Rapid MRSA Detection by a Latex Kit

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Methicillin resistant strains of *Staphylococcus aureus* (MRSA) are implicated in serious infections and nosocomial outbreaks, and show resistance to a wide range of antibiotics, thus limiting the treatment options. Therefore, rapid detection is clinically crucial for both treatment and infection control measures. This study assessed the performance of a rapid latex agglutination kit marketed to detect MRSA clinical isolates (MRSA-Screen test Denka Seiken Co Ltd, Tokyo, Japan) based on detecting a specific penicillin binding protein 2a (PBP2a) in comparison to the NCCLS oxacillin salt agar screen plate, the 1µg oxacillin disk diffusion test, and the oxacillin MIC by E-test. Testing was carried out on 133 isolates consisting of 99 MRSA and 34 methicillin sensitive strains of *S. aureus* (MSSA). Concordant results were observed between the latex kit and all the other tests for the 99 MRSA isolates. Only 1 of the 34 MSSA isolates gave a positive agglutination reaction in the latex kit. The kit sensitivity and specificity were determined to be 100% and 97%, respectively. This reliable performance indicates that the MRSA-Screen latex test is very useful test for the rapid detection of MRSA isolates in the clinical microbiology laboratory.

ABBREVIATIONS: CNS = coagulase negative staphylococci; MIC = minimum inhibitory concentration; MRSA = methicillin resistant *S. aureus*; MSSA = methicillin susceptible *S. aureus.*

INDEX TERMS: latex kit; methicillin resistance; rapid detection; *Stapyhylococcus aureus*.

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The increasing global encounter of methicillin resistant *Staphylococcus aureus* (MRSA) strains is causing a wide spectrum of hospital- and community-acquired infections. Moreover, the limitation incurred upon treatment options has been inflicting a substantial toll of morbidity and mortality.[1] Thus, rapid detection of MRSA strains is essential for proper treatment and specific infection control measures.[2] The conventional techniques of oxacillin disk diffusion, agar screen, and minimum inhibitory concentration (MIC) determinations take at least 24-hours for the results to be available. Moreover, their accuracy is influenced by several factors including inoculum size, incubation time and temperature, media, pH, and salt concentration.[3-5] Though molecular testing requires a shorter time, it is technically challenging and expensive.

To overcome the above noted testing problems, a latex agglutination kit (MRSA-Screen test Denka Seiken Co Ltd, Tokyo, Japan) was recently introduced for the rapid detection of MRSA isolates based on the detection of penicillin binding protein 2a (PBP2a) in these isolates.[6] The turnaround time for results by this test is within half an hour of acquiring a fresh isolate.

This study was undertaken to evaluate the performance of this kit in a clinical microbiology setting at a tertiary care medical center, in comparison with the conventional oxacillin disk diffusion and agar screen methods, and MIC determination by the E test.

MATERIALS AND METHODS

Isolates

A total of 133 consecutive clinical strains of *S. aureus* isolates representing 99 MRSA and 34 methicillin susceptible *S. aureus* (MSSA) were tested in this study. These were col-

lected between October 1999 and October 2000, and categorized based on the oxacillin disk and agar screen methods. The isolates were stored in brucella glycerin broth at –20 ^{0}C until simultaneously tested by the various methods discussed below. Prior to testing, each isolate was subcultured three times on trypticase soy agar containing 5% sheep blood to ensure purity and freshness.

Identification

Identification of staphylococcal isolates was based on standard procedures including colony morphology, Gram stain appearance, the catalase reaction, and the tube coagulase test. In addition, the mannitol salt fermentation test and the Slidex Agglutination Test (bioMerieux, France) were used to differentiate *S. aureus* from coagulase negative staphylococci (CNS). Additionally, the API Staph (bioMerieux, France) was used when test results were in doubt or not clear.

Oxacillin disk diffusion test

The 1µg oxacillin disk diffusion test, using Mueller-Hinton agar without supplementation with sodium chloride, was performed and interpreted according to the NCCLS guidelines.[3] After inoculation, the plates were incubated at 35 °C and results recorded after 24-hours of incubation. *S. aureus* isolates that showed inhibition zone size less than 13 mm or those showing mutants within the inhibition zone were considered resistant (less than or equal to 10 mm), or intermediate resistant (11 mm to 12 mm). Those with zone sizes greater than or equal to 13 mm were considered susceptible.

Oxacillin salt agar screen plate

Isolates inoculated on Mueller-Hinton agar plate containing 6 µg/mL oxacillin and 4% NaCl were incubated at 35 °C for 24-hours and interpreted according to reported procedure.[3,7] Growth indicated resistance, and absence of growth indicated susceptibility to methicillin.

E-test

The E-test strips (PDM-Epsilometer, AB Biodisk, Solna, Sweden) were used to determine the MICs of oxacillin for the *S. aureus* isolates.[8] The medium used was Mueller-Hinton agar supplemented with 2% NaCl. Inoculated plates were incubated at 35 °C for 24-hours, and readings of MICs were determined where the border of the elliptical inhibition zone intersected the scale on the oxacillin test strip. An MIC value of greater than or equal to 4 µg/mL was taken as resistant and an MIC less than or equal to 2 µg/mL was considered susceptible. An MIC value between "greater than 2 µg/mL" and "less than 4 µg/mL" was considered intermediate/borderline.

MRSA-screen test

Extraction of penicillin binding protein (known as PBP2a) from fresh isolates, after overnight growth, was carried out according

Table 1. Overall comparative findings of oxacillin assays versus MRSA-latex assay for the 99 MRSA and 34 MSSA isolates

Oxacillin assay (breakpoints)	Number tested	Number of isolates showing MRSA-latex:	
		Positive	Negative
Agar plate screen			
Resistant	99	99	0
Susceptible	34	1*	33
1 µg disk diffusion zone (mm)			
Resistant (<10)	99	99	0
Intermediate (11-12)	0	0	0
Susceptible (≥13)	34	1*	33
E test (µg/mL)	99		
Resistant (≥ 4)	99	99	0
Intermediate (2.1-3.9)	3	0	3
Susceptible (≤ 2)	31	1*	30

* Refers to isolate code number 84 (see Table 2 for its detailed susceptibility)

to the manufacturer's instructions (Denka Seiken Co Ltd, Tokyo, Japan). Briefly, 10 to 20 colonies were emulsified in four drops (200 µL) of Extraction Reagent 1, heated at 100 °C for three minutes in a heating block, neutralized after cooling with one drop (50 µL) of Extraction Reagent 2, and centrifuged at 1500g for five minutes. Then, 50 µL of the supernatant was mixed with 25 µL each of test and control latex separately. The mixtures of supernatant and latex on the circled test cards were rotated for 3-, 6-, and 10-minutes and examined for agglutination. A positive detection of MRSA PBP2a yielded agglutination with test latex but not control latex. Negative reaction was determined when no agglutination occured in either the test or control latex, while indeterminate results were noted when agglutination was observed with the control latex.

Quality control strains

The performance of these tests was monitored using quality control strains: MSSA (ATCC 25923) and an in-house determined MRSA.

Predictive indices

Predictive indices of sensitivity, specificity, positive and negative predictive values, and accuracy were calculated as reported previously.[9]

RESULTS

See Table 1 for results of the different tests for the 99 MRSA and the 34 MSSA isolates. All the MRSA isolates, as determined resistant by the oxacillin disk and agar screen methods, were uniformly resistant by the E-test (MICs greater than or equal to 4 µg/mL; mean = 108 µg/mL; range = 4 to greater than or equal to 256 µg/mL) and showed positive reactions by the MRSA-Screen latex kit within three minutes of testing.

Among the 34 MSSA isolates determined susceptible by the oxacillin disk and agar screen methods, 31 (91%) had MICs less than or equal to 2 µg/mL while the remaining three isolates showed MICs ranging between 2.1 and 3.9 µg/mL. The distribution of the MRSA-Screen latex kit reactions, within three minutes of testing, for the 34 MSSA isolates showed a clear negative for 27 isolates, a repeatedly strong positive for one isolate, and a weak positive for six isolates. Weak positive reactions were also observed at six and ten minutes rotation for one and three isolates, respectively. No autoagglutination was observed with test isolates and the control latex reagent. The results of the different tests for these MSSA isolates are presented in Table 2.

Table 2. Characteristics of MSSA isolates that showed strong false and weak positive reactions in MRSA-latex assay

MRSA-latex	Isolate	Oxacillin tests results at 24-hour incubation		
Reaction and time of agglutination (minutes)	Code	Agar plate	1 µg disk diffusion	E test (µg/mL)
Strong reaction at three minutes	84	S	S	2
Weak reaction at three minutes	18	S	S	2
	25	S	S	2
	26	S	S	3
	72	S	S	0.38
	112	S	S	0.75
	158	S	S	2
Weak reaction at six minutes	11	S	S	3
Weak reaction at ten minutes	12	S	S	2
	24	S	S	0.5
	69	S	S	0.38

S = Susceptible

DISCUSSION

In this study, concordant results were observed between the MRSA-Screen latex test and the conventional oxacillin disk diffusion test, agar screen test, and the E-test for the 99 MRSA isolates. Only one of the 34 MSSA isolates gave a discrepant result by showing a positive agglutination reaction in the latex test while being susceptible with the oxacillin tests. Thus, the MRSA-Screen latex kit had a sensitivity of 100% and a specificity of 97% as shown in Table 3. These predictive values fall within the previously reported ranges of 90% to 100% sensitivity and 94% to 100% specificity for this test kit. [7,10-16]

Though slide agglutination tests provide rapid results, they are susceptible to false positive and false negative reactions. In this study, a true false positive MRSA-Screen latex kit result, at three minutes rotation, was noted in 1of 34 (2.9%) MSSA isolates. This isolate however, showed changing susceptibility between 24- and 48-hours incubation in the other tests. At 24-hours the isolate was susceptible by the oxacillin disk and agar screen as well as by the E test MIC value (2 μg/mL). At 48-hours however, the oxacillin disk test showed appearance of mutants in the inhibitory zone, the oxacillin agar plate revealed growth of colonies, and the E test MIC value increased to 3 μg/mL, suggesting that this isolate could fall in the borderline resistance category. False positive MRSA-Screen latex kit results have been reported to be due to several factors including the use of heavy inoculum, underheating during PBP2a extraction, and prolonged reaction time.[10,11,16] Weak false positive agglutination reactions, due to the latter reason, were observed in four of our MSSA isolates when the reaction time was extended for 6 and 10 minutes (Table 2). To avoid these weak positive reactions, Marriott recommended including a positive and negative control in each test run, and noted also that more reproducible results were obtained when a heating block was substituted for a boiling water bath.[11]

False negative MRSA-Screen latex kit results were not encountered in this study. Though rare, false negative results have been reported to occur due to overheating during the extraction procedure, indicating that heating is considered a critical step in this assay.[11] Moreover, Van Leuwen found negative MRSA-Screen latex testing in the presence of positive mecA PCR.[13] To overcome this false negative reaction, they suggested inducing the mecA gene by exposing isolates to methicillin before performing the test. Other recommendations to improve the sensitivity of the assay included increasing the inoculum size, and prolonging the reaction time (up to 15 minutes) to reveal and make the agglutination reactions more pronounced.[7,12,14] This time prolongation, however, could lead to false positive weak reactions, as observed in some of our MSSA isolates (Table 2). Thus, as shown in our study and recommended by others only test cards showing a strong agglutination pattern within 3 minutes should be considered positive.[15]

In conclusion, the MRSA-Screen latex kit is an easily performed assay that is suited for clinical microbiology laboratories that provide rapid and reliable detection of MRSA, valuable information for proper treatment, and specific infection control measures.

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Table 3. Percent sensitivity, specificity, and predictive values positive (PPV) and negative (NPV) for the MRSA-Latex

Aspect*	Percent (%)
Sensitivity	100
Specificity	97
PPV	99
NPV	100

* Calculations were done based on the findings in Table 1 according to reference number 9.

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