Screening for methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: an evaluation of three selective media and Mastalex-MRSA latex agglutination

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ABSTRACT

Laboratory confirmation of MRSA is important for the implementation of infection control; conventional screening culture methods take up to five days for confirmation. The purpose of this study is to ascertain the efficiency of three selective media for growth of methicillin-resistant *Staphylococcus aureus* (MRSA) before and after enrichment in salt broth, and to evaluate the Mastalex-MRSA latex agglutination kit for detection of methicillin resistance. Screening swabs were collected from 63 patients, yielding125 *S. aureus* isolates and 40 coagulase-negative staphylococcus (CNS) isolates. Selective media used were mannitol salt agar (MSA), Baird-Parker agar with ciprofloxacin (BPC) and bromocresol purple (BCPA). Polymerase chain reaction (PCR) for *mec*A gene detection was used as the reference standard for evaluation of the Mastalex-MRSA assay, which was also evaluated on colonies of *S. aureus* from the selective media. No significant difference was found in efficiency of MRSA isolation among the selective media. Pre-enrichment in the salt broth did not enhance isolation of MRSA. Methicillin-sensitive *S. aureus* and CNS were significantly inhibited in all selective media ($P<0.05$). Only BPC significantly selected out methicillin-resistant CNS ($P<0.01$). Mastalex-MRSA was 97% specific and sensitive for the detection of MRSA. It was 65% sensitive and 100% specific in detecting methicillin resistance in CNS. In conclusion, all selective media performed equally well (MRSA isolation rate of approximately 80%). Mastalex-MRSA provided rapid and reliable detection of MRSA from selective media, reducing the time required for confirmation of this organism.

KEY WORDS: Genes, mecA. Methicillin resistance. Selective media. Staphylococcus aureus.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, first described in 1961, have emerged rapidly as a major epidemiological problem in healthcare settings.[1] In 1993/4, a UK national prevalence survey showed that MRSA colonisation had the highest relative risk for hospital-acquired infection. Screening for carriers (staff and patients), rather than simply identifying infected patients, has a major role in the control of an outbreak and reduces the number of infections.[2]

MRSA infection is usually preceded by MRSA carriage, and the rapid and sensitive identification of MRSA colonisation is important for the implementation of appropriate infection control measures. Current screening procedures in most hospitals require an additional 18-24 hours following isolation of suspect colonies before confirmation of MRSA can be obtained.[3] This adds to hospital costs due to prolonged patient isolation.

Screening for MRSA involves the testing of swabs taken from sites that may contain large numbers of contaminating flora. The use of selective and differential media increases the rate of recovery of MRSA isolates from sites that may contain contaminating flora, including coagulase-negative staphylococci (CNS).[4]

Heterogeneous expression of the methicillin-resistant phenotype depends on culture conditions such as increased salt concentration in the growth medium, incubation time and temperature,[5] and the selection of the resistant phenotype in the presence of an isoxazolyl penicillin.[6] Recent studies suggest that it is more appropriate to detect *mec*A or the protein product PBP2a directly to identify methicillin-resistance in staphylococci.[7,8]

Detection of the *mec*A gene by the polymerase chain reaction (PCR) will correctly identify even the most heterogeneous strains of MRSA and is widely recognised as the definitive method for the detection of methicillin-resistance in staphylococci.[5]

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This study compares the efficiency of different selective agar media for the isolation of MRSA as a preliminary step to testing the media directly on clinical specimens. The Mastalex-MRSA kit is evaluated for the rapid detection of PBP2a in isolates of *S. aureus* and CNS from selective and enriched media.

Materials and methods

Screening swabs were taken from 63 patients, blinded and tested in parallel with the routine laboratory screening

Table 1. Growth of staphylococci on CBA (control) and three selective screening media

Organism	Number of swabs showing heavy growth				
	MSA (%)	BPC (%)	BCPA (%)	CBA(%)	*P* value
MSSA	3 (8.1)	4 (10.8)	3 (8.1)	37 (100)	<0.005
MRSA	83 (94.3)	81 (92.1)	82 (93.2)	88 (100)	NS
CNS (methicillin-sensitive)	3 (15)	1 (5)	5 (25)	20 (100)	<0.05
CNS (methicillin-resistant)	15 (75)	4 (20)	19 (95)	20 (100)	NS,except for BPC (<0.01)

NS: not significant

method for MRSA. Patients were selected from different wards at University College London Hospitals (UCLH) and the National Hospital for Neurology and Neurosurgery (NHNN) over a two-year period, and included both UK and overseas cases attending tertiary referral facilities.

Media

Each swab was cultured on Columbia blood agar (CBA) as a control for purity and viability and on the following selective screening agar media: mannitol salt agar (MSA) containing 7.5% salt and 4 mg/L oxacillin (routine laboratory protocol), Baird Parker agar containing 8 mg/L ciprofloxacin (BPC) and bromocresol purple agar containing 75 mg/L aztreonam and 6 mg/L oxacillin (BCPA).

Isolates were plated onto media with and without overnight pre-enrichment in nutrient salt broth and tryptone T salt broth (7.5% and 6% salt, respectively). In addition, a CBA plate with a methicillin disc (5 μg) was inoculated and incubated at 30°C for 24 h. All other plates were incubated at 37°C for 24 and 48 h.

All media were obtained from Oxoid, Basingstoke, UK. A Macfarland 0.5 inoculum strength was used when plating liquid cultures. Suspect colonies were identified as *S. aureus* or CNS by Gram's stain, catalase, Staphaurex and DNase reactions.

Detection of methicillin/oxacillin resistance

Disc diffusion: Antibiotic susceptibility to methicillin (5 µg) and ciprofloxacin (5 µg) was performed using the Stokes' method[9] on lysed blood isosensitest agar. The control organism used was methicillin-sensitive *S. aureus* (NCTC 6571).

Mastalex-MRSA latex agglutination: Mastalex-MRSA latex agglutination (Mast Diagnostics, Derby Road, Bootle, Merseyside L20 1EA,UK) was performed according to the manufacturer's instructions on colonies of *S. aureus* and CNS from the CBA plate with methicillin disc and the three selective media at 24 h.

mecA detection: *mec*A PCR was performed on fresh subcultures from CBA plates of all *S. aureus* and CNS isolates. The PCR assay was standardised at the Laboratory of Hospital Infection, Central Public Health Laboratory, Colindale, London, and performed at UCLH using published methods.[10]

Table 2 Growth of staphylococci on BPC related to ciprofloxacin susceptibility

Organism	Growth on CBA	Ciprofloxacin -resistant (%)	Growth on BPC (%)
MSSA	37	4 (10.8)	4 (10.8)
MRSA	88	84 (95.5)	81 (92.1)
CNS (methicillin-sensitive)	20	2 (10)	1 (5)
CNS (methicillin-resistant)	20	15 (75)	4 (20)

Statistical analysis

Differences in the ability to detect MRSA and other staphylococci from the different screening media were evaluated by the χ^2 test. The sensitivity, specificity, positive and negative predictive values for the Mastalex-MRSA latex agglutination test and the Stokes disc diffusion method with reference to the *mec*A PCR for the detection of methicillin resistance in *S. aureus* and CNS were investigated.

Results

Twenty-five of the 63 patients screened were found to be MRSA-positive. CBA purity plates yielded heavy growths of *S. aureus* from 125 swabs and a predominance of CNS from 40 swabs. These specimens were used for parallel comparisons of growth on the screening media.

There were no significant differences between the three screening media (MSA, BPC, BCPA) and the CBA control in their ability to support MRSA growth. The results are described in Table 1. There was adequate growth at 24 h, with no advantage found in re-incubating for 48 h.

Methicillin-sensitive strains of *S. aureus* and CNS were significantly inhibited on all three media; however, in comparison to CBA, only BPC significantly inhibited the growth of methicillin-resistant CNS. Pre-enrichment in either salt broth did not improve the efficiency or speed of MRSA isolation.

BPC containing 8 mg/L ciprofloxacin was assessed for its usefulness in detecting methicillin-resistant organisms. The findings are presented with concomitant ciprofloxacin resistance by disc diffusion in Table 2.

Table 3. Mastalex-MRSA and Stokes' disc diffusion methods for the detection of methicillin resistance in *S. aureus* with reference to *mec*A PCR

Test		*mec*A PCR		Total
		Positive	Negative	
Mastalex -MRSA	Positive	87	1	88
	Negative	3	34	37
Total		**90**	**35**	**125**
Stokes' disc diffusion	Positive(resistant)	87	1	88
	Negative(sensitive)	3	34	37
Total		**90**	**35**	**125**

Sensitivity: 96.67%; Specificity: 97.14%

Positive predictive value: 98.86%; Negative predictive value: 91.89%.

Table 4. Mastalex MRSA and Stokes' disc diffusion methods for the detection of methicillin resistance in coagulase-negative staphylococci with reference to *mec*A PCR

Test		*mec*A PCR		Total
		Positive	Negative	
Mastalex -MRSA	Positive	17	0	17
	Negative	9	14	23
Total		**26**	**14**	**40**
Stokes' disc diffusion	Positive(resistant)	20	0	20
	Negative(sensitive)	6	14	20
Total		**26**	**14**	**40**

Mastalex-MRSA

Sensitivity: 65.38%; Specificity100%

Positive predictive value: 100%; Negative predictive value: 60.87%

Stokes' disc diffusion

Sensitivity: 76.92%; Specificity: 100%

Positive predictive value: 100%; Negative predictive value: 70.0%

Among the MRSA isolates in this study, 95% (84/88) were also resistant to ciprofloxacin, and 96% ciprofloxacin-resistant isolates (81/84) grew on the selective BPC agar.

Performance of the Mastalex-MRSA and Stokes' disc diffusion tests were evaluated against *mec*A PCR for sensitivity, specificity, positive and negative predictive values for the detection of methicillin resistance in 125 *S. aureus* isolates and in 40 CNS isolates. Colonies were taken from CBA plates (Tables 3 and 4).

Mastalex-MRSA was also assessed on isolates from the different selective media. All MRSA isolates from the three screening agar plates gave a positive agglutination with Mastalex-MRSA, and all were confirmed as MRSA by demonstration of the *mec*A gene using a PCR technique.

Discussion

The threat of potentially untreatable infection looms large with the increasing incidence of infection with drug-resistant organisms such as MRSA. Some countries (The Netherlands, in particular) have a low prevalence of MRSA, which is attributed to a stringent national policy of screening for, and treatment of, carriers.[11] In the UK, financial constraints have limited screening to high-risk areas such as intensive care, transplant and cardiovascular units.[12]

Effective screening, prevention and control of these organisms, however, depends on reliable and timely laboratory results.[13] Screening for the detection of MRSA at UCLH prior to the present study consisted of isolation of suspect colonies on MSA after 24 h pre-enrichment in salt broth – a process that took up to five days. No significant improvement on the current selective agar was found in this study, even with the use of a pre-enrichment broth, and no method was 100% successful. Reducing the oxacillin in the medium to 2 mg/L has been suggested; however, this medium too has yielded false-negative results.[14] BCPA medium was found to allow the over-growth of a variety of organisms, including *Proteus* spp., and this resulted in extra laboratory testing to detect MRSA.

The addition of ciprofloxacin as a selective agent in BPC agar was useful for those strains of MRSA that are ciprofloxacin-resistant. In the present study, 96% of ciprofloxacin-resistant MRSA isolates grew on BPC agar. The reason why a small number of these isolates failed to grow could be because the concentration of ciprofloxacin in the medium was close to the breakpoint for those isolates. Etest to ciprofloxacin would have confirmed this; however this was not investigated as part of the study. The medium was successful in inhibiting CNS and methicillin-sensitive *S. aureus*. Other workers have found this agar to be less reliable in selecting out MRSA, which could have been due to varying proportions of ciprofloxacin resistance in their test strains.[14]

Performance of the Mastalex-MRSA and Stokes' disc diffusion method with reference to the *mec*A PCR technique (Tables 3 and 4) reflected the heterogeneous nature of methicillin resistance, which is governed by many mechanisms and under the control of multiple genes.[5] Other workers have had similar experiences[15,16] and have recommended inducing methicillin resistance using a methicillin/oxacillin disc – the specificity of the Mastalex-MRSA test improved when tested on colonies taken from around the antibiotic disc. Mastalex-MRSA sensitivity for MRSA was better than for methicillin-resistant CNS; however, the test has been evaluated and marketed for the presumptive diagnosis of MRSA and hence cannot be used reliably for the detection of methicillin resistance in CNS.

Mastalex-MRSA proved successful on colonies from the screening agar plates, provided a dense suspension was used. Some workers have found colonies from MSA difficult to work with, due to the very sticky nature of the growth.[14] While this was also our experience, we found that use of a heavy inoculum and stringent adherence to the Mastalex-MRSA protocol in terms of heating (3 min), vortex-mixing and centrifugation to obtain a clear supernatant precluded these problems. This test allows a preliminary MRSA result to be available in 48 h, and a definitive one in 72 h - a sharp contrast to the four/five-day protocol followed in many laboratories.

Mastalex-MRSA proved extremely sensitive to temperature; and the heating time needs to be adhered to stringently. In the present study, extended heating at 100°C gave weak- or false-negative results. This has been reported in other studies.[15,17] The kit does not provide positive and negative controls; thus, *S. aureus* NCTC 6571, type strain,

and M34 (an in-house *mec*A PCR-positive control) were included in each batch of tests to avoid interpreting weak granulation as a false-positive result.

In conclusion, the use of selective screening media to detect MRSA remains problematic, resulting in a less than 100% yield. The present study was carried out on screening swabs from two tertiary referral hospitals and the results may be regarded as representative in this context. However, a larger study of patients harbouring different clones of MRSA is required to make changes in protocol more widely applicable as a routine diagnostic laboratory test.

Mastalex-MRSA provides reliable and rapid detection of MRSA when grown in pure culture on enriched media. Detection from selective media is also reliable provided a heavy inoculum is used and strict adherence to protocol followed. It is not recommended for the detection of methicillin resistance in CNS. Mastalex-MRSA is a reliable alternative to *mec*A PCR for the definitive diagnosis of MRSA, particularly in clinical situations where confirmation of methicillin resistance has urgent treatment and infection-control implications. □

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