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Improvement of a latex agglutination test for the evaluation of oxacillin resistance in coagulase-negative staphylococci

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Abstract

The "Slidex MRSA Detection" test (Denka Seiken, Japan) is a latex agglutination assay able to detect PBP2a. We evaluated its ability to differentiate *mecA*-positive from *mecA*-negative coagulase-negative staphylococci. We included 100 coagulase-negative staphylococci clinical isolates belonging to 9 species, 54 *mecA* positive and 46 *mecA* negative, as characterized by PCR. The specificity achieved using the manufacturer's instructions was 100%, but the sensitivity was only 57%. To increase sensitivity, we introduced modifications into the standard protocol. Using either large inocula or oxacillin induction before test performance, we achieved 100% sensitivity.

1. Introduction

Coagulase-negative staphylococci (CoNS) are common agents of bloodstream infection, and they tend to be more resistant to antimicrobial agents than *Staphylococcus aureus*, especially to methicillin (Hussain et al., 2000). The use of vancomycin as initial therapy for CoNS infections is a common practice worldwide. However, the Centers for Disease Control and Prevention (CDC) strongly discourage the use of this glycopeptide in such patients, to prevent the spread of vancomycin-resistant enterococci within the hospital (CDC, 1995). The potential appearance of vancomycin-intermediate *S. aureus* in patients treated with vancomycin (Walsh and Howe, 2002), in addition to the recent emergence of vancomycin-resistant *S. aureus* in the United States (CDC, 2004), strengthens the CDC's recommendation to limit the use of glycopeptides in hospital environments.

Therefore, the distinction between oxacillin-sensitive and -resistant CoNS is of great concern for clinical laboratories. The major mechanism of methicillin resistance (MR) is based on the acquisition of the *mecA* gene, which encodes a penicillin-binding protein named PBP2a, which possesses low affinity for β-lactam antibiotics (Chambers, 1988). The phenotypic detection of MR in staphylococci is complex, because of the heterogeneous expression of the *mecA* gene (Hussain et al., 2000). Several reports have indicated differences between oxacillin MICs and the presence or absence of the *mecA* gene in CoNS, when the oxacillin breakpoint for resistance is set at $\geq$4 μg/mL (Tenover et al., 1999). The current oxacillin breakpoint correlates with the presence or absence of the *mecA* gene in *S. epidermidis*, but does not allow the differentiation of *mecA*-positive and *mecA*-negative isolates in other species of CoNS, such as *S. saprophyticus, S. cohnii*, *S. lugdunensis*, *S. warneri*, and *S. simulans* (NCCLS, 2002; Hussain et al., 2000; Louie et al., 2001). Since 2002, the NCCLS has recommended direct detection of the *mecA* gene or the PBP2a in CoNS other than *S. epidermidis* involved in serious infections (NCCLS, 2002). According to data from a National Surveillance Network (WHONET Argentina), the rate of oxacillin resistance among *S. epidermidis* increased from 54% in 1994 to 78% in 2002 (unpublished data).

Molecular methods for the detection of the *mecA* gene are considered the "gold standard" for confirmation of MR.

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Table 1
Performance of the "Slidex MRSA Detection" test among 100 CoNS, under standard and modified assays

mecA PCR	Species	No. of isolates	Number of positive results				
			Standard assay	Modified assays			
				Agglutination time		Size of inoculum	Oxacillin induction
			3 loops of 1 μl	3 loops of 1 μl		6 loops of 1 μl	3 loops of 1 μl
			3 min	6 min	15 min	3 min	3 min
Positive (54)	*S. epidermidis*	38	20	28	31	38	38
	S. hominis	7	2	4	5	7	7
	S. haemolyticus	5	5	5	5	5	5
	S. simulans	4	4	4	4	4	4
	Total n (sensitivity %)	54	31 (57)	41 (76)	45 (83)	54 (100)	54 (100)
Negative (46)	*S. saprophyticus*	14	0	0	0	0	0
	S. epidermidis	11	0	0	0	0	0
	S. hominis	6	0	0	0	0	0
	S. auricularis	4	0	0	0	0	0
	S. simulans	2	0	0	0	0	0
	S. capitis	3	0	0	0	0	0
	S. cohnii	2	0	0	0	0	0
	S. haemolyticus	2	0	0	0	0	0
	S. warneri	2	0	0	0	0	0
	Total n (specificity %)	46	0 (100)	0 (100)	0 (100)	0 (100)	0 (100)

However, these methodologies are not available in many clinical laboratories, which could have access to other methodologies that allow detection of MR in CoNS other than *S. epidermidis*. The "Slidex MRSA Detection" test is a rapid slide agglutination assay designed to detect the presence of PBP2a in *S. aureus*. The aim of the present study was to improve the capability of the "Slidex MRSA Detection" test to detect PBP2a expression in a variety of species of CoNS.

A total of 100 consecutive CoNS clinical isolates were recovered between April and October 1997 at the Hospital Alvarez in Buenos Aires, Argentina. All isolates were identified to the species level by standard biochemical methods (Kloos and Bannerman, 1999) and tested using the Vitek Gram Positive Susceptibility 105 card (bioMerieux Vitek, Inc. Hazelwood, MO). This collection was representative of the CoNS species found in the hospital and comprised 49 *S. epidermidis*, 14 *S. saprophyticus*, 13 *S. hominis*, 7 *S. haemolyticus*, 6 *S. simulans*, 4 *S. auricularis*, 3 *S. capitis*, 2 *S. cohnii*, and 2 *S. warneri*. Unfortunately, no strains of *S. lugdunensis* were isolated.

PCR assays to detect the *mecA* gene were performed as previously described (Vannuffel et al., 1995). Amplification of 16S rRNA gene was also performed as a positive control of DNA extraction and DNA amplification for each strain. Of the 100 CoNS isolates tested, 54 were PCR positive for the *mecA* gene, and 46 were PCR negative.

By the agar dilution method (NCCLS, 2002), 32 *mecA*-negative strains were susceptible to oxacillin (Sigma, St. Louis, MO), with values ranging between 0.015 and 0.25 μg/mL. The remaining 14 isolates (13 *S. saprophyticus* and 1 *S. cohnii*) displayed MICs to oxacillin from 0.5 to 2 μg/mL. The 54 *mecA*-positive isolates displayed oxacillin MICs from 1 to 128 μg/mL.

The standard agglutination assay recommended by the manufacturer for *S. aureus* included an inoculum size of 3 times 1 μL loopful, an agglutination time of 3 min, and no oxacillin induction. Under these conditions, the agglutination test was negative for all 46 *mecA*-negative isolates, but was positive in only 31 out of 54 *mecA*-positive isolates (Table 1). The 23 *mecA*-positive strains that failed to agglutinate included 18 *S. epidermidis* and 5 *S. hominis* that showed oxacillin MICs in the range 1 to 128 μg/mL. Thus, the specificity of the standard assay was 100%, and the sensitivity was only 57%, as reported previously (Hussain et al., 2000; Louie et al., 2001; Horstkotte et al., 2001; Petinaki et al., 2002). These results indicated the need to optimize the parameters that influence the analytical sensitivity of the test. However, Ferreira recently reported a higher sensitivity using the standard assay (Ferreira et al., 2003).

In our work, three different variables of the "Slidex MRSA Detection" test were individually evaluated in a modified assay. When we increased the agglutination reading time from 3 min to 6 and 15 min, as recommended by other authors (Yamazumi et al., 2001), the sensitivity increased from 57% to 76% and 83%, respectively (Table 1). However, 9/54 *mecA*-positive isolates (7 *S. epidermidis* and 2 *S. hominis*) did not show agglutination even after 15 min of agglutination reading time.

Louie et al. enhanced the sensitivity using larger inocula of bacteria (Louie et al., 2001). To optimize the inoculum size, we selected 5/23 isolates (4 *S. epidermidis* and 1 *S. hominis*) that gave a negative result in the standard agglutination assay. The inocula tested were 4, 5, and 6 times 1 μL loopful, which resulted in, respectively, 2/5, 4/5, and 5/5 isolates showing agglutination. Subsequently, the complete group of 100 isolates was re-tested with 6 times 1 μL

loopful and a reading time of 3 min. The sensitivity increased to 100%, and the specificity remained 100%. Although some reports have described false positive results when the inoculum size is increased (Yamazumi et al., 2001; Zbinden et al., 2001; Hussain et al., 2000; Horstkotte et al., 2001), we did not observe a loss of specificity by using larger inocula.

Previous reports have shown improved performance of the agglutination test in CoNS when oxacillin induction was implemented before testing (Hussain et al., 2000; Zbinden et al., 2001; Petinaki et al., 2002). When oxacillin induction and the standard inoculum size were used, all of our 54 *mecA*-positive isolates showed agglutination within 3 min. Sensitivity increased from 57% to 100% (Table 1), while the specificity remained at 100%.

In conclusion, by either using larger inocula or by inducting oxacillin, all isolates positive for the *mecA* gene by PCR were detected as positive by the "Slidex MRSA Detection" test, and all strains negative for *mecA* remained negative in the agglutination assay. The data presented here indicate that the detection of MR in CoNS by the "Slidex MRSA Detection" test improves by either using a large inoculum (6 loops of 1 μL) or by inducing the expression of *mecA* gene with oxacillin before testing. Thus, the "Slidex MRSA Detection" test constitutes a good alternative to PCR in detecting MR among CoNS isolates.

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