Methicillin-resistant *Staphylococcus aureus* detection using chromogenic media: the Sheffield experience

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to cause major problems, both in hospitals and the community. Microbiology departments need to review their methodology regularly to ensure that they are contributing in the most appropriate manner to the battle against MRSA. Media employing chromogenic enzymes to aid the isolation and identification of MRSA is a relatively new approach. In this study, 192 swabs from 112 different patients were inoculated on two chromogen-containing media and four other commonly used solid MRSA media to determine which gave the appropriate combination of sensititivity, specificity and speed of result. Methicillin-resistant *S. aureus* was isolated on at least one of the six media from 102 of the 192 swabs. Both chromogenic media proved to be statistically significantly more sensitive than the other media after overnight incubation and had a sensitivity of 96% after 48 hours' incubation. The recent introduction of chromogen-containing MRSA media offers microbiology laboratories the opportunity to isolate and confirm the majority of MRSA infections/colonisations in 24 hours, which should result in better patient care. The possible slight increase in costs should not provide a valid excuse for using inferior methodologies.

KEY WORDS: Chromogenic media.
Methicillin.
Staphylococcus aureus.

Introduction

The fight against methicillin-resistant *Staphylococcus aureus* (MRSA) in the UK continues, both in the hospital and community environment. Mandatory reporting of *S. aureus* bacteraemia (including MRSA) was introduced in 2001. During the year from April 2006 to March 2007, a total of 6378 cases of MRSA bloodstream infection were reported, representing a decrease of 10% over the same period in 2005/06.[1] Thirty-eight per cent of the *S. aureus* isolated from blood cultures were methicillin resistant. This figure has remained at approximately 40% since 1999.[2] Methicillin-resistant *S. aureus* also continues to play an important role in surgical site infections, with recent data showing that 45% of infections are caused by *S. aureus*, of which 62% are MRSA.[3]

One characteristic that many microbiology departments commonly overlook is turnaround time. An accurate result obtained in the minimum time possible is what is required. Molecular techniques are now available with the required sensitivity and specificity to fulfil this requirement,[4–6] but at present the expertise and expense of providing such a service routinely on all screening swabs is prohibitive to most routine laboratories.

Therefore, routine culture remains the main means of MRSA detection, but there is disagreement about the optimum methodology. The use of overnight broth culture followed by subculture on routine media has been shown to increase MRSA detection,[7] but any increase in sensitivity achieved must be offset by the 24-hour delay that is often created in confirming both a positive and a negative result. Many laboratories continue to use mannitol fermentation and incorporate both salt and methicillin/oxacillin in the medium as the selective agents. Unfortunately, as with MRSA, many coagulase-negative staphylococci (CNS) are capable of fermenting mannitol and are also resistant to methicillin/oxacillin.[8]

The use of Baird-Parker plus ciprofloxacin (8 mg/L, BPC, Oxoid) has been recommended as the most appropriate medium when the epidemic strains EMRSA 15 and 16 predominate.[7] The use of nasal swabs inoculated directly on this medium was also found to be the most cost-effective screening procedure, after applying statistical analysis to a literature review of MRSA screening methods.[9]

Media containing chromogenic substrates that differentiate *S. aureus* from CNS have been recommended as an alternative to routine culture media for human clinical samples.[10] A previous study conducted in the authors' laboratory evaluated its suitability for MRSA screening by first incorporating methicillin in the medium, and then, as this was too inhibitory for MRSA, incorporating ciprofloxacin instead. However, the ciprofloxacin-containing chromogenic medium performed less efficiently than BPC.[11]

Developments in the antibiotic disc sensitivity field have provided an alternative approach. Recent studies have shown that antibiotic discs containing a cephamycin, or moxalactam, accurately confirm methicillin-resistance, without the need for either a reduction in incubation temperature or an increase in salt concentration.[12,13] Cefoxitin, a cephamycin, has since been recommended at a concentration of 4 mg/L for agar incorporation techniques in antimicrobial susceptibility testing.[14]

The present study evaluates two chromogenic media,

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Table 1. Ability of media evaluated to isolate colonies of MRSA (n=102).

	BPC		CHR-W		CHR-Y		MSO		ORSA		MSOEA	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
M±	2	8	9	9	8	11	1	10	4	8	1	6
M+	3	9	7	9	7	8	5	8	5	8	2	10
M++	8	12	6	6	9	9	13	21	6	7	9	16
M+++	38	59	74	74	69	70	46	52	59	63	25	48
Total	51	88	96	98	93	98	65	91	74	86	37	80

Scanty growth (M±) to heavy growth (M+++)

MRSA*Select* (CHR-W, Bio-Rad) and CHROMagar MRSA (CHR-Y, BioConnections), designed for the isolation of MRSA, both of which use cefoxitin as the selective agent. These are compared with four recommended MRSA isolation media, including BPC, mannitol salt agar plus oxacillin (4mg/L, MSO, Oxoid), oxacillin resistance screening agar base (ORSAB, Oxoid) and mannitol salt agar plus oxacillin (4 mg/L) containing egg yolk and 8 mg/L aztreonam (MSOEA).[15]

Materials and methods

The study took place in the autumn of 2005 and involved a total of 192 swabs from 112 different patients. As six different media were to be evaluated in the study and only a limited supply of some of the media was available, a decision was taken to incorporate swabs that had previously been shown to contain MRSA. As such, the majority of samples (n=130) were routine MRSA screening swabs, while 62 swabs were from samples known to contain MRSA from routine laboratory culture on non-MRSA media.

The swabs were distributed randomly by the lead author among the total number of swabs tested to ensure anonymity. Consequently, a total of 100 (52%) wound swabs were investigated, which is a greater number than to be found in most previous MRSA studies.[8,9,12] The remainder of the samples were 32 (17%) nasal swabs, 48 (25%) perineum/groin swabs, six eye swabs, four axilla swabs and two throat swabs.

With the exception of MSOEA, all media were prepoured commercially. The MSOEA was prepared using mannitol salt agar (Oxoid), 30% egg-yolk solution (Oxoid) and the relevant oxacillin and aztreonam Adatabs (Mast). The order of inoculation of the solid media was rotated after every 10 swabs in an attempt to standardise the inoculum.

All the media were incubated at 37°C in air. The solid media were examined at 24 and 48 hours. Confirmation of *S. aureus* was determined using a combination of Pastorex-Plus latex agglutination (Bio-Rad) and reaction on DNase agar (Oxoid) after overnight incubation. Confirmation of methicillin-resistance was determined by inoculation on DST agar (Oxoid) with 4 mg/L methicillin Adatabs and 2% salt. Any apparent discrepancy between the methicillin plate result and cefoxitin resistance seen on the chromogenic media was investigated using Mastalex (Mast) to determine the presence or absence of penicillin binding protein 2a (PBP2a) and hence methicillin resistance status.[17]

Analysis of the comparison of isolation rates was performed using the two-paired proportion method,[18] in which the number of specimens positive solely by one test alone were compared with the number positive solely by the other test.

Results

Of the 192 swabs tested, 102 were shown to contain MRSA using one or more of the media, giving a positivity rate of 53.1%. The results from the screening swabs alone were 42 positives from 130 swabs, a positivity rate of 32.3%. The results are summarised in Table 1.

The results demonstrate the relative sensitivity of the various screening media. After 48 hours' incubation these were shown to be: BPC (86%), CHR-W (96%), CHR-Y (96%), MSO (89%), ORSA (84%) and MSOEA (78%). The major benefit of the two chromogenic media, however, was more evident after 24 hours' incubation. The respective sensitivities were BPC (50%), CHR-W (94%), CHR-Y (91%), MSO (64%), ORSA (72%) and MSOEA (36%).

Using the two-paired proportion method, statistically significant improved results at 24 hours were demonstrated when either chromogenic medium was compared to any one the other media. The differences at 48 hours were not as marked. The calculated values are given in Tables 2 and 3. This form of statistical analysis allows each medium to be

Table 2. Significance of differences in performance (P values) of media for MRSA isolation after 24 hours' incubation (in order of success).

CHR-W					
0.45	CHR-Y				
<0.00006	0.0002	ORSA			
<0.00006	<0.00006	0.066	MSO		
<0.00006	<0.00006	0.00044	0.035	BCP	
<0.00006	<0.00006	<0.00006	<0.00006	0.044	MSOEA

Table 3. Significance of differences in performance (*P* values) of media for MRSA isolation after 48 hours' incubation (in order of success).

CHR-W					
1.0	CHR-Y				
0.023	0.023	MSO			
0.009	0.009	0.58	BCP		
0.003	0.0056	0.23	0.65	ORSA	
0.0001	0.0001	0.01	0.062	0.31	MSOEA

compared independently to another. At 24 hours' incubation (Table 2), both chromogenic media produced *P* values <0.001 when compared with the other solid media.

Ideally, screening media should be both sensitive and specific. Having demonstrated sensitivity, specificity can be evaluated by determining the number of isolates that grew on the respective media and were investigated further but proved not to be MRSA (i.e., false positives). These are shown in Table 4.

As expected, CNS proved the most problematic on all the media tested. The relative sensitivity and specificity of the various media are shown in Table 5. The chromogenic media, especially CHR-W, performed particularly well at 24 hours' incubation. Low specificity was seen with MSO, particularly at 48 hours' incubation, while MSOEA showed a low sensitivity, especially at 24 hours' incubation and despite its high specificity.

A final issue worthy of consideration is the ability to achieve pure cultures when performing sensitivity testing directly from the isolation medium. Once again, MSO performed poorly, as mixed sensitivities were often obtained (16%). The percentage of mixtures obtained for the other media were BPC (9%), CHR-W (4%), CHR-Y (6%), ORSAB (5%) and MSOEA (5%).

Discussion

In the population of MRSA strains studied here, the two media that contained chromogenic substrates gave significantly better isolation at 24 hours' incubation, demonstrating excellent sensitivity, specificity and speed. At 48 hours' incubation, despite the fact that each chromogenic medium detected 96% of the isolates, the advantages were not as marked.

While the detection rate is very impressive, it is an accepted fact that the greater number of media investigated the lower the sensitivity of each individual medium becomes, due to sampling error on swabs with low numbers of MRSA. Rotating the order of inoculation ensured that each medium was evaluated equally, but this cannot overcome the low inoculum effect.

Four MRSA isolates were not detected by the two chromogenic media. All were light growths. One was detected on CHR-W but not on CHR-Y, while for another the opposite occurred. One was detected on MSO only and one grew on both BPC and MSOEA. Therefore, all could be accounted for by the inoculum effect. Additionally, both media isolated a strain that was oxacillin- and ciprofloxacin-sensitive *in vitro*, which proved to be MRSA, using DNase testing, Pastorex Plus agglutination and by detecting the presence of PBP2a.

The only slight hesitation the authors would have in recommending CHROMagar MRSA medium to be used alone is due to the appearance of variable results in individual batches when the medium was prepared in-house (unpublished data). However, this did not appear to be a problem in the study and may demonstrate the greater consistency of commercially prepared prepoured plates.

Interestingly, the majority of false-positive isolates on CHR-W were obtained from the final batch of swabs, and the plates had less than two weeks to their expiry date. This may indicate a problem with the recommended shelf life, rather than with the medium itself, but does suggest that more organism types are capable of breaking down the chromogenic substrates as they age within the media.

The use of chromogenic media to detect MRSA is supported by other workers,[19–21] although slightly variable results have been reported. This may depend on whether or not the CHROMagar MRSA was prepared in house.

Table 4. Number and types of organisms investigated that proved not to be MRSA.

	BPC		CHR-W		CHR-Y		MSO		ORSA		MSOEA	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
CNS	2	6	1	13	6	2	7	13	12	1	2	1
MSSA	1	–	–	–	–	–	–	–	–	–	–	–
DIP	–	–	1	–	–	1	–	–	–	–	–	–
FST	–	–	1	1	–	–	–	–	–	–	–	–
Mixed	–	–	–	–	–	–	7	–	–	2	–	–
Total	3	6	3	14	6	3	14	13	12	3	2	1

CNS: coagulase-negative staphylococci, MSSA: methicillin-sensitive *Staphylococcus aureus*,
DIP: *Corynebacterium* species, FST: *Enterococcus* species.

Table 5. Sensitivity and specificity of media evaluated.

	After 24 hours		After 48 hours	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
BPC	50.0	96.7	86.3	90.0
CHR-W	94.1	96.7	96.1	84.4
CHR-Y	91.2	94.0	96.1	90.0
MSO	63.7	84.4	89.2	70.0
ORSA	72.6	86.7	84.3	83.3
MSOEA	36.3	97.8	78.4	96.7

The BPC performed particularly poorly in this study and was evident in the fact that only 50% of strains were detected at 24 hours, as compared to 81% previously.[7] Five of the MRSA strains isolated proved to be lipase-negative. This will have affected the efficiency of the BPC media, as such strains were not detected in a previous MRSA population studied. Furthermore, the fact that the pH of the basal Baird-Parker medium has been adjusted to pH 6.8, instead of pH 7.2, may have contributed to the poor results obtained on this occasion.

Both MSO and ORSAB gave similar results, which is not surprising as they rely on mannitol fermentation; however, the former gave the better sensitivity and the latter the better specificity. Both gave much improved results in the present study than were obtained by the authors previously,[7] even though an MRSA strain that failed to ferment mannitol was detected in the present study.

The MSOEA medium showed the highest specificity (96.7%) but had a sensitivity of only 78.4%; this is unacceptable, particularly as the majority of isolates needed 48 hours. The fact that both lipase-negative and mannitol fermentation-negative strains were detected will have had a detrimental effect on the efficiency of this medium.

The emergence of vancomycin-resistant MRSA[22] has heightened fears that a fully-resistant *S. aureus* is a probability rather than a possibility. Government figures suggest that hospital-associated infections, of which MRSA is the most notorious, result in approximately 5000 deaths annually, cost the NHS around £1 billion per year,[23] and are the cause of untold misery for patients and their relatives. As a consequence, MRSA screening numbers have increased dramatically in line with Department of Health recommendations.[24] These numbers will increase further if Lord Darzi's report is implemented, as it recommends the introduction of MRSA screening for all elective admissions next year, and for all emergency admissions as soon as possible over the next three years.[25]

It is in everyone's interest that greater effort be made to win the battle against MRSA. Both chromogenic media investigated in this study performed admirably, but a multicentre study is required to compare these two media against the two other chromogenic media (MRSA ID [bioMérieux] and ChromMRSA [Oxoid]) available in the UK.

The use of molecular techniques will become more widespread in the future. Until then, however, the use of isolation media that give accurate and fast results is essential. The present study demonstrates that both MRSA*Select* and CHROMagar MRSA offer this opportunity. □

References

1 Health Protection Agency. MRSA bacteraemia, *Clostridium difficile* and GRE bacteraemia. *Health Protection Report* 2007; **1** (30). Published 27 July 2007.

2 Health Protection Agency. *Staphylococcus aureus* bacteraemia: voluntary reporting in England, Wales and Northern Ireland: January to December 2006. *Health Protection Report* 2007; **1** (38). Published 21 September 2007

3 Third Report of the Mandatory Surveillance of Surgical Site Infection in Orthopaedic Surgery - April 2004 to March 2007 www.hpa.org.uk/infections/topics_az/surgical_site_infection/documents/SSI3rdMandatory01-11-07.pdf

4 Kearns AM, Seiders PR, Wheeler J *et al*. Rapid detection of methicillin-resistant staphylococci by multiplex PCR. *J Hosp Infect* 1999; **43** (2): 33–7.

5 Huletsky A, Giroux R, Rossbach V *et al*. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* 2004; **42**: 1875–84.

6 Wren MWD. Carder C, Coen PG, Gant V, Wilson APR. Rapid molecular detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005; **44** (4): 1604–5.

7 Davies S, Zadik PM. Comparison of methods for the isolation of methicillin-resistant *Staphylococcus aureus. J Clin Pathol* 1997; **50**: 1–3.

8 Zadik PM, Davies S, Whittaker S *et al*. Evaluation of a new selective medium for methicillin-resistant *Staphylococcus aureus. J Med Microbiol* 2001; **50**: 476–9.

9 Kunori T, Cookson B, Roberts JA *et al*. Cost-effectiveness of different MRSA screening methods. *J Hosp Infect* 2002; **51**: 189–200.

10 Gaillott O, Wetsch M, Fortineau N *et al*. Evaluation of CHROMagar *Staph aureus*, a new chromogenic medium for isolation and presumptive identification of *Staphylococcus aureus* from human clinical specimens. *J Clin Microbiol* 2000; **38**: 1587–91.

11 Davies S, Zadik PM, Mason CM *et al*. Methicillin-resistant *Staphylococcus aureus*: evaluation of five selective media. *Br J Biomed Sci* 2000; **57**: 269–72.

12 Felten A, Grandy B, Lagrange PH *et al*. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system and the MRSA-Screen latex agglutination test. *J Clin Microbiol* 2002; **40** (8): 2766–71.

13 Skov R, Smyth R, Clausen M *et al*. Evaluation of a cefoxitin disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus. J Antimicrob Chem* 2003; **52**: 204–7.

14 Woodford N, Livermore D. And a 'new' classical method: cefoxitin screen for MRSA. *Antibiotic Resistance Monitoring and Reference Laboratory Newsletter* 2003; Summer: 4.
15 Winstanley TG, Eggington R, Spencer RC. Selective media for MRSA. *J Clin Pathol* 1993; **46**: 1140.
16 Wood W, Harvey G, Olson ES *et al*. Aztreonam selective agar for Gram-positive bacteria. *J Clin Pathol* 1993; **46**: 769–71.
17 Cavassini M, Wenger A, Jaton K *et al*. Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus. J Clin Microbiol* 1999; **37** (5): 1591–4.
18 Altman DG. *Practical statistics in medical research*. London: Chapman and Hall, 1991: 235–9.
19 Perry JD, Davies A, Lynne A *et al*. Development and evaluation of a chromogenic agar medium for methicillin-resistant *Staphylococcus aureus. J Clin Microbiol* 2004; **42** (10): 4519–23.
20 Dierden B, van Duijn I, van Belkum A *et al*. Performance of CHROMagar MRSA medium for detection of methicillin-resistant *Staphylococcus aureus. J Clin Microbiol* 2005; **43** (4): 1925–7.
21 Hedin G, Fang H. Evaluation of two new chromogenic media, CHROMagar MRSA and *S. aureus* ID, for identifying *Staphylococcus aureus* and methicillin-resistant *S. aureus*. *J Clin Microbiol* 2005; **43** (8): 4242–4.
22 Centres for Disease Prevention and Control. *Staphylococcus aureus* resistant to vancomycin – United States. *JAMA* 2002; **288**: 824–5.
23 National Audit Office. *Improving patient care by reducing the risk of hospital-acquired infection*: *a progress report*. Report by the Controller and Auditor General. HC876 Session; 2003–2004: 14 July 2004.
24 Department of Health. *Screening for MRSA colonisation: a summary of best practice*. A report by the Chief Medical Officer and Chief Nursing Officer. 16 November 2006. www.dh.gov.uk/en/Publicationsandstatistics/Lettersandcirculars
25 Department of Health. *Our NHS, our future.* Lord Darzi's interim report. 4 October 2007. www.networks.nhs.uk/news.php?nid=1802