JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2008, p. 4116–4117 Vol. 46, No. 12
0095-1137/08/$08.00+0 doi:10.1128/JCM.01146-08

Failure of the BD GeneOhm StaphS/R Assay for Identification of Australian Methicillin-Resistant *Staphylococcus aureus* Strains: Duplex Assays as the "Gold Standard" in Settings of Unknown SCC*mec* Epidemiology▽

Identification and susceptibility testing of presumed staphylococci in blood cultures require >24 h by routine phenotypic methods. Delayed appropriate antibiotic therapy results in increased mortality in critically ill patients, while unnecessary vancomycin use may result in suboptimal therapy and unnecessary selection for resistant organisms (1, 6, 10).

We tested the BD GeneOhm StaphS/R assay, a rapid nucleic acid test to differentiate coagulase-negative *Staphylococcus* (CoNS) isolates from methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MSSA and MRSA, respectively) isolates, with samples drawn immediately from 145 consecutive positive blood culture bottles (Bactec 9240; BD Systems) which contained gram-positive cocci in clusters visible by microscopy. There were no discordant results between the BD GeneOhm StaphS/R assay, culture, coagulase testing (as previously described), and susceptibility testing (by the CLSI disc diffusion method) in 46 MSSA isolates and 87 CoNS isolates (2, 7). However, only 50% (12/24) of the MRSA isolates identified by culture were correctly identified as MRSA by the BD GeneOhm assay. Genotyping of these by using published typing methods (5) showed that 11 of the 12 isolates corresponded to the predominant Australian nosocomial clone (AUS2/3 clone; ST 239-MRSA-III) and that one corresponded to a community-acquired clone prevalent in eastern Australia (South West Pacific clone; ST 30-MRSA-IV) (9).

We modified a published assay targeting *mecA* and *nuc*, including an internal control comprising a unique probe-binding region (based on *Drosophila melanogaster*; GenBank accession no. AY119135; nt 144 to 175) flanked by binding sites for our *nuc* primers (11). Using 5 μl of extracted DNA (BioRobot EZ1 QIAamp tissue protocol), this *S. aureus* methicillin resistance assay (SAM2-PCR) correctly identified 98.6% (216/219) of the *S. aureus* isolates (including 64/65 culture-proven MRSA isolates) within 2 hours of a positive Gram stain. Incorrect labeling caused two errors, and failed extraction caused another. No true assay failures occurred, with 100% concordance on repeat testing.

SAM2-PCR correctly identified 100% (439/439) of all non-*Staphylococcus aureus* isolates as being *nuc* negative (Table 1).

TABLE 1. Results of SAM2-PCR compared with those of culture and susceptibility identification

Isolate identification by culture and susceptibility testing	No. of isolates identified by PCR with indicated result[b]			
	nuc+ *mec*+	*nuc*+ *mec*−	*nuc*− *mec*+	*nuc*− *mec*−
MSSA (n = 154)	1	152	0	1
MRSA (n = 65)	64	0	0	1
MSSE (n = 138)	0	0	8	130
MRSE (n = 274)	0	0	265	9
Other[a] (n = 27)	0	0	0	27

[a] Nonstaphylococcal (*Micrococcus* spp., *Streptococcus* spp., *Enterococcus* spp.).
[b] +, positive; −, negative.

In eight methicillin-susceptible CoNS (MSSE) isolates, *mecA* was detected, while *mecA* was not detected in 9/274 methicillin-resistant CoNS (MRSE) isolates. These SAM2-PCR results were confirmed as being correct by an independent PCR for all eight MSSE isolates and five of nine MRSE isolates (8). Three of the four remaining discordant specimens had no DNA present (extraction failures), with one assay failure occurring (0.2% [1/658]). Determination of methicillin resistance in CoNS isolates using the SAM2-PCR was therefore more accurate than that using direct susceptibility testing, which was incorrect in 3.2% (13/412).

The BD GeneOhm StaphS/R assay targets the right extremity (i.e., junctional target) of the SCC*mec* region. False negatives have been documented secondary to variations in this region, new variants of the SCC*mec* (type IV and V) and nontypeable strains (3, 4). This approach will always be vulnerable to epidemiological variations. A *nuc*/*mec* assay with an internal control to detect inhibition (not observed in our study) seems robust and reliable, outperforming the culture methods in our hands. It may, therefore, be prudent for simple duplex assays to remain the "gold standard" for rapid detection of *S. aureus* and MRSA from blood culture bottles.

We acknowledge Lin Cai for assistance with genotyping of the MRSA isolates and Andrew Ginn for help with the cloning of the internal control.

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▽ Published ahead of print on 22 October 2008.