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Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA)

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These evidence-based guidelines have been produced after a literature review of the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). We have considered the detection of MRSA in screening samples and the detection of reduced susceptibility to glycopeptides in *S. aureus*. Recommendations are given for the identification of *S. aureus* and for suitable methods of susceptibility testing and screening for MRSA and for *S. aureus* with reduced susceptibility to glycopeptides. These guidelines indicate what tests should be used but not when the tests are applicable, as aspects of this are dealt with in guidelines on control of MRSA. There are currently several developments in screening media and molecular methods. It is likely that some of our recommendations will require modification as the new methods become available.

Keywords: MRSA identification, MRSA susceptibility testing, MRSA screening, MRSA molecular methods, GISA, meticillin

Contents

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1. Recommendations

1.1 Identification

1.1.1. Tube coagulase tests or a latex agglutination test should be used for routine identification of *S. aureus*. Other tests are acceptable if they give equivalent or better performance. (Category 1b)

1.1.2. Identification using DNase tests and negative results in slide coagulase tests should be confirmed with a tube coagulase or latex agglutination test. (Category 1b)

1.1.3. In most routine diagnostic laboratories use of current molecular tests for identification of *S. aureus* is unlikely to be justified unless they are used for confirmation of equivocal results or where clinical samples have a high suspicion of MRSA infection, in which case identification is likely to be combined with confirmation of methicillin resistance. (No recommendation)

1.2 Susceptibility testing

1.2.1. A standard recognized method, such as those published by the British Society for Antimicrobial Chemotherapy (BSAC) or the USA National Committee for Clinical Laboratory Standards [NCCLS; now known as Clinical Laboratory Standards Institute (CLSI)], should be used for routine susceptibility testing of *S. aureus.* Other tests are acceptable if they give equivalent or better performance. (Category 1b)

1.2.2. Latex methods detecting penicillin binding protein 2a (PBP 2a) and/or polymerase chain reaction (PCR) methods detecting the *mecA* gene may be used for confirmation of equivocal results. (Category 1b)

1.3 Screening for MRSA

1.3.1. Comparative evaluations of screening media are limited. Baird Parker medium with ciprofloxacin (BPC) has good overall performance for the recovery of ciprofloxacin-resistant MRSA from screening swabs. The growth of most MRSA on this medium within 24 h offers the advantage of early recognition. The use of this medium, nevertheless, is limited to the detection of ciprofloxacin-resistant isolates. Isolates susceptible to this agent will thus be inhibited and an alternative medium without ciprofloxacin is necessary if ciprofloxacin-susceptible isolates are to be detected. (Category 1b). MSA or ORSAB media may be used and early data on newer chromogenic media appear promising, but there is insufficient evidence to recommend any particular medium among these.

1.3.2. Enrichment of screening swabs is more sensitive than direct plating and may be particularly useful for screening in some high-risk groups of patients and in screening for clearance of MRSA carriage. (Category 1b). Comparative evaluations of enrichment media are limited

1.3.3. Molecular methods for processing screening swabs are a potentially valuable development to reduce test time. A number of methods have been developed or are in the process of development, which will require review probably within the next 2 years. It is most important that any method should accurately distinguish between MRSA and mixtures of methicillin-susceptible *S. aureus* with methicillin-resistant coagulase-negative staphylococci present in the sample. Cost-effectiveness data are awaited but we would anticipate a progressive introduction of this technology into clinical practice. (Category 2)

1.4 Glycopeptide resistance

1.4.1. Laboratory detection of glycopeptide resistance in *S. aureus* is problematic. Disc diffusion methods are not able to detect GISA/hGISA and may give equivocal results with VRSA. (Category 1a)
For detection of vancomycin-resistant *S. aureus* (VRSA), isolates should be screened on Brain Heart Infusion (BHI) agar containing vancomycin 6 mg/L. (Category 1b)

1.4.2. For detection of glycopeptide-intermediate *S. aureus* (GISA), isolates should be screened on BHI agar containing vancomycin 6 mg/L or Mueller-Hinton (MH) agar containing vancomycin or teicoplanin 5 mg/L. (Category 1b)

1.4.3. For detection of hetero-GISA (hGISA), isolates should be screened with the macro Etest method. (Category 2)

1.4.4. In order to confirm VRSA and GISA, those isolates that are positive in screening tests should be further tested by an MIC method, using a standard Etest or agar dilution on MH or BHI agar. Isolates with vancomycin or teicoplanin MICs >4 mg/L are likely to be VRSA or GISA and should be sent to a reference laboratory for confirmation. (Category 1b)

1.4.5. In order to confirm hGISA, those isolates that are positive in screening should be tested using a population analysis profile (PAP) method as glycopeptide MICs for hGISA strains may be no different from those that are susceptible. (Category 2)

1.4.6. If patients on glycopeptide treatment fail to respond and *S. aureus* is repeatedly isolated, the isolates should be investigated by the macro Etest method. (Category 2)

1.5 Future research

1.5.1. Comprehensive comparative evaluations of currently available direct plating and enrichment media for screening are required.

1.5.2. Current methods and those developed in the future should be carefully evaluated in the clinical setting for sensitivity and specificity and for their ease of use and cost effectiveness.

1.5.3. Healthcare providers should be aware of new technological approaches to the detection and confirmation of MRSA in clinical samples, and ensure that adequate health technology evaluation programmes are in place.

2. Introduction

2.1 Background

Methicillin-resistant *Staphylococcus aureus* (MRSA) were first reported in 1961[1] and have since become a major nosocomial pathogen worldwide.[2,3] In the UK, the mean incidence of MRSA bacteraemia is about 40% of *S. aureus* bacteraemia. An additional concern is the emergence of vancomycin-intermediate *S. aureus* (VISA) and more recently vancomycin-resistant *S. aureus* (VRSA).[4,5] The reservoir of MRSA is infected and colonized patients,[6] and the major mode of transmission from patient to patient is on the contaminated hands of healthcare workers.[6,7] It is axiomatic that the sooner an MRSA infection is diagnosed, and the susceptibility to antimicrobial agents established, the sooner appropriate therapy and control measures can be initiated. Laboratory diagnosis and susceptibility testing are crucial steps in treating, controlling and preventing MRSA infections.

Guidelines for the control of MRSA infections in the UK have been previously published by a joint Working Party of the British Society for Antimicrobial Chemotherapy (BSAC), and the Hospital Infection Society (HIS) in 1986[8], 1990[9] and together with the Infection Control Nurses Association (ICNA) in 1998.[10] The Department of Health Special Advisory Committee on Antimicrobial Resistance (SACAR) asked the three Societies to revise the guidelines. Unlike the previous reports, which focussed on the prevention and control of MRSA infections, SACAR requested that guidelines should be extended to cover prophylaxis and therapy of MRSA infections and also the laboratory diagnosis and susceptibility testing of MRSA. Members of the Working Party were representatives of the BSAC, HIS and ICNA. This report deals with the laboratory diagnosis and susceptibility testing of MRSA in the UK (guidelines for the prophylaxis and therapy of MRSA infections are due to be published in *JAC* and guidelines for the control and prevention of MRSA in hospitals are due to be published in the *Journal of Hospital Infection*).

2.2 Reference search strategy and evidence grading

On-line literature searches were conducted to December 2004 using MEDLINE and EMBASE and were restricted to publications in English. The subject headings (MeSH headings or Emtree terms) used by MEDLINE or EMBASE indexers respectively have been used. Where no satisfactory MeSH or Emtree heading existed, textword searching was done.

Each recommendation is graded using the US Centers for Diseases Control and Prevention (CDC) system, and is categorized on the basis of existing scientific data, theoretical rationale, applicability and economic impact. The CDC/HICPAC system for categorizing recommendations is as follows:

Category 1a. Strongly recommended for implementation and strongly supported by well-designed experimental, clinical or epidemiological studies.

Category 1b. Strongly recommended for implementation and strongly supported by certain experimental, clinical or epidemiological studies and a strong theoretical rationale.

Category 1c. Required for implementation, as mandated by federal or state regulation or standard. (The UK equivalent is to operate within EU or UK Health & Safety Legislation).

Category 2. Suggested for implementation and supported by suggestive clinical or epidemiological studies or a theoretical rationale.

No recommendation. Unresolved issue. Practices for which insufficient evidence exists or no consensus regarding efficacy exists.

2.3 What is an MRSA?

S. aureus is a Gram-positive coccus where the round cells, approximately 1 μm in diameter, form grape-like (Greek *staphyle*) clusters indicative of the ability to divide in more than one plane. They are capable of both aerobic and anaerobic respiration and most strains ferment mannitol anaerobically. On blood agar they form characteristic golden (Latin *aureum*) or white colonies. They produce catalase, coagulase and an extracellular cell clumping factor, and some strains produce capsules.[11]

Virtually all MRSA produce an additional penicillin-binding protein, PBP2a or PBP2′[12,13] which confers resistance to all currently available β-lactam agents. PBP2a is encoded by the *mecA* gene.[14] Additional genes, which are also found in susceptible isolates, can affect the expression of methicillin resistance in *S. aureus*, resulting in heterogeneity of resistance and making detection of resistance difficult.[15,16]

Methicillin-resistant isolates with alterations to existing PBPs have been described.[16–18] These isolates have been termed 'moderately resistant *S. aureus*' (MODSA). They are not frequently reported, the resistance is low-level and their clinical significance is unclear. Under some test conditions, low-level resistance may also be seen in isolates which produce large amounts of penicillinase (penicillinase hyper-producers).[18,19] These isolates have been referred to as 'borderline oxacillin-resistant *S. aureus*' (BORSAs). However, animal model experiments indicate that their clinical significance is doubtful[20] and there are no reports of failure of treatment with penicillinase-resistant penicillins in infections with such isolates.

Although methicillin is now not used in treatment, it was the first penicillinase-resistant penicillin to be used in the 1960s and was recognized at that time as the most reliable agent for routine susceptibility testing. Hence resistant strains were termed 'methicillin-resistant *S. aureus*' (MRSA). Later use of oxacillin as an alternative to methicillin in susceptibility tests resulted in the term 'oxacillin-resistant *S. aureus*' (ORSA). These designations are used interchangeably in the literature and are synonymous.

3. Identification of *S. aureus*

Speciation of isolates is essential to distinguish *S. aureus* from coagulase-negative staphylococci (CoNS). Various tests can be used to identify *S. aureus*, including production of protein A, cell-bound clumping factor, extracellular coagulase and heat-stable nuclease. In addition, molecular methods have been developed more recently. Comparison of the performance of tests in various studies can be difficult because different strains of *S. aureus* and different species of CoNS have been included and may behave in different ways. Quality control of tests should be by use of positive and negative control strains, or as directed in commercial systems.

3.1 Tube coagulase test

Free (extracellular) coagulase clots plasma in the absence of calcium. The tube coagulase test with rabbit plasma and examination of tubes after incubation for 4 h and 24 h[21,22] is the standard test for routine identification of *S. aureus.* Tests negative at 4 h should be re-examined at 24 h because a small proportion of strains

require longer than 4 h for clot formation. Some other species of staphylococci, including *Staphylococcus schleiferi* and *Staphylococcus intermedius*, may also give positive results in tube coagulase tests but are not common isolates from human infections. In addition, rare strains of *S. aureus* are negative in coagulase tests. For routine testing more rapid tests are now widely used, particularly latex agglutination tests.[22]

3.2 Slide coagulase test

Clumping factor (bound coagulase) differs from free coagulase in that it is cell-bound and requires only fibrinogen.[21] The slide agglutination test for clumping factor is very rapid but up to 15% of *S. aureus* strains are negative,[11] so isolates negative in slide tests should be confirmed with a tube agglutination test. Some less common species of staphylococci, including *S. schleiferi* and *Staphylococcus lugdunensis*, may give positive results in the slide coagulase test. The test is unsuitable for isolates that are not easily emulsified and clumping factor can be obscured by large amounts of capsule.

3.3 Latex agglutination tests

Early versions of commercial latex agglutination tests for *S. aureus* detected protein A and/or clumping factor. These tests had problems with some MRSA which produce little or no clumping factor and protein A.[23] Later formulations of latex tests include protein A and/or clumping factor but also detect various surface antigens, which improved the sensitivity of the tests but at some expense to specificity due to cross-reaction with CoNS.[24–26] In addition, any test including clumping factor may give false-positive results with *S. lugdunensis* and *S. schleiferi*.[22,27]

3.4 DNase and heat-stable nuclease tests

Deoxyribonuclease (DNase) plates can be used to screen isolates but, as various amounts of DNase are produced by CoNS, positives should be confirmed with an additional test. Heat-stable nuclease tests can be used to identify *S. aureus*,[28] although some rare coagulase-negative species can be positive. The metachromatic agar diffusion method for heat-stable nuclease has been particularly used in direct tests on blood cultures[29] but this method is medium dependent.[30] A latex agglutination test based on heat-stable nuclease has also been described.[11]

3.5 Commercial biochemical tests

There are many commercial kits and automated instruments which include identification of *S. aureus*.[31–34] While performance of these tests may be good, they are slower, technically more time-consuming or more expensive than tests such as coagulase and latex agglutination. Hence they are less likely to be used for specific identification of *S. aureus*. In contrast, the Staphychrom II test is a 2 h chromogenic test based on prothrombin and protease inhibitors. In one study a sensitivity of 98.6% and specificity of 100%, better than tube coagulase, were reported.[35]

3.6 Molecular tests

Occasional isolates of *S. aureus* give equivocal results in coagulase or other biochemical tests, and there is a need for confirmation of identity by an alternative method. In addition, the results of susceptibility testing to methicillin/oxacillin may be equivocal and again in this instance further molecular testing is appropriate. Rapid molecular tests for determining susceptibility to methicillin/oxacillin (see section 4.9) can be combined with simultaneous detection of an *S. aureus*-specific target to allow rapid identification of MRSA isolates.

Most molecular methods for identification of *S. aureus* have been PCR based. Early tests required the Southern blotting of amplified products to confirm their identity,[36] but a range of primers designed to amplify species-specific targets have now been developed. Such targets include the nuclease (*nuc*), coagulase (*coa*), protein A (*spa*), *femA* and *femB*, *Sa442*, 16S rRNA and surface-associated fibrinogen-binding protein genes.[37–43] Preliminary investigations with a commercially available real-time PCR kit (Roche Applied Science, Penzberg, Germany), which detects a specific sequence within the internal transcribed spacer (ITS) region of *S. aureus*, have proved successful,[44] and a novel molecular approach utilizing isothermal signal-mediated amplification of mRNA transcribed from the *coa* gene in a colorimetric microwell format has been shown[45] to offer an attractive alternative to PCR in routine laboratories that lack real-time PCR equipment. These, and other alternative molecular methods for combined identification and susceptibility testing (see below), have been developed in recent years for possible use in routine diagnostic laboratories, but few studies have been published regarding their performance in the routine diagnostic situation. However, it is clear that at present, given the additional costs of these tests and the vast numbers and range of clinical samples that arrive in diagnostic laboratories, it is only feasible to identify *S. aureus* by molecular methods for specific confirmation purposes when other methods give equivocal results, or with clinical samples where there is a high suspicion of MRSA infection. Commercial PCR tests provide appropriate controls; for those protocols developed within-laboratory, inclusion of control strains both positive and negative for the target gene(s) is essential, as is a reagent control.

4. Methicillin (oxacillin) susceptibility testing

The literature on methicillin susceptibility testing is extensive, and often conflicting in recommendations regarding the most reliable method for routine use. This is partly because the various studies of phenotypic methods have included different strains, which may differ significantly in heterogeneity,[46] and behave differently under particular test conditions. Also the factors affecting the expression of resistance may interact in different ways, e.g. the effect of changing the NaCl concentration may depend on the basal medium and the temperature of incubation. Optimal conditions for detection of resistance vary among strains, although current UK 'epidemic' types 15 and 16 are less affected than others. No single set of test conditions is suitable for detection of all resistant strains.

In assessing the performance of susceptibility testing methods the MIC determined by a dilution method has traditionally been the reference method; but methicillin MICs are affected by test conditions and some reports of erroneous results in studies of MRSA detection methods may actually be due to failure to detect resistance with the reference MIC tests. MIC methods have now been replaced as the reference method by molecular methods, which detect the *mecA* gene. Disc diffusion methods remain the most widely used in routine clinical laboratories, although some commercial systems for detection of methicillin resistance are available and automated methods are increasingly used.

With phenotypic tests, *in vitro* conditions, such as test agent, temperature and NaCl concentration, have long been known to affect the expression of resistance[47–50] and have been reviewed previously.[51]

Test agent. There is cross-resistance among β-lactam agents with MRSA, although β-lactam agents with activity against MRSA are being developed.[52] Traditionally, methicillin or oxacillin have been tested and results are representative of all β-lactam agents.[53] However, methicillin is no longer being manufactured. Cefoxitin has recently been investigated as an alternative agent for detection of resistance by disc diffusion and all studies indicate that tests are more reliable than those with oxacillin.[54–57] It has been suggested that no special test conditions are needed when testing cefoxitin but the range of conditions tested has been very limited and results with one set of conditions should not be extrapolated to others without investigation to ensure that resistant and susceptible populations are adequately discriminated.

Medium. Older reports indicate that MRSA are more clearly distinguished on Mueller-Hinton (MH) than on Iso-Sensitest, Diagnostic Sensitivity Test (DST) and PDM media.[58,59] Columbia agar has also been reported to be superior to Iso-Sensitest agar.[60,61] Variation in methicillin susceptibility test performance with MH medium from different manufacturers[62,63] and of different batches from the same manufacturer,[64] have been reported. No recent data are available for performance of different media.

Addition of up to 5% NaCl to MH, Columbia and DST media improves detection of resistance,[60,61,65] but some strains are adversely affected by NaCl, particularly with Iso-Sensitest agar.[65] The effect of NaCl appears dependent on the combination of NaCl concentration, medium, incubation temperature, inoculum density and test format. In the United States the NCCLS[66] recommends addition of 2% NaCl to MH media for dilution tests only, while the BSAC recommends 2% NaCl in Columbia agar or MH agar for dilution and disc diffusion methods.[67]

Inoculum. A larger inoculum increases the chances of detecting the minority of cells which appear resistant with strains that are particularly heterogeneous in the expression of resistance. Increasing the inoculum will not, however, always increase the reliability of testing, particularly if other test conditions are favourable for expression of resistance. A heavy inoculum can cause problems on media containing 5% NaCl as with these conditions in dilution tests it can be difficult to read endpoints for susceptible strains. In disc diffusion tests with higher concentrations of NaCl, a heavy inoculum may also lead to increased false-resistant reports, particularly with oxacillin.[68] Hence the inoculum used must be appropriate for the other test conditions and should be standardized in density.

Incubation. Detection of resistance is generally more reliable at lower incubation temperatures.[49,58,69,70] Rarely, strains grow poorly at 30°C, particularly on media with 5% NaCl, and resistance may be missed. Resistant sub-populations of some heterogeneous strains grow slowly and, depending on other test conditions, extension of incubation to 48 h may improve reliability of detection. BSAC and NCCLS methods require that tests should be incubated for 24 h rather than 16–20 h used for other tests.[66,67]

Reading tests. In standardized disc diffusion tests with methicillin or oxacillin,[66,67] most strains show no zone of inhibition. With some strains resistance is seen as reduced zone sizes, colonies varying in size and number within zones of inhibition, colonies reducing in size towards the disc, or concentric rings of inhibition and growth around discs. In agar dilution MIC tests trailing endpoints are not uncommon, making discrimination of endpoints difficult.

In all methods the use of control strains is required to ensure that the method is performing correctly, and both BSAC and NCCLS standardized methods include recommendations for susceptible and resistant control strains.[66,67] In addition, participation in an external quality assessment scheme will provide an independent assessment of performance.

4.1 Dilution methods

Agar dilution. Tests on MH or Columbia agars with 2% NaCl and an inoculum of 10^4 cfu/mL will distinguish most resistant from susceptible strains.[66,71] With the BSAC method either medium may be used;[51,67] with the NCCLS method only MH with 2% NaCl is permitted.[66] Both methods require incubation for 24 h. The BSAC requires incubation at 30°C, while the NCCLS requires 33–35°C. In both methods an oxacillin MIC of ≤ 2 mg/L indicates that the strain is susceptible and >2 mg/L resistant. In the BSAC method a methicillin MIC of ≤4 mg/L indicates that the strain is susceptible and >4 mg/L resistant, while NCCLS breakpoints are one dilution higher. Cefoxitin MIC breakpoints are yet to be designated.

Broth microdilution. The NCCLS method, which requires the use of MH broth with 2% NaCl, an inoculum of 5×10^5 cfu/mL and incubation at 33–35°C for 24 h, is the only defined method in general use.[66]

4.2 Etest method

The Etest method (AB Biodisk, Solna, Sweden) gives an MIC result and is affected by test conditions in a similar way to other MIC and diffusion methods. The test conditions recommended by the manufacturer are based on providing results comparable with NCCLS methods and include MH agar with 2% NaCl, an inoculum density equivalent to 0.5–1.0 McFarland standards, application of inoculum with a swab and incubation at 35°C for 24 h. Evaluations comparing the Etest with dilution MIC and molecular methods have generally found good essential agreement.[68,72–74] The Etest has an advantage over other MIC methods in that it is as easy to set up as a disc diffusion test.

4.3 Breakpoint methods

Breakpoint methods include both agar and broth methods and are essentially similar to dilution MIC methods but test only the breakpoint concentration (2 mg/L oxacillin, 4 mg/L methicillin).

4.4 Agar screening method

This method has been recommended for screening colonies isolated on routine media and for confirmation of suspect resistance seen in disc diffusion tests. The method recommended by the NCCLS[66] requires suspending the test organism to the density of a 0.5 McFarland standard and inoculating MH agar containing 4% NaCl and 6 mg/L oxacillin with a spot or a streak of the organism. Plates are incubated at 35°C or less for 24 h and any growth other than a single colony is indicative of resistance.

4.5 Disc diffusion

Many different combinations of conditions have been recommended for disc diffusion. Standardized methods have, however, been defined by the BSAC[51,67] and the NCCLS.[75,76]

The recent development of cefoxitin disc diffusion tests is likely to alter the recommendations for these methods as studies all suggest that tests with cefoxitin are more reliable than those with oxacillin.[54–57] It is suggested that no special medium or incubation temperature is required with cefoxitin,[54,55] although some effect of temperature has been reported,[57] there may be medium effects and the effects of inoculum have not been reported.

Resistance of MRSA isolates that are *mecA*-positive but are highly heterogeneous in expression of resistance can be very difficult to distinguish from low-level resistance in 'BORSA' and 'MODSA' strains. In disc diffusion tests, hyper-producers of penicillinase may show small methicillin or oxacillin zones of inhibition, whereas most true methicillin-/oxacillin-resistant isolates give no zone. Resistance mediated by *mecA* may be confirmed by PCR or latex methods. Some hyper-producers of penicillinase give no zone, particularly with oxacillin, and will therefore be falsely reported as MRSA. Tests with cefoxitin do not appear to be affected to the same extent as oxacillin by hyper-production of penicillinase.

4.6 Latex agglutination

A rapid (10 min for a single test) slide latex agglutination test based on detection of PBP2a[77] is commercially available as a kit from several suppliers. The method involves extraction of PBP2a from suspensions of colonies and detection by agglutination with latex particles coated with monoclonal antibodies to PBP2a. The test is very sensitive and specific with *S. aureus*,[78–86] but may not be reliable for colonies grown on media containing NaCl.[87] The method requires no special equipment and is suitable for confirmation of resistance or equivocal tests in routine clinical laboratories. Isolates producing small amounts of PBP2a may give weak agglutination reactions or agglutinate slowly. Reactions tend to be stronger if PBPa production is induced by growth in the presence of a penicillin. Rare isolates may give negative reactions.

4.7 Automated methods

Automated systems including Vitek/Vitek2 (bioMérieux), Phoenix (Becton Dickinson) and Microscan (Dade Behring) include tests for methicillin/oxacillin susceptibility and are generally reported to be reliable for *S. aureus* although a small number of incorrect results, mostly false resistance, have been reported.[32,34,88–90]

4.8 Quenching fluorescence method

With the Crystal MRSA method (Becton Dickinson) inhibition of growth of an isolate by oxacillin is indicated by the quenching of fluorescence of an oxygen-sensitive fluorescent indicator by oxygen remaining in the broth. The method is reasonably reliable but requires several hours of incubation.[80,84,86,91,92]

4.9 Molecular methods

The fact that high-level resistance to penicillinase-resistant penicillins is generally related to the presence of the *mecA* gene means that a genotypic method for the detection of *mecA* allows rapid and unambiguous characterization of this resistance mechanism. The earliest molecular methods for the detection of *mecA* relied on either radiolabelled or digoxigenin (DIG)-labelled DNA probes.[93] The non-radioactive DIG-labelled probe performed as well as the radioactive label, enabling the safer utilization of the test system in a diagnostic laboratory, but even when used in either a dot blot or colony blot format, DNA probing involves a number of time-consuming manipulations resulting in delayed reporting. These early probe studies also highlighted occasional discrepancies, in that borderline-resistant *S. aureus* isolates with a methicillin MIC of 8 mg/L were sometimes probe-negative, but produced large amounts of β-lactamase, which accounted for the elevated methicillin MIC.[94]

More recently, PCR-based methods have been used routinely by reference laboratories as their standard method for detecting the *mecA* gene.[95] Occasional susceptible strains carrying a non-functional or non-expressed *mecA*, will also be detected, but the presence of *mecA* is generally considered to indicate a potential for resistance and is used as a marker to identify MRSA. Borderline resistance, which is not mediated by *mecA*, will not be detected and although they are uncommon such discrepancies have been highlighted in several studies.[96,97] Discrepant results can also arise because of a locally present strain which may lack the target sequence. It is important therefore that thorough local evaluation is made in different geographical regions. This comment also applies to molecular screening methods (section 5.2).

Generally speaking, MRSA PCR assays that use a single amplification step are both robust and simple to perform. However, simple assays of this type are vulnerable to the presence of inhibitors, which will lead to a false-negative result, and the addition of a second set of primers to amplify a gene which is always present within staphylococci has been a very common control method. Primers directed against the *nuc*, *coa* and *gyrA* genes have been used for this purpose.[42,98,99] An alternative internal control involves the amplification of *S. aureus*-specific 16S rRNA.[100] This basic principle of the development of assays was applied to a new sensitive and specific molecular assay which has recently been described for the direct detection of MRSA (see section 5.2).[101]

Commercial kits are available that successfully identify the *mecA* gene in organisms previously identified as *S. aureus*,[102] but these generally work only with purified cultures or enrichment screening broths[44,45] (see section 5.2).

4.9.1 Direct identification of MRSA in blood cultures. In most clinical microbiology laboratories, positive blood cultures identified by automated systems are first examined microscopically for Gram-positive cocci in clusters (GPCC), followed by conventional culture, identification and susceptibility tests to detect the presence of MRSA. A number of studies have examined the use of molecular methods for direct detection of MRSA in blood cultures positive by microscopy for GPCC in order to facilitate rapid diagnosis of MRSA and enable appropriate therapeutic decisions to be made in a timely manner. Such methods have included gel-based and real-time PCR,[103–109] DNA probes[110,111] and peptide nucleic acid probes[112] but usually require specialized equipment and expertise. A relatively simple commercial kit, suitable for use in a diagnostic laboratory, based on a colorimetric gene probe hybridization assay for staphylococcus-specific 16S rRNA, *mecA* and *nuc* gene sequences in a microwell strip format (EVIGENE kit; Statens Serum Institut, Copenhagen, Denmark), has been shown to identify definitively MRSA in positive blood cultures within 7 h, without a requirement for conventional culture or any of the cross-contamination drawbacks (e.g. potential for cross-contamination of amplicons) associated with PCR.[113]

4.9.2 Identification of MRSA in endotracheal aspirates and other clinical samples. Rapid and specific detection of MRSA colonization of the upper and lower respiratory tract is of particular importance for critically ill mechanically-ventilated patients in intensive care units. A 6 h multiplex PCR procedure (targeting the *femA* and *mecA* genes) has been used successfully to identify MRSA in endotracheal aspirates from mechanically-ventilated patients.[114] Interestingly, the data suggested that molecular detection of MRSA was particularly valuable for samples co-infected by fast-growing Gram-negative bacteria, such as *Pseudomonas aeruginosa*, which are a potential cause of overgrowth and false-negative results using standard cultural methods. A nested multiplex PCR detecting *mecA* and the gene encoding TSST-1 and requiring a single 1 mL blood sample, was described by Kitagawa *et al*.[104] This assay gave a result in 4 h and was applied to a total of 35 patients with pyrexia or watery diarrhoea following major gastro-intestinal surgery. The PCR gave positive products from both sets of primers in 12 patients from whom MRSA was also cultured; none of the patients or healthy volunteers gave a positive result. This illustrates the advantage that PCR-based methods can have over conventional culture, in enabling a rapid intervention in a difficult clinical situation.

5. Detection of MRSA in screening samples

5.1 Conventional approaches

Numerous publications outline methods that include solid agar media, with or without prior broth enrichment, for the detection of MRSA from screening samples. Enrichment media are used to enhance the detection of MRSA by overnight incubation before plating on solid agar. However, comparative studies of currently available media assessed with clinical specimens are very limited.

5.1.1 Solid agar media. Most media contain an indicator to distinguish *S. aureus*, inhibitory substances to aid the selection of *S. aureus* from other organisms and methicillin, oxacillin or, more recently, cefoxitin to select methicillin-resistant strains. Inhibitory substances have included NaCl, ciprofloxacin, polymyxin B, aztreonam, tellurite and desferrioxamine.[115–121] Most screening media utilize an indicator system comprising a carbohydrate, most commonly mannitol, and a pH indicator, traditionally phenol red, to highlight potential MRSA colonies. More recently, chromogenic indicators have been used. The time and temperature of incubation used vary among the studies. Comparative studies undertaken with pure cultures of organisms can be useful in indicating the selective nature of media and in testing whether different strains will grow, although many such studies do not address the latter. However, studies with pure cultures are not a substitute for evaluations with clinical specimens. Details of a range of media and reported sensitivity and specificity for studies with clinical specimens are summarized in Table 1.

Care must be taken in interpreting the data on sensitivity and specificity of screening media in comparative studies. Calculations of performance are based on the assumption that 100% MRSA are isolated on the combination of media included in the study. Hence, the reported performance of any medium will depend on the comparators, and a medium that performs well in one study might appear less effective in another. A single strain may be prevalent locally and this may bias the results of comparative studies, e.g. a selective medium containing ciprofloxacin may perform well in a location where prevalent strains are ciprofloxacin resistant but poorly in locations where ciprofloxacin-susceptible strains are more common. There are also differences in the design of studies, with some preliminary studies including only pure isolates, some plating the swabs on several media in succession (some indicating that the order of inoculation is randomized) and others suspending organisms from the swab in liquid medium followed by inoculation of fixed amounts of suspension on different media (giving similar inoculum on different plates but introducing a change in method from that used in routine tests).

Mannitol salt agar (MSA) or variations of this medium have been extensively used as the primary screening medium for MRSA. The reported sensitivity of these media has varied widely (Table 1). The addition of lipovitellin to detect lipase production may markedly increase the sensitivity of MSA.[115]

In studies of oxacillin resistance screening agar ORSAB the sensitivity has varied (Table 1). While specificity was high in some studies,[118,121,122] others reported that 26-47% of blue colonies (typical of MRSA) growing on ORSAB may be other organisms, particularly *S. epidermidis*.[120,123]

Three studies from the same laboratory[116,119,124] have found Baird Parker medium with ciprofloxacin (BPC) to have high sensitivity and good specificity. Tellurite reduction, proteolysis and production of lipase are used to aid the identification of *S. aureus* in BPC medium and permitted recognition of presumptive MRSA isolates after 24 h most often on BPC. In another study[125] the sensitivity of BPC and several other direct plating media was markedly lower than comparator enrichment methods.

The use of desferrioxamine together with oxacillin, tellurite and egg yolk in a mannitol salt agar base (DOTEMSA) has been compared with BPC.[116] DOTEMSA had the advantage of being more inhibitory to coagulase-negative staphylococci. DOTEMSA and BPC had high sensitivity (89%) after 48 h, but DOTEMSA had a sensitivity of only 51% at 24 h compared with 81% for BPC. A further study[117] utilizing desferrioxamine together with amphotericin B, polymyxin B and oxacillin in a Columbia agar base (CODAP) showed good inhibition of pure cultures of non-MRSA isolates but had low sensitivity for MRSA.

The recently developed chromogenic agars for identifying *S. aureus* have been utilized for the detection of MRSA. Chromogenic agars with various selective agents had variable sensitivity and specificity in screening for MRSA when compared with some of the more traditional media,[119,121,122] but performance was good when cefoxitin was used as a selective agent.[121] There are several recently marketed chromogenic media containing cefoxitin (e.g. MRSA Select, Bio-Rad; CHROMagar MRSA, BioConnections; MRSA ID, BioMerieux) and the manufacturers claim good performance with a high proportion of positives detected after incubation for 24 h. Independent comparative studies are, however, urgently needed.

There is no single medium that will recover all MRSA strains. A large number of variations have been used to greater or lesser effect. The complicated formulations of some make them expensive, less cost effective and impractical for routine use in many laboratories unless media are available commercially as pre-poured plates. The limited published evidence suggests that BPC is an effective medium for recovery of ciprofloxacin-resistant MRSA from screening swabs if a high proportion of isolates are ciprofloxacin resistant. The growth of most MRSA on this medium within 24 h offers the advantage of early recognition of MRSA, and rapid latex tests can be performed without interference from

Table 1. Solid agar selective media used for screening for MRSA in screening swabs

Ref.	Medium	Abbreviation	Selective agents[a]	Indicator system	Total MRSA isolates/no. of samples	Comparator media (enrichment media)	Sensitivity (%) after incubation for		Specificity (%) after incubation for	
							18–24 h	42–48 h	18–24 h	42–48 h
124	Mannitol Salt Agar Methicillin	MSAM	NaCl 4 mg/L methicillin	mannitol/phenol red	134/402	Mannitol Salt Agar BPC (Tryptone-T broth + 6% NaCl)	66	84	NA	NA
119	Mannitol Salt Agar Oxacillin	MSAO	7% NaCl 2 mg/L oxacillin	mannitol/phenol red	191/719	BPC Chromogenic MMSAO HMO	47	61	NA	NA
116	Mannitol Salt Agar Oxacillin	MSAO	7% NaCl 2 mg/L oxacillin	mannitol/phenol red	184/540	BPC DOTEMSA	42	60	NA	NA
118	Mannitol Salt Agar Oxacillin	MSAO	7.5% NaCl oxacillin disc (1 µg)	mannitol/phenol red	236/579	ORSAB	53.8	65.7	92.7	91.8
115	Mannitol Salt Agar Oxacillin	MSAO	NaCl oxacillin	mannitol/phenol red	43/816	Mannitol Salt Agar MHAO MSALO (Tryptone soya broth + 6% NaCl)	NA	65	NA	89
130	Mannitol Salt Agar Oxacillin	MSAO	NaCl 4 mg/L oxacillin	mannitol/phenol red	43/1124	BPC CIP ATM (Mannitol broth + 2.5% NaCl) (Nutrient broth + 20 mg/L aztreonam)	5	NA	NA	NA
119	Mannitol Salt Agar Oxacillin	MMSAO	3% NaCl 2 mg/L oxacillin	mannitol/phenol red	191/719	MSAO MMSAO BPC CHRAC	23	46	NA	NA
115	Mannitol Salt Agar Lipovitellin Oxacillin	MSALO	NaCl oxacillin disc	mannitol phenol red lipovitellin	43/816	MSA MSAO MHAO (Tryptone soya broth + 6% NaCl)	NA	90	NA	93
118	Oxacillin Resistance Screening Agar	ORSAB	5.5% NaCl 2 mg/L oxacillin 50 000 U/L polymyxin B	mannitol/aniline blue	236/579	MSAO	50.8	62.8	95.6	94.5

Table 1. (*continued*)

Ref.	Medium	Abbreviation	Selective agents[a]	Indicator system	Total MRSA isolates/no. of samples	Comparator media (enrichment media)	Sensitivity (%) after incubation for		Specificity (%) after incubation for	
							18–24 h	42–48 h	18–24 h	42–48 h
120	Oxacillin Resistance Screening Agar	ORSAB	5.5% NaCl 2 mg/L oxacillin 100 000 U/L polymyxin B	mannitol/aniline blue	196/1427	(Mueller-Hinton broth + 4.5% NaCl + 6 mg/L oxacillin)	NA	74	NA	NA
121	Oxacillin Resistance Screening Agar	ORSAB	5.5% NaCl 2 mg/L oxacillin 50 000 U/L polymyxin B	mannitol/aniline blue	85/747	MRSA ID CHROMagar MRSA (Selective mannitol broth)	62	78	97.9	93.1
119	Baird Parker medium with ciprofloxacin	BPC	8 mg/L ciprofloxacin tellurite lithium chloride	egg yolk tellurite	191/719	MSAO MMSAO CHRAC HMO	80	94	NA	NA
116	Baird Parker medium with ciprofloxacin	BPC	8 mg/L ciprofloxacin tellurite lithium chloride	egg yolk tellurite	184/540	MSAO DOTSEMA	81	89	NA	NA
124	Baird Parker medium with ciprofloxacin	BPC	8 mg/L ciprofloxacin tellurite lithium chloride	egg yolk tellurite	134/402	Mannitol Salt Agar MSAM (Tryptone-T broth + 6% NaCl)	73	86	NA	NA
130	Baird Parker medium with ciprofloxacin	BPC	8 mg/L ciprofloxacin tellurite lithium chloride	egg yolk tellurite	43/1124	MSAO CIP ATM (Mannitol broth + 2.5% NaCl) (Nutrient broth + 20 mg/L aztreonam)	61	NA	NA	NA
130	Columbia blood agar with ciprofloxacin	CIP	8 mg/L ciprofloxacin	none	43/1124	MSAO BPC ATM (Mannitol broth + 2.5% NaCl) (Nutrient broth + 20 mg/L aztreonam)	51	NA	NA	NA
119	CHROMagar *Staph.aureus* with ciprofloxacin	CHRAC	8 mg/L ciprofloxacin	chromogen	191/719	BPC MSA MMSAO HMO	61	70	NA	NA

Table 1. (*continued*)

Ref.	Medium	Abbreviation	Selective agents[a]	Indicator system	Total MRSA isolates/no. of samples	Comparator media (enrichment media)	Sensitivity (%) after incubation for		Specificity (%) after incubation for	
							18–24 h	42–48 h	18–24 h	42–48 h
121	S aureus ID with cefoxitin	MRSA ID	4 mg/L cefoxitin	chromogen	85/747	ORSAB CHROMagar MRSA (Selective mannitol broth)	80	89	99.5	85.6
121	CHROMagar MRSA	none	not stated	chromogen	85/747	ORSAB MRSA ID (Selective mannitol broth)	59	72	99.3	92.1
115	Mueller-Hinton Oxacillin	MHAO	oxacillin	none	43/816	MSA MSAO MSALO (Tryptone soya broth + 6% NaCl)	NA	65	NA	88
116	Desferrioxamine Oxacillin Tellurite Mannitol Salt Agar	DOTEMSA	7.0% NaCl 2 mg/L oxacillin 100 mg/L desferrioxamine 30 mg/L potassium tellurite	egg yolk	184/540	BPC MSA	51	89	NA	NA
119	Halifax MRSA medium with oxacillin	HMO	2 mg/L oxacillin 10 mg/L aztreonam 75 000 U/L polymyxin B 100 000 IU/L nystatin	DNase	191/719	BPC MSA MMSAO CHRAC	25	56	NA	NA

NA, not available.
[a]Concentrations of selective agents given where available.

this medium. Not all MRSA strains, however, are resistant to ciprofloxacin and ciprofloxacin-susceptible strains will not be detected. Currently in the UK most hospital isolates are ciprofloxacin resistant (94.6% of blood culture isolates in a bacteraemia resistance surveillance study in 2001–2002).[126] This may not be the case in other settings, such as the community,[127] and may differ among hospitals, in which case the use of a medium without ciprofloxacin, such as ORSAB, MSA or a chromogenic medium, is required. There is currently insufficient evidence to recommend any one of these above the others.

5.1.2 Enrichment. Enrichment broths have commonly been used to increase the sensitivity of screening by allowing small numbers of MRSA to grow during overnight incubation before subculture on a screening agar medium. The improved sensitivity of enrichment may be desirable, particularly with high-risk groups of patients, and in screening for clearance of MRSA carriage. Enrichment broths have also been widely used as 'multibroths', where multiple swabs from the same patient are included in a single enrichment broth, providing substantial savings in media and time compared with processing swabs individually.

Enrichment media generally contain additional NaCl and may also contain methicillin, oxacillin or, more recently, cefoxitin to add selectivity. There is some evidence that some strains of EMRSA-16 may not tolerate the higher NaCl concentrations used in some screening media[128] and this should be taken into consideration when using enrichment broths containing NaCl. Broth bases used have included nutrient broth, tryptone soya broth, MH broth, BHI broth and Robertson's cooked meat medium. Most studies report an increase in isolation rate when an enrichment broth is used before plating.[115,118–120,125,129,130]

Indicator-enrichment media (broth containing carbohydrate, indicator and antibiotics) may be useful in providing an indication of the presence of MRSA after overnight incubation and, if the medium has very good sensitivity, will reduce the number of enrichment broths requiring subculture. The use of an indicator-enrichment medium has shown a higher sensitivity than BPC and MSA agar media for MRSA but a lower specificity.[130] On a similar theme, one study investigated the use of a semi-solid MSA as a bedside screening test.[131] This medium, which included 10% NaCl and cloxacillin as selective agents, gave a high sensitivity and specificity for nose, oropharyngeal and rectal swabs, but comparison was limited to direct plating on media without specific selection for MRSA. Other commercially produced indicator-enrichment media have been marketed recently but there are currently no published data on performance.

The use of enrichment will increase the isolation rate of MRSA but, in the absence of a reliable indicator system, will increase the length of time to detection and the cost of processing individual screening swabs. Conversely, if multiple swabs are cultured in single enrichment broths the costs are reduced compared with plating individual swabs.

5.2 Molecular methods

A number of different molecular methods for the rapid detection of MRSA in screening samples have been described in the last 10 years. The majority of these have relied on multiplexed PCR primers to detect genes which identify strains as *S. aureus* (*nuc* and *fem* are frequently used) and *mecA*.[132] However, the methods are generally only applicable to identification of purified cultures of staphylococci, and therefore they should not be used directly on samples. Commercial test kits include appropriate controls; locally developed assays should use control material which is both positive and negative as described in section 3.6. In addition, these earlier assays lack sensitivity when used directly on clinical material and have not proved useful in screening nose and other swabs due to the presence of CoNS carrying the methicillin resistance gene *(mecA)* in the vast majority of hospital patients. Because the *mec* gene present in CoNS is structurally identical or very similar to the *mecA* gene found in MRSA, the presence of small numbers of clinically insignificant methicillin-resistant CoNS can give a false result in all of these earlier PCR assays. This is reported to be a problem with the multiplex microwell plate PCR system recently marketed by Biologische Analysensytem GmbH as it does not link the presence of *mecA* to an *S. aureus*-specific DNA sequence. Although the occurrence in blood cultures of mixed infections is relatively low (2–3%), the false positive rate is acknowledged as a problem by some.[113]

A variety of strategies have been attempted to reduce false positives. These include adding oxacillin to broth enrichments when these have been carried out prior to PCR,[41,133] and the use of assays to detect the *nuc* gene (which is present only in *S. aureus)* in broth pre-enrichment samples prior to going on to use a specific *mecA* primer for those samples which are *nuc* positive.[134] All these assays could still be confounded by the presence of *mecA*-positive CoNS on the screening swab, although when artificial mixed inocula were used, such false positives were not seen.[133]

Only a small number of assays have so far been developed for use directly on screening swabs, although this application represents the most exciting in terms of control of MRSA. In addition to multiplex PCR,[41,133] an interesting isothermal signal amplification method (CytAMP®—British BioCell International, Cardiff, UK) has been recently applied to the detection of MRSA from patient screening swabs.[45] This assay targets the coagulase and *mecA* genes, thereby simultaneously identifying *S. aureus* and methicillin resistance. An evaluation of this method in direct comparison with conventional multiplex PCR and culture using 100 enrichment broths which had contained MRSA screening swabs found that the isothermal amplification method and culture had similar sensitivities and specificities relative to those of PCR, but that the molecular approach enabled definitive results to be reported within 3 h following overnight enrichment in broth containing oxacillin.

All systems that rely on broth enrichment (incorporating oxacillin) introduce a delay to the production of results. Broth enrichment systems are also unreliable in specimens containing both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant CoNS (MRCoNS). In order to increase the speed of diagnosis, real-time PCR has recently been applied to the detection of MRSA.[43] However, if directly used on specimens rather than on cultured bacteria, this assay is again incapable of differentiating between mixed cultures of MSSA and MRCoNS. More recently, Fang and Hedin[134] have used a system involving enrichment of samples and subsequent use of real-time PCR to pre-screen for the presence of the *nuc* gene, thus indicating the presence of the *S. aureus*; the pre-screen was followed by a further PCR to detect *mecA* and *femB* genes. However, this assay is only capable of providing results following overnight incubation and, therefore, cannot be considered as rapid.[134] An approach to counter this problem in screening samples has been described recently.[135] An immunomagnetic enrichment step is first used to selectively capture *S. aureus* using a monoclonal antibody raised against

S. aureus protein A. The captured DNA is then released and used in a quantitative branch chain assay. In this preliminary report the assay appears to offer good sensitivity although the protocol takes 6 h.[135]

The only currently commercially available amplification assay which may reliably detect *mecA* in *S. aureus* is the IDI-MRSA™ assay (Infectio diagnostic, Ste-Foy, Quebec),[101] which amplifies only part of the SCC*mec* element from *S. aureus* using primers to all the different right-hand flanking sequences in SCC*mec* and a single primer in *orfX*. The assay is based on a real-time PCR format and can be completed in 2 h from sample acquisition. A preliminary evaluation indicated 100% sensitivity and 97.9% specificity compared with primary isolation and broth enrichment techniques for the detection of MRSA.[136] A subsequent evaluation with 1657 isolates of MRSA[101] found a sensitivity of 98.7% and a specificity of 98.4%. This assay has also been tested against nasal swabs from 288 patients processed with a combined direct plating on MSA agar and another swab enriched in TSB with salt followed by screening of all presumptive *S. aureus* on 6% oxacillin screening agar.[137] Values of 91.7% sensitivity, 93.5% specificity, 82.5% positive predictive value and 91.7% negative predictive value were obtained compared with the combined results of culture methods. Six samples which were positive by at least one of the culture methods were negative by PCR. Four isolates from the six samples were available for further testing and three were shown to lack the *mecA* gene. The *mec* region can undergo deletion *in vivo*[138] and there has been a recent report in which the deletion of the SCC*mec* region has not been complete and some elements have been found to remain within the genome.[139] Should this sequence to the far right of the SCC*mec* element remain, there is a small chance that false-positive results could be obtained with the IDI assay. Apart from speed, another advantage of using direct PCR tests targeted at *mecA* detection in an *S. aureus* background is that they can detect markedly heteroresistant strains, which may be difficult to detect in phenotypic tests, as recently recognized in the Netherlands.[140] This is an area that is developing fast and we anticipate will require reviewing again within the next 2 years.

6. Reduced susceptibility to glycopeptides in *S. aureus*

Owing to the increasing numbers of infections caused by multi-resistant MRSA, the glycopeptides vancomycin and teicoplanin have become the drugs of choice for treatment of staphylococcal nosocomial infections.[141,142] Acquired resistance to vancomycin and teicoplanin was first reported in enterococci in 1989[143] and although vancomycin-resistant enterococci (VRE) and MRSA share a similar ecological niche and transfer of the resistance gene between these species was demonstrated *in vitro*[144] the first case of vancomycin-resistant MRSA (VRSA) was not reported until 2002.[145] However, in 1997 a clinical isolate of MRSA with reduced susceptibility to vancomycin was reported from Japan.[146] These isolates, commonly designated vancomycin-intermediate *S. aureus* (VISA), have caused considerable debate amongst microbiologists both as to their clinical significance and their detection in the laboratory.[147–149]

6.1 Terminology

There is some confusion regarding the definition and nomenclature of reduced susceptibility to glycopeptides in *S. aureus* which is compounded by differences in breakpoints. The first *S. aureus* with reduced susceptibility to vancomycin (MIC 8 mg/L)[146] was designated vancomycin-resistant (VRSA) by BSAC breakpoints (Table 2). The first USA report referred to these isolates as vancomycin-intermediate (VISA) according to NCCLS breakpoints (Table 2).[150] The broader generic term glycopeptide-intermediate *S. aureus* (GISA) was introduced as these isolates also had reduced susceptibility to teicoplanin,[151] although some have reduced susceptibility to teicoplanin only (TISA).[152,153] In addition, two resistance phenotypes, heterogeneous and homogeneous, have been recognized. Isolates with heterogeneous intermediate resistance (hGISA or hetero-GISA) appear susceptible to glycopeptides (vancomycin MIC ≤ 4 mg/L) but contain subpopulations of cells at frequencies of $>10^{-6}$ that exhibit reduced susceptibility (vancomycin MIC 8–16 mg/L).[154,155] The term *S. aureus* with reduced vancomycin susceptibility (SA-RVS) has been suggested for isolates with vancomycin MICs of ≥ 4 mg/L, which may more closely resemble GISA than glycopeptide-susceptible *S. aureus* (GSSA).[151,156]

Table 2. Glycopeptide MIC breakpoints values

Agent	Guideline	Susceptible (mg/L)	Intermediate	Resistant (mg/L)
Vancomycin	NCCLS	≤4	8–16 mg/L	≥32
	BSAC	≤4	–	>4
Teicoplanin	NCCLS	≤8	16 mg/L	≥32
	BSAC	≤4	–	>4

These definitions are largely phenotypic. We recommend that the terms VISA, TISA and GISA are used for isolates with homogeneous low-level resistance; hVISA, hTISA and hGISA for isolates with heterogeneous low-level resistance; and VRSA for isolates with higher levels of resistance to vancomycin (MIC ≥32 mg/L). It should be appreciated, however, that infections caused by strains with homogeneous low-level resistance are unlikely to respond to therapy with glycopeptides in serious infections and this may also be the case with some heterogeneous low-level resistant isolates (see section 6.4).[157,158]

6.2 Mechanisms of resistance

The resistance mechanisms in VRSA and GISA/hGISA are different. VRSA have acquired resistance mediated by the *vanA* gene cluster.[159–161] In contrast the GISA/hGISA have what resembles mutation-directed resistance. Although the genetic mechanism has not been clearly determined several changes in cell wall structure and biosynthesis have been reported. It is thought that these changes result in 'affinity trapping' of vancomycin which prevents the molecules from reaching their target of action on the cytoplasmic membrane.[162–164]

6.3 Prevalence

Three VRSA have been reported, all of which were MRSA and from the USA.[145,160,161] The isolates were epidemiologically unrelated and vancomycin MICs were ≥32 mg/L, while teicoplanin susceptibility was variable. There have been only a few reports worldwide of GISA[165] and these have typically been in patients on prolonged vancomycin therapy. In contrast, hGISA are more

common, although data are difficult to interpret as the screening methods, definitions and interpretive criteria used are not standardized.[165] Published prevalence rates range from 0–74%.[166]

6.4 Clinical significance

While the clinical significance and the need to detect VRSA is without question, and probably so for GISA,[165] this is not the case for hGISA, although there have been several reports of glycopeptide treatment failure associated with hGISA.[5,165–172] One report concluded that a significant risk for vancomycin treatment failure in MRSA bacteraemia begins to emerge with increasing vancomycin MICs (>0.5 mg/L) well within the susceptible range.[158] Other confounding factors may, however, be responsible for clinical failures in some published cases.[173] It seems likely that reduced glycopeptide susceptibility in hGISA may be significant in serious infections.[157,158,169] Several studies have shown that GISA can be derived from hGISA by selection with glycopeptides *in vitro*,[154,174] suggesting that hGISA may be precursors of GISA.[175,176] 'Reversion' of a GISA strain to hGISA has also been reported[177] and less fit, slower growing GISA may revert to the fitter hGISA state when glycopeptide selection pressure is removed.[176]

6.5 VRSA detection

Two of the three VRSA reported to date were not reliably detected by automated MIC methods including the Vitek, Vitek 2 and Microscan Systems.[161,178] It is therefore important that either a screening plate (BHI agar supplemented with 6 mg/L vancomycin), or a non-automated MIC method (Etest, broth microdilution or agar dilution) incubated for a full 24 h, be used. With one of the three VRSA isolates (vancomycin MIC 32 mg/L) the Etest showed a double zone of inhibition with small colonies growing inside the inner zone up to 32 mg/L. In disc diffusion tests there was less dense growth almost up to the disc within a zone of inhibition around a 30 μg disc.[161,179]

6.6 GISA/hGISA detection

Laboratory detection of these isolates is problematic. Disc diffusion methods are not able to detect either GISA or hGISA[151] and it is possible that slow growing GISA with atypical colony morphology may not be detected on the primary specimen plate unless incubated for 48 h.[180]

Detection usually involves screening followed by a confirmatory test, however, there is a great deal of variation in the methods used by different groups and no agreed standards. Screening methods include BHI or MH agar supplemented with 2–6 mg/L vancomycin or 2–8 mg/L teicoplanin. Commercial BHI agar plates supplemented with 6 mg/L vancomycin gave more consistent results than in-house prepared BHI agar plates for detection of GISA.[151] A later study found that in-house prepared MH plates supplemented with 5 mg/L vancomycin also gave consistent results.[181] The addition of 2–4% NaCl may also affect the results.[182,183] Questions have been raised regarding the sensitivity and specificity of screening methods using agar plates supplemented with glycopeptides. In one study only 4 out of 14 screen-positive isolates were positive on repeat testing.[181] BHI supplemented with 4 mg/L vancomycin, the screening method used in the initial reports of GISA, gave high false-positive rates in three large studies.[174,184,185] BHI or MH supplemented with higher concentrations of glycopeptides (5–6 mg/L of vancomycin or teicoplanin) will also give some false-positive results.[153,181,186–188] False-negative results reported with these latter screening methods are associated mainly with hGISA rather than GISA.[189] The two national GISA screening guidelines published to date highlight the lack of consensus in this area. CDC (USA) recommend BHI agar supplemented with 6 mg/L vancomycin, an inoculum of equivalent density to a 0.5 McFarland standard and 24 h of incubation,[190] whereas the French Microbiology Society recommend MH agar supplemented with 5 mg/L teicoplanin, an inoculum adjusted to the density of a 2 McFarland standard and 48 h of incubation.[175] The latter method has been adopted in the European Antimicrobial Resistance Surveillance Scheme.[191] *S. aureus* ATCC 29213 and *E. faecalis* ATCC 51299 are recommended as quality control isolates for vancomycin screening plates.[151]

A screening method based on antagonism between vancomycin and various β-lactams has been used with varying success.[175,192] It is now clear that such antagonism is characteristic of some but not all GISA/hGISA.[176]

Non-automated MIC methods, such as the Etest or broth microdilution, have been used to confirm GISA/hGISA status. However, most hGISA will not be detected by the standard Etest or broth microdilution.[189] Interestingly, agar dilution was reported to be more reliable than broth microdilution.[189] CDC recommend the standard Etest method (MH agar, an inoculum with the density of a 0.5 McFarland standard and a full 24 h of incubation), which will reliably detect GISA but not hGISA isolates.[190] Bolmstrom *et al.*,[193] found that GISA and hGISA detection with the Etest was more efficient with BHI agar, an inoculum with the density of a 2 McFarland standard and 48 h of incubation (the 'macro Etest'). Walsh *et al.*[189] also found that the 'macro Etest' was more sensitive (96%) and specific (97%) than the standard Etest (82% and 93%, respectively) for detecting GISA/hGISA. The recommended interpretative criteria for the 'macro Etest' were ≥8 mg/L for both vancomycin and teicoplanin or >12 mg/L for teicoplanin alone.[189]

The population analysis profile (PAP) method is regarded by many as the 'gold standard' for distinguishing hGISA and GISA isolates. A PAP area under the curve (PAP-AUC) ratio of the test strain to the Mu3 reference hGISA strain has been suggested, with ratios ≤0.90 indicating glycopeptide-susceptible *S. aureus*, >0.90–1.3 indicating hGISA and >1.3 indicating GISA.[194] This method is time-consuming and therefore not practical for use in most routine laboratories.

7. Developments

Conventional media will always be limited by the incubation period required for organisms to grow, in most cases overnight. Future development of solid screening media is directed at facilitating the recognition of MRSA colonies after overnight incubation while maintaining or improving the sensitivity and specificity of the media. Developments of enrichment media aim to provide high sensitivity, possibly combined with an indicator system that would reliably allow negative cultures to be distinguished without further tests after overnight incubation. Enrichment systems may be combined with a rapid test for MRSA so that positive specimens might also be reliably detected after overnight incubation.

Future developments of molecular methods will be directed at ensuring that, whichever assay methodology is used, there is a high

degree of specificity for MRSA, and interference from *mecA* in CoNS is eliminated. There are suggestions regarding improved ways of detecting PCR product in real-time as an alternative to the use of fluorescent molecular beacons, as is the current practice in platforms such as the LightCycler and SmartCycler. Fluorescence polarization, which is currently widely used in microbiology laboratories for the assay of antimicrobials, has been applied to the detection of PCR products generated from the *mecA* gene.[195] Potentially this would obviate the need to use fluorescently labelled primers, and might well produce a machine which could provide near-patient testing.

An interesting recent development is the detection of PCR product by capture of that product by specific oligonucleotides which are bound to an optically coated silicon-based surface. The nucleic acid hybrid, which is produced in real-time during the PCR reaction, is enzymatically transduced on to the molecular thin film which can then be visually detected in white light.[196] The authors described an application of this system to detect the *mecA* gene present in MRSA strains. Although considerable optimization would be necessary to produce a viable assay, the fact that the positive result can be obtained in a quantitative form reflecting the production of the PCR product, and is easily obtained using a charged coupled device to measure the changes in the visual signal, would appear to offer a system which might be both robust and suitable for near-patient testing.

In time, it seems likely that in clinical microbiology laboratories the chip arrays which are currently used mainly in a research setting will be developed for the rapid mass screening of bacterial isolates for both identity and antimicrobial resistance genes. Currently, it remains doubtful as to whether it would be economically viable to have a very small chip that could be used purely for the detection of MRSA in screening swabs.

There have been a number of interesting developments in the field of biosensors, including the possible use of ATP bioluminescence methods to detect viable bacteria. None has yet been applied directly to the detection of MRSA in screening swabs.

A related approach has been used to develop a new system recently (BacLite *Rapid* MRSA, Acolyte Biomedica Ltd, UK). The system takes bacteria from a swab which are initially enriched by a short culture step during which they are exposed to methicillin, *S. aureus* are specifically captured on magnetic beads and detected using ATP bioluminescence. An evaluation in peer-reviewed journals is awaited.

Lastly, it has been known for many years that bacteria which are dead have a change in their surface charge which results in altered behaviour when exposed to non-uniform alternating current electric fields, the so-called dielectrophoresis effect. A recent study showed that an *S. epidermidis* exposed to streptomycin had markedly different dielectrophoretic properties to those that had not been exposed and were alive.[197] This study provided only proof of principle but, as in some other applications, dielectrophoresis has been used for the differentiation of biological molecules, and this may provide another avenue for the research and development of rapid, near patient testing for the carriage of MRSA.

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Transparency declarations

All authors have declared that during the preparation of this document they were not in the employment of, nor received funding from, any pharmaceutical firm or other organization that may have resulted in a conflict of interest.

Comment on editorial process

This Working Party Report was put out for consultation on 28 March 2005 (consultation period closed on 22 April 2005) and amended in light of the comments prior to its submission to the Journal. This national consultation exercise amongst major stakeholders and other interested parties replaced the Journal's peer review process.

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