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Brief reports

Evaluation of the Mastalex latex agglutination test for methicillin resistance in *Staphylococcus aureus* grown on different screening media

Derek F. J. Brown* and Enid Walpole

Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QW, UK

The Mastalex MRSA latex agglutination method was evaluated with 52 methicillin-resistant and 27 methicillin-susceptible strains of *Staphylococcus aureus* grown on various media. All tests were correct with colonies grown on blood agar with or without oxacillin 2 mg/L. Tests on colonies grown on mannitol salt agar were less reliable, although addition of oxacillin 2 mg/L improved performance. One of the 26 MRSA which grew on Baird–Parker medium with 8 mg/L ciprofloxacin gave a false-negative result. Agglutination was faster when strains were grown on media with oxacillin. The method would be particularly useful for urgent confirmation of resistance.

Introduction

Infections with methicillin-resistant *Staphylococcus aureus* (MRSA) have caused problems in hospitals worldwide and have increased dramatically in the UK in recent years.[1] The effectiveness of infection control measures is enhanced by early detection of resistant isolates. This is dependent on the time taken to isolate and identify MRSA in specimens taken from infected patients and in screening specimens taken to identify patients colonized with MRSA.

Conventional disc diffusion and dilution tests for the detection of methicillin resistance in *S. aureus* require overnight incubation, while rapid methods—such as Vitek,[2] Rapid ATB Staph[2] and Crystal MRSA[3]—still require several hours of incubation. Almost all MRSA have an additional penicillin-binding protein, PBP2a, mediated by the *mecA* gene.[4] PCR methods detecting the *mecA* gene will provide results within a few hours, but are not available in most laboratories and test costs are relatively high. A rapid latex agglutination test based on detection of PBP2a has been described.[5] The method employs latex particles coated with monoclonal antibodies to PBP2a, which is extracted from test colonies. With colonies of a wide range of MRSA grown on blood agar, a sensitivity of 98.5–100% and a specificity of 100% have been reported.[6–8] MRSA screening swabs are, however, commonly plated on selective media and the presence of selective agents such as NaCl, oxacillin or methicillin in these media may affect the reliability of the test with some strains, particularly those which are markedly heterogeneous and do not grow well on selective media.

In this study, we tested the performance of a latex agglutination method, the Mastalex MRSA screening test, with isolates from a wide variety of sources grown on blood agar and a variety of selective media used in screening for MRSA.

Materials and methods

Organisms

Fifty-two MRSA, including representatives of epidemic strains (EMRSA) 1–16, were from various sources in the UK, USA, France, Japan and Australia. Twenty-seven methicillin-susceptible isolates of *S. aureus* were recent clinical isolates or susceptible control strains. Resistance was confirmed by testing for *mecA*.[9]

Media

Colonies were grown on the following media: Columbia agar (Oxoid, Basingstoke, UK) with 5% horse blood (blood agar); blood agar containing oxacillin 2 mg/L; mannitol salt agar (MSA) from Mast (Bootle, UK); Mast MSA

*Corresponding author. Tel: +44-1223-257020; Fax: +44-1223-242775; E-mail: dfjb2@cam.ac.uk

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containing oxacillin 2 mg/L; MSA from Oxoid; Oxoid MSA containing oxacillin 2 mg/L; and Baird–Parker agar (Oxoid) containing ciprofloxacin 8 mg/L. Plates were incubated for 18 h in air at 37°C except for blood agar with oxacillin 2 mg/L, which was incubated for 18 h at 30°C.

Mastalex MRSA latex agglutination method

The Mastalex MRSA method (Mast) was used according to the manufacturer's instructions. Briefly, 10 colonies were suspended in 200 μL 'extraction reagent 1' and heated in a boiling water bath for 3 min. Tubes were cooled and 50 μL 'extraction reagent 2' was added. Tubes were centrifuged for 1 min in a microcentrifuge. Fifty microlitres of supernatant were mixed with 50 μL sensitized latex suspension and rotated manually for 3 min while looking for agglutination. The supernatant was tested simultaneously with a negative control latex suspension. The time at which agglutination was visible by eye was recorded.

Statistical analysis

Differences in detection of methicillin resistance with the Mastalex method when colonies were grown on different media were tested by the χ^2 test.

Results and discussion

All methicillin-susceptible isolates were *mecA* negative. Most did not grow on media containing oxacillin or ciprofloxacin (Table). No methicillin-susceptible isolate, including the isolates that grew on media containing oxacillin, was positive with the Mastalex test.

All MRSA were *mecA* positive. Mastalex tests were 100% correct with colonies of MRSA grown on blood agar and blood agar with oxacillin (Table). This is in agreement with previous reports on the method.[5–7] All agglutination reactions with colonies from blood agar were positive within the 3 min specified by the manufacturer, which contrasts with previous reports suggesting that either a small proportion of agglutination reactions took up to 6 min[6] or that rotation of slides for up to 15 min was necessary.[7] These studies used mechanical agitation of slides whereas we rotated slides manually, which may give more efficient mixing and hence more rapid agglutination. However, the full 3 min of rotation was needed for some strains and this is tedious if done manually.

Tests with four (7.7%) and 16 (30.8%) of the 52 MRSA gave false-negative results with colonies from the Mast and Oxoid MSA media without oxacillin, respectively. These tests were significantly less reliable than tests on colonies from blood agar ($P < 0.05$ and $P < 0.001$ in comparisons with Mast and Oxoid MSA, respectively). Tests on colonies grown on Oxoid MSA without oxacillin were also significantly less reliable than tests on colonies grown on any other medium tested ($P < 0.001$ when compared with blood agar with or without oxacillin and Mast MSA with oxacillin; $P < 0.01$ when compared with other media). Colonies grown on MSA were frequently sticky, making it difficult to pick up colonies on a loop and to suspend growth evenly; these difficulties were more marked with

Table. Effect of growth medium on detection of methicillin resistance in *S. aureus* by the Mastalex method

Medium	*MecA* status	Number of isolates tested	Number of strains growing	Number Mastalex positive	Mean time to agglutination (s)
Blood agar					
without oxacillin	+	52	52	52	46
	–	27	27	0	–
+ oxacillin 2mg/L	+	52	52	52	22
	–	27	5	0	–
Mast MSA					
without oxacillin	+	52	52	48	91
	–	27	27	0	–
+ oxacillin 2 mg/L	+	52	50	49	76
	–	27	9	0	–
Oxoid MSA					
without oxacillin	+	52	52	36	95
	–	27	27	0	–
+ oxacillin 2 mg/L	+	52	51	46	82
	–	27	9	0	–
Baird–Parker agar +	+	52	26	25	46
ciprofloxacin 8 mg/L	–	27	5	0	–

Oxoid MSA, possibly because the Oxoid medium has 7% added NaCl, as opposed to 3% in the Mast medium.

There was no correlation between EMRSA type and false-negative results. Because of their current prominence, 20 of the 52 MRSA tested were EMRSA types 15 and 16. False-negative results with these types were recorded with two isolates grown on Mast MSA, one on Mast MSA with oxacillin, six on Oxoid MSA and two on Oxoid MSA with oxacillin. This was not significantly different from results with the 32 other MRSA isolates, where false-negative results were recorded with two isolates grown on Mast MSA, none on Mast MSA with oxacillin, 10 on Oxoid MSA and three on Oxoid MSA with oxacillin.

Addition of oxacillin to MSA improved the reliability of tests, but false-negative results were still found in tests on colonies from one and five isolates grown on Mast and Oxoid media, respectively. Also the growth of two isolates on Mast MSA and one on Oxoid MSA was inhibited. In contrast, Willey *et al.*[8] found results on MSA with oxacillin 2 mg/L to be reliable.

The mean times to read a positive result (Table) were markedly lower for tests on colonies from blood agar and Baird–Parker agar than from those on MSA. Agglutination times were also reduced on all media to which oxacillin was added. This is presumably related to induction of PBP2a production by growth in the presence of oxacillin, leading to a higher concentration of PBP2a in the agglutination reaction. The blood agar plates with and without oxacillin were incubated at 30 and 37°C, respectively, and the lower temperature of incubation of the plates with oxacillin may have contributed to better expression of PBP2a and consequently more rapid agglutination results with colonies from this medium.

Baird–Parker agar containing ciprofloxacin is a useful medium for isolation of MRSA in situations where ciprofloxacin-resistant strains are endemic.[10] However, half of the strains tested in this study did not grow on the medium. With strains that did grow, growth was good and agglutination was rapid. The cause of the single false-negative result (confirmed on retesting) with colonies from Baird–Parker agar with ciprofloxacin is not obvious.

The Mastalex test procedure is straightforward but relatively labour intensive, especially the agglutination reaction. A single test took 10 min whereas a batch of 10 tests took 24 min. The use of a rotating platform or shaker might be considered, but this is likely to extend the agglutination time. Other factors that may affect the speed and reliability of the test are the density of the cell suspension, which varies depending on how many colonies are available, how good the growth is on the test medium and how easy it is to pick up colonies on the loop, particularly from media containing NaCl.

In conclusion, the Mastalex latex agglutination test for methicillin resistance in *S. aureus* is highly reliable and reasonably rapid if colonies are grown on blood agar, with or without added oxacillin. Caution is needed when the method is used with colonies grown on MSA or Baird–Parker agar with ciprofloxacin. The method would be particularly useful when confirmation of resistance is urgently required.

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