guidelines (1).

Special media with specific chromogenic substrates and incorporated antibiotics permitting the detection and identification of MRSA have been available for quite some time (12). We investigated *S. aureus* isolates that were positive only by MRSA ID chromogenic medium and negative by cefoxitin disc diffusion. Previous studies have reported false-positive results with chromogenic medium, but the isolates were never investigated any further (12).The MRSA ID chromogenic medium targets the α-glucosidase enzyme of *S. aureus* in the presence of cefoxitin (4 μg/ml), and positive isolates are visualized as green colonies. The MRSA ID chromogenic medium has a sensitivity of 96% and a specificity of 99.5% after 24 h of incubation. Because of the ability to induce the *mecA* gene, cefoxitin has been found to be superior to the incorporation of oxacillin in culture medium for the detection of MRSA (13). Quality control testing was performed on each plate of chrome agar and oxacillin screening agar by inoculating ATCC 29213 as a negative control and ATCC 43300 as a positive control.

Of the 30 *S. aureus* isolates that showed discordant results, only 2 (6.6%) were confirmed to be MRSA by *mecA* PCR (11). The MICs of oxacillin for two isolates (O-1102 and P-417) were in the susceptible range by both Etest and the Vitek 2 AST-GP67 automated system (Table 1). Both isolates were susceptible to cefoxitin by disc diffusion and Etest, while isolate O-1102 was identified as MRSA by the Vitek cefoxitin screening system. Further screening showed that both isolates were positive for PBP2a by latex agglutination. O-1102 was isolated from an nonhealing diabetic foot ulcer that was successfully treated with levofloxacin, while isolate P-417 was isolated from pus from a middle ear infection that was successfully treated with high-dose (1 g twice a day) ciprofloxacin. The antibiotic susceptibility patterns of these OS-MRSA isolates showed that isolate O-1102 was positive for inducible clindamycin resistance and resis-

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TABLE 1 Phenotypic and genotypic characterization of OS-MRSA

Test	Result with isolate/source: P-417/pus	O-1102/tissue
β-Lactamase production	Positive	Positive
Screening for PBP2a (latex agglutination)	Positive	Positive
MRSA ID	Positive	Positive
Cefoxitin screening		
Disc diffusion (zone size in mm)	Sensitive (30)	Sensitive (25)
Chrome agar MRSA ID (4 μg/ml)	Positive	Positive
Vitek	Negative	Positive
Etest (μg/ml)	0.5	4
Oxacillin screening		
Screening agar (6 μg/ml)	Positive	Positive
Disc diffusion (zone size in mm)	Sensitive (11)	Sensitive (12)
Vitek (μg/ml)	1	1
Etest (μg/ml)	0.125	0.5
Genotypic screening		
16S rRNA gene	Positive	Positive
PVL	Positive	Negative
mecA/SCC*mec* type	Positive/III	Positive/III
aacA-aphD	Positive	Positive
erm(A)	Positive	Negative
erm(C)	Positive	Positive
tetM	Negative	Negative
tetK	Positive	Positive

tant to more than three classes of antibiotic, making it multidrug resistant (MDR). Although isolate P-417 was not MDR, it was resistant to linezolid, with an unusually high MIC (>8 μg/ml).

The identities of MRSA isolates were further confirmed by amplifying the 16S rRNA gene of the genus *Staphylococcus* specific for *S. aureus* (11) since latex agglutination has been reported to yield less consistent results with coagulase-negative *Staphylococcus*. A multiplex PCR (11) was done to simultaneously detect genes responsible for resistance to three classes of antibiotics, namely, *aacA* to *aphD*, *erm*(C) and *erm*(A), and *tetK* and *tetM*, which encode resistance to aminoglycosides, erythromycin, and tetracycline, respectively. The presence of the toxin Panton-Valentine leukocidin (PVL) was tested for by a separate PCR (15), and isolate P-417 was found to be positive. SCC*mec* typing showed that both of the isolates were type III (8).

In conclusion, ours is the first study to report the usefulness of chromogenic medium for the detection of OS-MRSA. Our result show that *S. aureus* isolates that are positive on MRSA ID plates but negative by cefoxitin screening and susceptible to oxacillin should be investigated further for the presence of PBP2a by latex agglutination or by PCR for the *mecA* gene. The use of a chromogenic medium in combination with latex agglutination is a simple and effective method to detect OS-MRSA, which will otherwise be classified as methicillin-susceptible *S. aureus* because of its susceptibility to oxacillin and cefoxitin. Though recent studies have shown that OS-MRSA does respond to treatment with beta-lactams (14), they are best reserved for non-life-threatening infections, as there is always a danger of rising MICs during treatment, leading to failure of therapy.

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