Methicillin-resistant *Staphylococcus aureus*: laboratory detection methods in use in the Republic of Ireland and Northern Ireland

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ABSTRACT

There is no universally agreed laboratory protocol for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and hence a variety of approaches are used. As part of an all-island survey of MRSA in the Republic of Ireland (the South) and Northern Ireland (the North), a questionnaire was circulated to 14 participating laboratories in the North and 49 in the South, to determine the methods used to isolate MRSA from clinical specimens, identify *S. aureus* and test for susceptibility to methicillin. Almost two-thirds (64%) of laboratories in the North but only 16% of laboratories in the South use enrichment culture. There is heavy reliance on commercial kits to confirm the identification of *S. aureus* in the South but all laboratories in the North use the staphylocoagulase test. More than 90% of all laboratories use a disc method for susceptibility testing and 71% of laboratories in the North supplement this with the E-test; however, a range of methicillin disk concentrations are in use. There is a need to review current laboratory methods used to detect MRSA, with follow-up audit on their implementation. Additional resources may be needed in some laboratories to comply with revised guidelines, and reference facilities are required to assess new commercially available techniques and to confirm the identification of unusual or difficult strains.

KEY WORDS: Laboratory techniques and procedures.
Methicillin resistance.
Staphylococcus aureus.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an increasingly important nosocomial pathogen. Although most patients with MRSA are colonised, invasive infections such as bacteraemia, endocarditis and pneumonia can occur in susceptible patients. Current methods of control in hospitals include the prudent use of antibiotics and hand washing, and the screening of patients (and occasionally staff) to facilitate prompt isolation of those with MRSA, adequate cleaning of clinical areas, and the eradication of MRSA using topical antimicrobial agents.[1-3]

Critical to any successful control strategy is the early detection of MRSA. Non-sterile sites in which normal flora may be present hamper easy recovery of the organism, while the heterogeneous expression of methicillin complicates detection. A balance has to be achieved between reliable and accurate detection of MRSA and the timeliness of the result in the clinical context. Detection of *mecA*, the gene encoding methicillin-resistance, is now regarded as the 'gold standard' but this test is not available in most diagnostic laboratories.[4,5] Other approaches such as the *in vitro* detection of the *mecA* gene product PBP2a are becoming increasingly available with the advent of commercial kits.[6]

In 1998, the Department of Health and Children in Dublin commissioned a study on the prevalence of MRSA in the Republic of Ireland (the South) following an earlier survey in 1995.[7] The 1999 study was carried out in cooperation with the Northern Ireland MRSA Working Party (the North), and set out to describe the epidemiology of MRSA, hospital infection control procedures, the methods used by laboratories to detect MRSA antibiotic resistance amongst MRSA, and the strains circulating on the island. Full details of the study can be found elsewhere.[8] Here, we summarise the information received on laboratory methods used to detect and confirm the identity of MRSA.

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Materials and methods

Following a pilot study, a questionnaire seeking details of laboratory practice was circulated in January 1999 to all laboratories providing diagnostic microbiology services in the North and in the South. Information was sought on the methods used to isolate MRSA from screening specimens, methods used to identify *S. aureus* and methods used to detect methicillin resistance. Details requested on screening

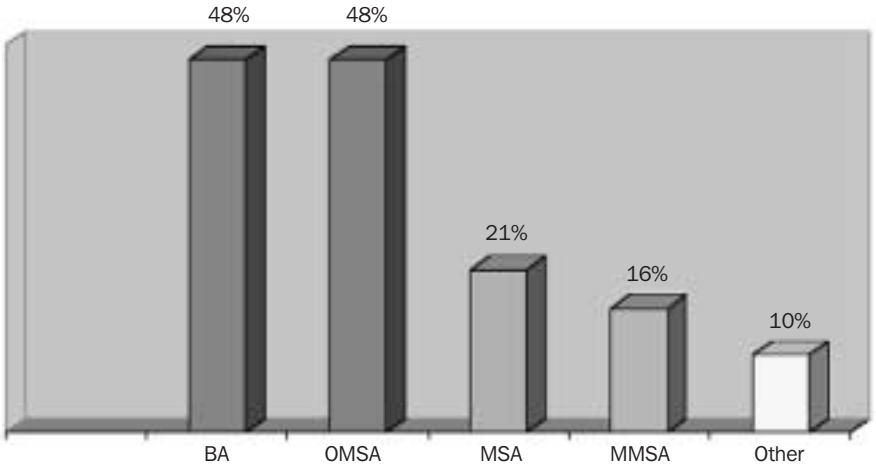

Fig. 1. Laboratories (%) using culture media for direct plating of screening specimens. BA: blood agar, OMSA: oxacillin mannitol salt agar, MSA: mannitol salt agar, MMSA: methicillin mannitol salt agar.

specimens included whether direct plating or enrichment culture (or both) were undertaken, the types of media used, and the temperature and duration of incubation. Participants were asked to specify whether they identified *S. aureus* by standard methods (staphylocoagulase, clumping factor, DNase) and/or by commercial kits. Finally, details of the methods used to detect methicillin resistance (method, antibiotic, disk concentration, media, temperature and duration of incubation) were queried. Data were analysed using Epi Info software.[9]

Results

All 14 laboratories in the North and all 49 laboratories in the South participated, with one laboratory (in the South) providing incomplete data.

Isolation of MRSA from screening specimens

Blood agar and oxacillin mannitol salt agar (OMSA) were the media most commonly used for direct plating (Figure 1). However, 57% of laboratories in the North and 35% of laboratories in the South used more than one medium. Among the laboratories using OMSA, 86% in the North and 70% in the South incubated the plates for 48 h at either 35˚C or 37˚C. The most common antibiotic concentration in OMSA was 4 mg/L (used by 70% of laboratories). Among laboratories ($n=10$) using methicillin mannitol salt agar (MMSA), 30% used a methicillin concentration of 4 mg/L and 30% used a methicillin concentration of 5 mg/L. No laboratory in the North used MMSA.

Table 1. MRSA isolation methods for screening specimens

Culture method	Laboratories ($n=63$)
Direct	46 (73%)
*Enrichment	6 (10%)
*Enrichment and direct plating	11 (17%)

**Number of laboratories using each type of medium – nutrient broth (8), NaCl nutrient broth (3), cooked meat (3), NaCl/cooked meat (3).*

Twenty-seven per cent of laboratories (64% of laboratories in the North and 16% in the South) used an enrichment culture technique, with or without direct plating, for the isolation of MRSA (Table 1). Two-thirds of laboratories in the North used salt enrichment (with either nutrient broth or cooked meat broth), but only 25% of laboratories in the South used salt enrichment.

Identification of S. aureus

A variety of methods were used to confirm the identification of *S. aureus* (Table 2). In the South, 31% of laboratories used a commercial kit as the sole method of identifying *S. aureus*, while 44% used a kit in combination with another method(s). All laboratories in the North used staphylocoagulase, with or without another method for confirmation.

Detection of methicillin resistance

The disc diffusion method was the most frequently used technique (more than 90% of all laboratories). Disc diffusion was supplemented by the E-test in 71% of laboratories in the North but in only 20% of laboratories in the South. Blood agar or diagnostic sensitivity test agar were the media used most commonly with the disc diffusion technique across the island; however, a greater range of other media, such as Iso-Sensitest, Columbia and 5% salt agar, was used in the South.

Seventy-one per cent of all the laboratories surveyed incubated sensitivity plates for 24 h, 5% of laboratories incubated plates for less than 18 h and 7% incubated for more than 24 h. Methicillin was the antibiotic most frequently used (in 96% of laboratories that specified the antibiotic used). Twenty-eight per cent of laboratories used a methicillin disc concentration of 10 mg (18% in the South and 54% in the North). In the South, 25 µg was the methicillin concentration most frequently used (by 62% of laboratories).

Discussion

There is no single agreed method for the detection and confirmation of MRSA. The results from this study indicate that a variety of approaches and methods are used in Ireland. Most guidelines recommend the use of salt enrichment culture because this method increases the

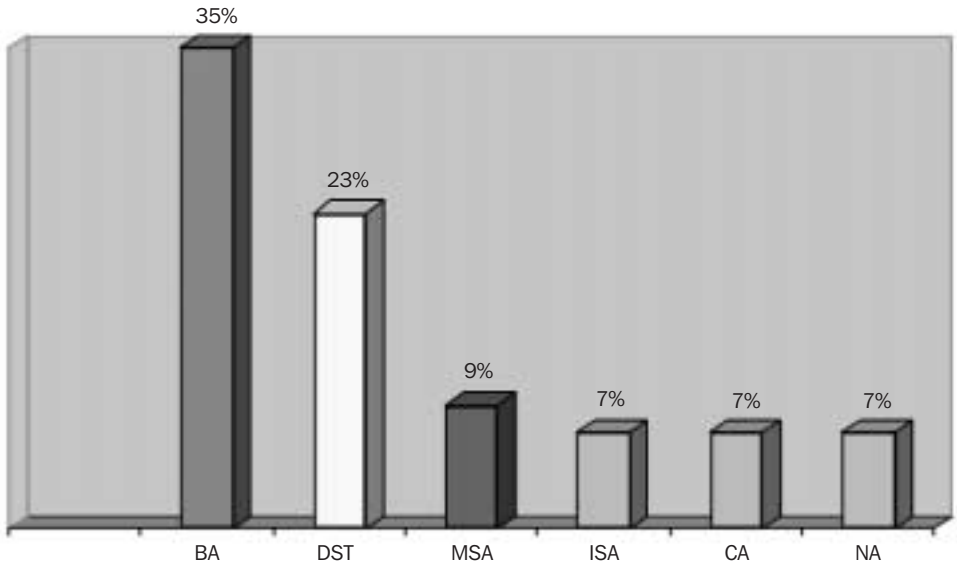

Fig. 2. Laboratories (%) using the following range of culture media with the disc diffusion method to detect methicillin resistance. BA: blood agar, DST: diagnostic sensitivity test medium, MSA: mannitol salt agar, ISA: Iso-Sensitest agar, NA: nutrient agar, CA: Columbia agar.

sensitivity of screening,[3,10] although other considerations such as salt tolerance of local strains and increased turnround time should be taken into account.[11]

The majority of laboratories in the Republic of Ireland employ direct plating and may therefore fail to identify some patients carrying MRSA. Differences in detection methods hamper efforts to compare prevalence rates between different hospitals and between different regions. Similarly, laboratories that incubate mannitol salt agar plates for periods of 24 h only (29% of laboratories in this study) also may fail to detect MRSA.

A number of new media for the rapid detection of MRSA have been developed and look promising; however, studies to evaluate them do not compare direct plating on these media with the results obtained following enrichment culture.[12-14] A study in 1999 that compared several selective media and a number of enrichment culture methods found that enrichment culture was more sensitive than direct plating.[15]

Assessment of the susceptibility of *S. aureus* to methicillin is influenced by the susceptibility test medium used, the disc concentration of antibiotic, and the duration and temperature of incubation. Incorporation of methicillin in culture media and its widespread use for disc diffusion susceptibility testing will cause problems because the manufacture of methicillin has ceased.

Interestingly, laboratories currently using oxacillin in culture media tend to use an oxacillin concentration of 4 mg/L, yet the UK Public Health Laboratory Service recommends 2 mg/L.[10] One study has investigated the effect of replacing methicillin (4 mg/L) with oxacillin (2 mg/L) in a solid selective screening medium.[12] These workers found that the medium containing oxacillin was less sensitive than its methicillin-containing counterpart (recovering only 73% of MRSA isolates).

In the future, the detection of genes unique to *S. aureus* may become the recommended standard for identification. In the meantime, a variety of approaches are in use, including detection of staphylocoagulase and the presence of DNase, or the use of commercial kits. It is worrying that a significant proportion of laboratories in the South rely on kits alone to identify *S. aureus*, as these kits may fail to detect all isolates or give false-positive results.[16-18] Recently available kits (Staphaurex Plus, Pastorex Staph Plus and Slidex Staph Plus) perform better than the earlier ones but false-positive results may be obtained with non-*S. aureus* staphylococcal species.[17] To date, the tube coagulase test remains the preferred method of confirmation, even if the result is not available on the same day. However, a preliminary result can be made available to laboratory users, pending the result of the tube coagulase test.

Most laboratories use some form of disc diffusion method for detecting methicillin resistance, but a variety of media are used. The methodologies recommended in the 1995 MRSA guidelines in the South (25 µg methicillin strip, blood or nutrient agar and overnight incubation at 30˚C) need to be updated.[1] Best correlation between phenotypic and genotypic resistance has been shown to occur with either 1 µg oxacillin or 10 µg methicillin discs.[19,20]

Recent efforts to detect MRSA using a duplex-PCR technique to detect *mecA* and *femB* (one of the so-called factors essential for methicillin resistance) have potential but are likely to remain a research tool until the technique becomes more cost-effective.[21,22] Another method used to detect *mecA* employs cycling probe technology, which has

Table 2. Identification methods used to confirm the identify of *S.aureus*

Method *	Laboratories (*n*=62)
Kit only	15
Kit/staphylcoagulase	10
Kit/DNase	8
Staphylocoagulase only	6
Kit/DNase/staphylcoagulase	5
Staphylocoagulase/clumping factor	4
Other combinations	14

** Many laboratories used more than one method*

the advantage of being quicker and does not require DNA amplification.[23] An alternative approach is the detection of PBP2a (the product of the *mecA* gene), which may be more appropriate for the routine diagnostic laboratory because simple PBP2a detection methods are now available commercially and recent studies have been shown that these are useful, rapid techniques.[6,24,25]

It is clear from the results of this study that clarification is required of the optimal methods for the isolation, identification and antibiotic susceptibility testing of MRSA, with ongoing audit of methods and results. It is likely that this will confirm a continuing need for enrichment culture – the value of which, and the additional time required before results are available, needs to be communicated to users of the laboratory service.

The appearance in some countries of *S. aureus* strains that show reduced susceptibility to glycopeptides such as vancomycin means that it is important not to rely solely on disc susceptibility methods, but to determine minimum inhibitory concentrations (MIC) of vancomycin. Ideally, therefore, MIC should be determined for all MRSAs when isolated from a patient for the first time.

Some of the problems in the laboratory approach to MRSA diagnosis highlighted in this study may be due to inadequate resources or the use of outdated guidelines. However, there is also a need for reference facilities to confirm the identification of unusual strains of MRSA and to assess rapid methods of identification at both genotypic and phenotypic levels. □

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