A cost-effective protocol for screening patients for methicillin-resistant *Staphylococcus aureus*

E. M. DICKSON, M. M. DAVIDSON, A. J. HAY and D. O. HO-YEN

Department of Microbiology, Raigmore Hospital, Old Perth Road, Inverness IV2 3UJ, United Kingdom

Accepted: 29 April 2011

ABSTRACT

The incidence of hospital-acquired infection with methicillin-resistant *Staphylococcus aureus* (MRSA) is rising worldwide. Rapid identification of MRSA carriers is an important step in reducing the risk of transmission to other patients. Molecular methods are increasingly popular but are technically demanding and expensive. This study assesses the modification of one of the commercially available latex agglutination tests (Mastalex-MRSA) for the identification of penicillin-binding protein 2′ on known strains of MRSA as well as other organisms identified from chromogenic agar plates. A total of 3050 patients with unknown MRSA status were processed through the routine laboratory during the investigation period and 73 of these were presumptive positive following overnight incubation. Of 70 patients who could be evaluated, 32 (43.8%) specimens would be suitable for use with the kit directly from overnight incubation on chromogenic agar, and the other 38 (52.1%) would be suitable following four hours' incubation on blood agar. The cost of one positive MRSA test with the inclusion of this test is €15.15 compared with published reports of €35.00 for a commercial polymerase chain reaction (PCR) test. This protocol would allow the reporting of presumptive positive MRSA results approximately 24 hours earlier than currently achieved.

KEY WORDS: Agglutination. Latex fixation tests. Methicillin-resistant Staphylococcus aureus. Penicillin binding proteins.

Introduction

The incidence of hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) infection is rising worldwide, although this varies between countries, partly depending on the approach to detection.[1] The United Kingdom is reported to have one of the highest rates of MRSA infection in Europe,[2] where 31% of *S. aureus* isolates causing invasive infection are methicillin-resistant;[3] however, a decrease in rates first observed in 2006 appears to be ongoing.[3]

Active surveillance with screening to identify MRSA carriers and treating them by isolation and eradication to reduce the risk of transmission is of great importance to the continued decline in rates of MRSA infection.[4] Owing to the risk of transmission of MRSA from infected patients, it is important to obtain rapid results on patients in identified risk groups.[5] Any delay could result in cross-infection from MRSA-positive patients and facilitate the spread of MRSA through a hospital.[6]

Various methods are available for use in the diagnostic laboratory which can improve turnaround time for MRSA identification.[7,8] Culture methods are used widely and are recommended in the most recent guidelines for laboratory diagnosis of MRSA.[7] The development of chromogenic agars for the detection of MRSA has provided laboratories with the ability to identify presumptive positive MRSA colonies after incubation for 24 hours.[8]

In recent years, attention has turned to the development of molecular techniques for the detection of MRSA, which offer much more rapid results than conventional culture.[9] There are many reports of 'in house' molecular methods for the detection of various MRSA genes, such as *mecA*, *nuc* or *orfX*,[10,11] or multiplex assays for the detection of the most common *SCCmec* types.[12] Commercial real-time assays are also available that identify MRSA from clinical samples.[13,14] The problems with these methods include higher cost and greater technical complexity.

Current practice in the authors' laboratory is to perform further confirmatory tests of presumptive MRSA colonies from chromogenic agar, with a presumptive report at 48 hours and final report at 72 hours. This delay in producing a confirmed result prompted the authors to seek another method to improve turnaround time on MRSA screening samples. Almost all MRSAs have an additional penicillin-binding protein, PBP2′, mediated by the *mecA* gene,[15] and commercially available rapid latex agglutination tests have been described, based on the detection of PBP2′.[16–18]

The aim of this study is to adapt one of these kits (Mastalex-MRSA, Mast Diagnostics, Bootle, UK) for use on presumptive MRSA colonies from screening samples, and assess whether or not this technically simple test could provide same-day results in patients with unknown MRSA status.

Materials and methods

The study was undertaken at Raigmore Hospital in Inverness, Scotland, which is a 577-bed district general hospital that provides services to a population of some 299,000 residents covering the north of Scotland. Specimens

Correspondence to: Elizabeth Dickson
Department of Microbiology, Royal Infirmary of Edinburgh
51 Little France Crescent, Old Dalkeith Road, Edinburgh EH16 4SA
Email: elizabeth.dickson2@nhs.net

for MRSA identification were received from patients whose MRSA status was unknown, as well as from patients who were previously known carriers. The former were taken either from the community, upon emergency admission to hospital, at pre-operative clinics, or admission to the intensive therapy unit (ITU). Specimens received were site-specific swabs such as nose, axillae or perineum, together with some endotracheal aspirates and urine. The swabs were collected into Amies medium and were transported and processed within six to 48 hours of collection.

Data collection from current practice
The MRSA requests for the year were examined. From April to August 2009, all consecutive unselected swabs requesting MRSA were processed using current practice. Briefly, individual swabs from patients previously known to have MRSA were incubated overnight at 37°C in nutrient broth with 7.5% salt (Oxoid, Basingstoke, UK), then subcultured on *Brilliance* MRSA agar (Oxoid) for a further overnight incubation at 37ºC. Swabs received from patients of unknown MRSA status were pooled and directly plated on *Brilliance* MRSA agar and incubated overnight at 37°C. Possible MRSA colonies with distinct denim blue colour on *Brilliance* MRSA agar following 18–24 h incubation were recorded as follows: mixed growth; 1–9 colonies of presumptive MRSA; and >10 colonies of presumptive MRSA. Isolates were then processed for identification by standard procedures including Prolex StaphXtra test (Prolab, Cheshire, UK) and inoculation for DNase and a Methi Test–cefoxitin strip (Medical Wire & Equipment, Wiltshire, UK) as well as identification and sensitivity testing by Vitek2 (bioMérieux, Basingstoke, UK). Final results were reported and recorded along with data collected on growth characteristics.

Latex kit modification
The Mastalex-MRSA latex agglutination kit recommends the use of approximately 10 medium colonies (1 mm diameter). Frequently, fewer than five colonies are isolated from MRSA screening samples. In order to determine if this kit could be adapted for use on these samples, two dominant UK epidemic strains of MRSA, EMRSA 15 and EMRSA 16,[19] and two common local strains of MRSA, SMRSA 111 and SMRSA 106 (typing by Scottish MRSA Reference Laboratory) were used. These strains were processed in duplicate, using a 1 µL loopful, or 20, 10, 8, 6, 4, 2 and 1 colonies. Each reaction was observed for agglutination after 3 min and strength of reaction was scored according to the manufacturer's instructions. Organisms other than MRSA isolated from nasal swabs and with the ability to grow on *Brilliance* MRSA agar were tested in duplicate. These were methicillin-sensitive *S. aureus, Enterococcus faecalis, Bacillus* spp., methicillin-sensitive coagulase-negative strains of staphylococci, *S. warneri* and *S. cohnii*, and methicillin-resistant coagulase-negative strains of staphylococci, *S. haemolyticus* and *S. epidermidis*. A 1 µL loopful or 20, 10 and 5 colonies were observed for agglutination and the strength of reaction recorded. Reaction mixes of EMRSA 15 and individual organisms, identified above, were tested in varying quantities in duplicate. As a control, a single suspension of the organism was used to check the agglutination result of that organism alone. In order to perform MRSA confirmation with Mastalex-MRSA in samples with less than five colonies after 24 h incubation, one EMRSA 15 colony was picked from a *Brilliance* MRSA agar plate and subcultured on a blood agar plate. Growth was recorded following incubation at 37°C for 4 h, 5 h and 6 h. Where sufficient growth was detected, the Mastalex-MRSA test was carried out along with the standard identification procedures.

Financial considerations
The cost for the current routine method for patients of unknown MRSA status was determined. This included the cost of a transport swab, a *Brilliance* MRSA agar plate and a plastic loop for all specimens. This was the total cost if a specimen was negative. Specimens producing blue colonies required further processing and the additional cost of confirmatory tests, as detailed above. The cost of performing a Mastalex-MRSA agglutination test was calculated, although the figures do not take into consideration the additional labour cost associated with this work.

Proposed protocol
Using the results from the above methods, a protocol was developed for the routine use of the Mastalex-MRSA kit in the diagnostic laboratory service in order to achieve a faster turnaround time for positive patients.

Statistical analysis
The results for patients of unknown MRSA status from the test period were compared to the results obtained between November 2008 and October 2009 using an unpaired *t*-test.

Results

Current practice
During the 20-week period of data collection, samples from a total of 4034 distinct patients were processed (Table 1). Of these, 984 were previously known to harbour MRSA and not considered further in the context of this study. The remaining 3050 patients were of unknown MRSA status. When compared with the entire year, there was no statistical difference with specimen numbers during this period ($P=0.48$). Breakdown by source showed that 60% of specimens were from nasal swabs alone, 29% from pooled sites, 8% from urine and 1.6% from endotracheal aspirates. The remaining 1.4% were from miscellaneous individual sites or sputum samples. Samples from 73 patients produced blue colonies on *Brilliance* agar following overnight

Table 1. Breakdown of patient status and results for specimens received in the microbiology department at Raigmore Hospital for identification of MRSA.

	Known MRSA patients (%)	Patients with unknown MRSA status (%)	Total (%)
Negative	460 (46.7)	2977 (97.6)	3437 (85.2)
Presumptive positive	524 (53.3)	73 (2.4)	597 (14.8)
Total	984	3050	4034
Confirmed positive	444 (45.1)	47 (1.5)	491 (12.2)

incubation and were further processed overnight for preliminary MRSA identification. Of these, 47 (64%) were confirmed as MRSA on final report.

Latex kit modification

All EMRSA and SMRSA strains produced denim blue colonies on *Brilliance* MRSA agar and a positive result with as few as two colonies. When examining *Brilliance* agar plates there is often mixed growth present that may affect the interpretation of the result. Blue colonies produced by a methicillin-sensitive *S. aureus* (MSSA) clinical isolate, *E. faecalis, Bacillus* spp., and mixed-colour colonies of methicillin-sensitive coagulase-negative staphylococci did not produce any reaction with the Mastalex-MRSA kit. Two strains of methicillin-resistant coagulase-negative staphylococci (CNS) produced a positive reaction with the Mastalex-MRSA kit; however, more that 10 colonies were required for a strong reaction. The same organisms were used to identify interference with the kit, but none was observed. Following 4 h incubation of one MRSA colony on blood agar at 37°C, it was possible to obtain sufficient growth to perform a Mastalex-MRSA latex agglutination. This also provided sufficient growth for standard *S. aureus* identification and further MRSA confirmatory tests, as described above.

Proposed protocol

Using the results from current practice, a protocol was developed for earlier confirmation of MRSA in specimens from patients with unknown MRSA status (Fig 1). Only 70 patients could be evaluated because the original *Brilliance* plates from three were not seen by the assessors to record growth details. Using this protocol, 32 (45.7%) of the 70 presumptive positive patients evaluated whose specimens produced >5 colonies and no mixed growth could have been identified using the kit. For the 38 (54.3%) patients with <5 colonies or a mixed growth, an additional incubation step could have provided enough growth to perform the latex agglutination test.

Financial considerations

In the authors' laboratory, the cost of processing a negative sample is €0.77, and a presumptive positive costs €9.29. The cost of performing the current protocol during the study period was €2970.46. The additional cost of running the Mastalex-MRSA kit would have been €5.86 per isolate, including a positive control, making the cost of processing a positive MRSA patient €15.15. The cost of processing the samples during the study period using the Mastalex-MRSA kit in addition to the current method would have been €3398.24.

Discussion

A rapid result on patients with unknown MRSA status is important for the management of this organism among vulnerable patients in hospitals.[20] Currently, in the authors' laboratory, swabs are cultured from patients with unknown MRSA status directly on chromogenic agar, with a negative result available after 24 h. Other studies have evaluated the use of chromogenic agars to produce a faster result, with favourable outcomes.[21,22] However, in the authors' laboratory,

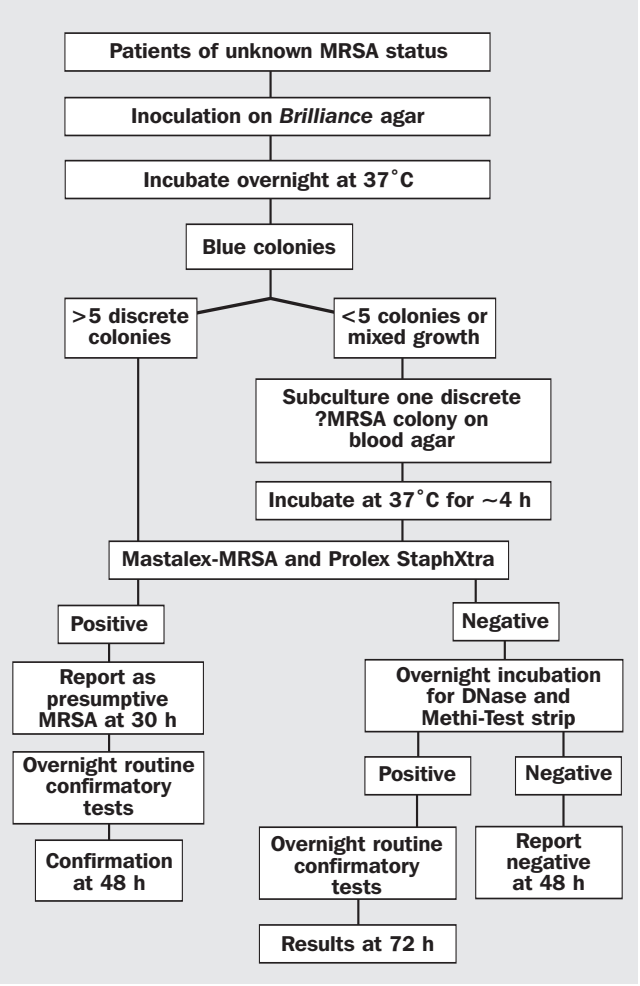

Fig. 1. Prospective algorithm for processing presumptive MRSA specimens through routine laboratory practice.

a presumptive positive is not reported until 48 hours as further testing requires overnight incubation, with final confirmation at 72 hours.

The ability of the epidemic strains, EMRSA 15 and 16, and SMRSA strains used in this study to produce a strong reaction with as few as two colonies using the Mastalex-MRSA kit confirms induction of the *mecA* gene[23] and increased production of PBP2′ with the surrogate marker in *Brilliance* agar. Five colonies were chosen as the cut-off value to provide sufficient colonies for further confirmatory tests as well as the Mastalex-MRSA test. Positive results with CNS are expected as some methicillin-resistant CNS also carry the *mecA* gene.[24] However, a greater number of colonies (>10) were required to produce a strong reaction, and this agrees with the findings of Cavassini and colleagues.[25]

Distinct colony morphology (mostly white colonies) also permits the differentiation from MRSA. Where methicillin-resistant CNS produce blue colonies, a negative Prolex StaphXtra test confirmed the identification. Utilising a latex agglutination test for PBP2′ and implementing the proposed protocol, even specimens that produce fewer than five colonies on the *Brilliance* plate can be processed and a presumptive report issued on the same working day.

Plans for a national rollout of MRSA screening[26] will put an extra burden on laboratories to provide a rapid result on a greater number of specimens. Therefore, the ability of this

protocol to provide a presumptive result within 24 h and a final report in 48 h is of critical importance.

Molecular techniques have been introduced in some laboratories, with sensitivity and specificity increased by linking the polymerase chain reaction (PCR) to an end-stage hybridisation/enzyme-linked immunosorbent assay (ELISA) or real-time detection step.[13,14] Although the result is more rapid than culture techniques, this is at a higher financial and technical cost.[27] Indeed, this laboratory has reported on the use of the commercial IDI-MRSA assay and found it to be workable and able to achieve same-day results.[28] However, under current financial restrictions, this PCR system could not be sustained within the authors' laboratory.

Other studies have determined that MRSA screening reduces the burden on the healthcare system and that culture-based[29,30] and PCR-based techniques[31] can be cost-effective. Studies using PCR-based methods vary in respect of the associated costs reported. Schulz and colleagues[31] view PCR as cost-effective, with a cost per test of €35 using BD Gene Ohm (IDI-MRSA). In the present study, the cost of performing the Mastalex-MRSA test was lower, and this can be performed only on the specimens that are presumptive positive.

In summary, the Mastalex-MRSA kit is a rapid and easy-to-use test that can be performed on as few as five presumptive MRSA colonies from *Brilliance* agar. In cases that produce insufficient colonies, a 4 h incubation on blood agar can be used. Thus, a presumptive positive result can be issued on patients after 24 h (not the current 48 h). This provides a better service for the patients and clinical staff within the authors' district general hospital. It may also be possible to use other commercial latex kits in a similar manner.

The authors would like to thank staff at the Scottish MRSA Reference Laboratory for typing the MRSA isolates, and the biomedical scientists in the bacteriology department of Raigmore Hospital, in particular Andrea Hull and Julius Msangi, for their assistance with data collection.

References

1 Humphreys H. Can we do better in controlling and preventing methicillin-resistant *Staphylococcus aureus* (MRSA) in the intensive care unit (ICU)? *Eur J Clin Microbiol Infect Dis* 2008; **27**: 409–13.

2 Gould IM. The clinical significance of methicillin-resistant *Staphylococcus aureus*. *J Hosp Infect* 2005; **61**: 277–82.

3 European Antimicrobial Resistance Surveillance System annual report. 2008. *Antimicrobial Resistance in Europe*. Ch 5, Subsection 5.3: *Staphylococcus aureus*, 55–8.

4 Department of Health. *Screening for MRSA colonisation: a strategy for NHS trusts*. London: NHS, 2006.

5 Rajan L, Smyth E, Humphreys H. Screening for MRSA in ICU patients. How does PCR compare with culture? *J Infect* 2007; **55**: 353–7.

6 Aldeyab MA, Kearney MP, Hughes CM *et al.* Can the use of a rapid polymerase chain screening method decrease the incidence of nosocomial meticillin-resistant *Staphylococcus aureus*? *J Hosp Infect* 2009; **71**: 22–8.

7 Brown DFJ, Edwards DI, Hawkey PM *et al*. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). *J Antimicrob Chemother* 2005; **56**: 1000–18.

8 Perry JD, Davies A, Butterworth LA, Hopley ALJ, Nicholson A, Gould KF. Development and evaluation of a chromogenic agar medium for methicillin-resistant *Staphylococcus aureus. J Clin Microbiol* 2004; **42**: 4519–23.

9 Struelens MJ, Hawkey PM, French GL, Witte W, Tacconelli E. Laboratory tools and strategies for methicillin-resistant *Staphylococcus aureus* screening, surveillance and typing: state of the art and unmet needs. *Clin Microbiol Infect* 2009; **15**: 112–9.

10 Hagen RM, Seegmüller I, Navai J, Kappstein I, Lehn N, Miethke T. Development of a real-time PCR assay for rapid identification of methicillin-resistant *Staphylococcus aureus* from clinical samples. *Int J Med Microbiol* 2005; **295**: 77–86.

11 Nilsson P, Alexandersson H, Ripa T. Use of broth enrichment and real-time PCR to exclude the presence of methicillin-resistant *Staphylococcus aureus* in clinical samples: a sensitive screening approach. *Clin Microbiol Infect* 2005; **11**: 1027–34.

12 Renwick L, Hardie A, Girvan EK *et al*. Detection of meticillin-resistant *Staphylococcus aureus* and Panton-Valentine leukocidin directly from clinical samples and the development of a multiplex assay using real-time polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 2008; **27**: 791–6.

13 Cunningham R, Jenks P, Northwood J, Wallis M, Ferguson S, Hunt S. Effect on MRSA transmission of rapid PCR testing of patients admitted to critical care. *J Hosp Infect* 2007; **65**: 24–8.

14 Wolk DM, Picton E, Johnson D *et al*. Multicenter evaluation of the Cepheid Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) test as a rapid screening method for detection of MRSA in nares. *J Clin Microbiol* 2009; **47**: 758–64.

15 Matsuhashi M, Song MD, Ishino F *et al*. Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to beta-lactam antibiotics in *Staphylococcus aureus. J Bacteriol* 1986; **167**: 975–80.

16 Nakatomi Y, Sugiyama J. A rapid latex agglutination assay for the detection of penicillin-binding protein 2′. *Microbiol Immunol* 1998; **42**: 739–43.

17 Van Leeuwen WB, Van Pelt C, Luijendijk A, Verbrugh HA, Goessens WHF. Rapid detection of methicillin resistance in *Staphylococcus aureus* isolates by the MRSA-Screen latex agglutination test. *J Clin Microbiol* 1999; **37**: 3029–30.

18 Chapin KC, Musgnug MC. Evaluation of penicillin binding protein 2a latex agglutination assay for identification of methicillin-resistant *Staphylococcus aureus* directly from blood cultures. *J Clin Microbiol* 2004; **42**: 1283–4.

19 Khandavilli S, Wilson P, Cookson B, Cepeda J, Bellingan G, Brown J. Utility of spa typing for investigating the local epidemiology of MRSA on a UK intensive care ward. *J Hosp Infect* 2009; **71**: 29–35.

20 Harbarth S. Control of endemic methicillin-resistant *Staphylococcus aureus* – recent advances and future challenges. *Clin Microbiol Infect* 2006; **12**: 1154–62.

21 Louie L, Soares D, Meaney H, Vearncombe M, Simor AE. Evaluation of a new chromogenic medium, MRSA Select, for detection of methicillin-resistant *Staphylococcus aureus. J Clin Microbiol* 2006; **44**: 4561–3.

22 Carson J, Lui B, Rosmus L, Rennick H, Fuller J. Interpretation of MRSASelect screening agar at 24 hours of incubation. *J Clin Microbiol* 2009; **47**: 566–8.

23 Nonhoff C, Denis O, Brenner A *et al.* Comparison of three chromogenic media and enrichment broth media for the detection of methicillin-resistant *Staphylococcus aureus* from mucocutaneous screening specimens. *Eur J Clin Microbiol Infect Dis* 2009; **28**: 363–9.

24 Ibrahem S, Salmenlinna S, Virolainen A *et al*. Carriage of methicillin-resistant staphylococci and their SCCmec types in a long-term-care facility. *J Clin Microbiol* 2009; **47**: 32–7.
25 Cavassini M, Wenger A, Jaton K, Bille J, Blanc DS. Authors' reply. Further evaluation of the MRSA-Screen kit for rapid detection of methicillin resistance. *J Clin Microbiol* 1999; **37**: 3784.
26 Reilly JS, Stewart S, Christie P *et al*. Universal screening for meticillin-resistant *Staphylococcus aureus*: interim results for the NHS Scotland pathfinder project. *J Hosp Infect* 2010; **74**: 35–41.
27 Conterno LO, Shymanski J, Ramotar K *et al*. Real-time polymerase chain reaction detection of methicillin-resistant *Staphylococcus aureus*: impact on nosocomial transmission and costs. *Infect Control Hosp Epidemiol* 2007; **28**: 1134–41.
28 Davidson MM, Evans R, Hay AJ. Same-day detection of meticillin-resistant *Staphylococcus aureus* from screening swabs by real-time polymersase chain reaction. *J Hosp Infect* 2006; **63**: 484–5.
29 Clancy M, Gaepler A, Wilson M, Douglas I, Johnson J, Price CS. Active screening in high-risk units is an effective and cost-avoidant method to reduce the rate of methicillin-resistant *Staphylococcus aureus* infection in the hospital. *Infect Control Hosp Epidemiol* 2006; **27**: 1009–17.
30 Gavaldà L, Masuet C, Beltran J *et al*. Comparative cost of selective screening to prevent transmission of methicillin-resistant *Staphylococcus aureus* (MRSA), compared with the attributable costs of MRSA infection. *Infect Control Hosp Epidemiol* 2006; **27**: 1264–6.
31 Schulz M, Nonnenmacher C, Mutters R. Cost-effectiveness of rapid MRSA screening in surgical patients. *Eur J Clin Microbiol Infect Dis* 2009; **28**: 1291–6.