Screening methods for meticillin-resistant *Staphylococcus aureus*

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The UK National External Quality Assessment Service (UK NEQAS) distributes clinically relevant and educational specimens for external quality assessment (EQA). The aim of this report was to assess the suitability of using a freeze-dried specimen format for the EQA of conventional and rapid methods and to review the methods used by participants to screen for meticillin-resistant *Staphylococcus aureus* (MRSA). Of the 714 laboratories that returned a result, 678 reported the presence of MRSA, and results showed a mean of 73 c.f.u. per 25 µl and a median of 50 c.f.u. per 25 µl confirming that the specimen was homogeneous. Four different approaches to MRSA screening were used routinely, including: (i) liquid culture; (ii) direct plating onto conventional media; (iii) direct plating onto chromogenic media; and (iv) rapid methods. A wide variety of methods were used within each of these four categories to screen for MRSA, and many laboratories reported using more than one method. Attempts should be made to determine the most appropriate approach to MRSA screening and to standardize the protocols across the UK.

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INTRODUCTION

Colonization provides a reservoir from which bacteria, such as *Staphylococcus aureus*, can be introduced when host defences are breached and increases the risk for subsequent infection. Individuals with *S. aureus* infections are generally infected with their colonizing strain. Colonization also allows *S. aureus* to be transmitted among individuals in both healthcare and community settings and is a significant contributor to prolonged hospital stay, poor clinical outcomes and increased healthcare costs amongst surgical patients (Gordon & Lowy, 2008).

The transmission of meticillin-resistant *S. aureus* (MRSA) and the risk of MRSA infection (including MRSA bacteraemia) in hospitals and care facilities can be addressed if MRSA carriers are identified and treated. This requires screening of patient populations for MRSA carriage either before or on admission to identify carriers and implement a decolonization regimen (Department of Health, 2006). Evidence from the literature shows that active screening for MRSA and subsequent control measures lead to a reduction in the incidence of MRSA infections. Screening is the only strategy with proven efficacy in high endemic settings (Kluytmans, 2007).

The UK has one of the highest rates of infection and colonization of MRSA within Europe and the need for improved intervention has been highlighted by experts in infection control (Hardy *et al.*, 2007). The Department of Health made a commitment in the 2008/2009 Operating Framework to support National Health Service (NHS) trusts in introducing MRSA screening for all elective patients by the end of March 2009. Universal screening is only mandatory implemented in England, while other guidance is followed in Wales, Scotland and Northern Ireland. Hospital trusts have been requested to review their screening and decolonization policies and implement a decolonization regimen to reduce the risk of infection and the spread of MRSA to vulnerable patients (Department of Health, 2006).

There has been little consistent or definitive advice to the NHS on which patients to screen, how to screen them and when. There is also a high degree of variability in practice in NHS trusts and there is no single recommendation with a strong and incontrovertible evidence base that can be recommended uniformly for all NHS trusts. Currently, the choice is a matter for local assessment (Department of Health, 2006).

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Abbreviations: EQA, external quality assessment; MRSA, meticillin-resistant *Staphylococcus aureus*; NHS, National Health Service; UK NEQAS, UK National External Quality Assessment Service.

Table 1. MRSA screening methods used

Method	Laboratories		
	All (%)	UK	Overseas
Broth			
Brain Heart Infusion	8 (6.5)	0	8
Cooked Meat Medium	4 (3.2)	0	4
In-house	12 (9.7)	0	12
In-house*	14 (11.3)	0	14
Mast*	7 (5.6)	6	1
MRSA-Bouillon*	3 (2.4)	0	3
Nutrient broth with 3–7 % NaCl	34 (27.4)	13	21
Other	10 (8.1)	3	7
Other*	9 (7.3)	1	8
Oxoid Selective Mannitol broth*	12 (9.7)	12	0
Phenol Red Mannitol broth*	3 (2.4)	0	3
Todd–Hewitt broth	5 (4.0)	0	5
Tryptone Soy broth	3 (2.4)	0	3
Total	124		
Solid agar media			
Baird Parker Medium	1 (0.7)	1	0
Blood-based	9 (6.6)	2	7
Blood-based†	3 (2.2)	0	3
Chocolate-based	4 (2.9)	0	4
In-house	11 (8.0)	0	11
In-house†	3 (2.2)	0	3
Mannitol Salt agar	50 (36.5)	6	44
Mueller–Hinton agar	19 (13.9)	1	18
Other	8 (5.8)	0	8
Other†	5 (3.6)	1	4
Oxacillin Resistant Screening agar	24 (17.5)	1	23
Total	137		
Chromogenic media			
CHROMagar	23 (6.3)	2	21
Colorex MRSA (E&O)	4 (1.1)	4	0
In-house	2 (0.5)	0	2
MRSA Ident (Heipha)	8 (2.2)	0	8
MRSA Select (Bio-Rad)	36 (9.9)	12	24
MRSA-ID (bioMérieux)	161 (44.2)	69	92
Other	7 (1.9)	0	7
Oxoid Chromogenic MRSA agar	112 (30.8)	94	18
SA-ID (bioMérieux)	11 (3.0)	0	11
Total	364		
Molecular and immunological methods			
BD GeneOhm MRSA	20 (20.4)	11	9
GenoQuick MRSA (Hain)	5 (5.1)	3	2
GenoType MRSA Direct (Hain)	2 (2.0)	0	2
GenoType Staphylococcus (Hain)	2 (2.0)	0	2
In-house	33 (33.7)	1	32
MASTALEX-MRSA (Mast)	3 (3.1)	2	1
MRSA Screen (Denka Seiken)	2 (2.0)	0	2
Other	6 (6.1)	0	6
Slidex MRSA (bioMérieux)	3 (3.1)	0	3
Xpert MRSA (Cepheid)	22 (22.4)	5	17
Total	98		

*Selective.

†With susceptibility testing.

freeze-dried specimen format for the EQA of conventional and rapid methods and to review the methods used by participants to screen for MRSA.

METHODS

The UK NEQAS for Microbiology General Bacteriology EQA scheme has 742 participating laboratories from 16 EU and 19 non-EU countries. In January 2009, the General Bacteriology scheme, distribution 2395, included a specimen containing MRSA (10^3 c.f.u. ml⁻¹) suitable for detection by direct culture on an MRSA-selective agar, broth enrichment followed by subculture and rapid (molecular and immunological) techniques. The specimen was distributed with a supplementary form asking participants for information on the methods and media that they used for MRSA screening as well as their results from the conventional and rapid methods used.

RESULTS AND DISCUSSION

Of the 742 laboratories participating in the scheme, 714 laboratories returned a result. In total, 678 laboratories reported the presence of MRSA, 26 reported a negative result or MRSA not detected, and 10 (all non-UK laboratories) reported *S. aureus* detected. Of the 26 laboratories that failed to detect MRSA, 10 completed the supplementary form and four stated that they used Oxoid Selective Mannitol broth containing ciprofloxacin (Kelly *et al.*, 2004).

Overall, 12 of the 714 laboratories stated that they used Oxoid Selective Mannitol broth. Ten of the 12 laboratories that used Oxoid Selective Mannitol broth failed to isolate MRSA with this medium as the strain was ciprofloxacin-sensitive. Five of the laboratories that failed to detect MRSA using Oxoid Selective Mannitol broth used alternative methods to isolate the pathogen, for example direct plating onto chromogenic agar. It is unlikely that this level of investigation would be applied to all routine specimens and ciprofloxacin-sensitive MRSA would not be detected using Oxoid Selective Mannitol broth. In the UK, most hospital isolates are ciprofloxacin-resistant but this is not necessarily the case for community-acquired isolates and types prevalent in other countries (Brown *et al.*, 2005).

The results of the questionnaire showed that a wide range of screening methods were used including liquid culture (enrichment and selective) media (29 %; 124/424) and culture, conventional solid agar (32 %; 137/424), chromogenic media (86 %; 364/424) and rapid methods (23 %; 98/424). Nutrient broth was the most commonly used enrichment broth (34/124) but approximately 45 other commercial or in-house broths were reported (Table 1).

Mannitol Salt agar, Mueller–Hinton agar and Oxacillin Resistant Screening agar are the most common conventional solid agars used although again a wide variety were

reported (Table 1). The most common approach was to use chromogenic media. Analysis of a previous UK NEQAS questionnaire sent to participants in 2007 showed MRSA-ID (now known as bioMérieux chromID MRSA) and Chromogenic MRSA agar (Oxoid) were the most popular chromogenic agars and the results of this survey show that this is still the case (Table 1) (Tande *et al.*, 2008; Carson *et al.*, 2009; van Hal *et al.*, 2009).

Participants were requested to reconstitute the freeze-dried specimen in 1 ml broth and apply 'a drop from a pasteur pipette or dipped swab as the inoculum'. Pre-distribution tests performed in-house showed colony counts of between 38 and 50 c.f.u. per 'drop' (a 'drop' is defined here as 25 µl). Participants' results showed a mean of 73 c.f.u. per 25 µl and a median of 50 c.f.u. per 25 µl confirming that the specimen was homogeneous.

Ninety-seven laboratories (21 of which were in the UK) used rapid methods, with Cepheid Xpert MRSA (22) and BD GeneOhm (20) being the most popular commercial methods, although 33 participants reported using an in-house assay (Table 1) (van Hal *et al.*, 2009; Wolk *et al.*, 2009; Svent-Kucina *et al.*, 2009). The UK NEQAS questionnaire in 2007 showed that 32 laboratories (three of which were UK laboratories) were using molecular methods, illustrating that the number of laboratories implementing molecular methods has increased. Seventy-three (17 of which were UK laboratories) of the 97 laboratories who reported that they routinely use a molecular assay reported a result. Sixty-eight of these reported the correct result. The five incorrect results were not associated with the use of any particular assay (four were commercial assays, whilst one was an in-house assay). Therefore, 93% of laboratories using rapid methods reported the correct result, which compares favourably with the percentage of laboratories that reported a correct result using conventional methods (95%).

The data from the supplementary questionnaire demonstrated that four different approaches to MRSA screening were used routinely, including: (i) liquid culture; (ii) direct plating onto conventional media; (iii) direct plating onto chromogenic media; and (iv) rapid methods. Liquid culture was used for detection, selection and enrichment in combination with either conventional media or chromogenic media. A wide variety of methods were used within each of these four categories to screen for MRSA, and many laboratories reported using more than one method.

The UK Department of Health has recommended that hospital trusts should review their policies for MRSA

screening to determine the most appropriate initial approach for their patient population. EQA can assist laboratories in reviewing their screening procedures, deciding which assay to adopt and determine whether a robust screening programme is in place. In addition, many laboratories are implementing molecular assays and EQA can contribute to the development and validation of these novel molecular protocols.

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