

Detection of methicillin/oxacillin resistance in staphylococci

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Methicillin/oxacillin-resistant staphylococci are heterogeneous in their expression of resistance to β -lactam agents and the test conditions have a major effect on the expression and therefore the detection of resistance. Conflicting recommendations regarding the most reliable method for routine use are partly related to differences between strains and there may be a variable interaction between the factors affecting the expression of resistance, including the agent tested, the medium, the NaCl concentration, the inoculum, temperature and period of incubation and the reading of endpoints. 'Borderline' resistant strains may have altered PBPs or be penicillinase hyperproducers, and these can be difficult to distinguish from resistant strains that carry the *mecA* gene. Recommended methods for MIC and disc diffusion testing are described, although it is unlikely that any single method will detect all resistant strains. Some rapid and/or automated methods are also available, including latex agglutination techniques for the detection of PBP2a. The gold standard method for the detection of resistance mediated by *mecA* is PCR, which is most commonly used as a reference method at present.

Introduction

Soon after the first reports of methicillin resistance in *Staphylococcus aureus* in 1961^{1,2} the unusual behaviour of the strains in susceptibility tests was noted. Early reports indicated that methicillin-resistant *S. aureus* (MRSA) were heterogeneous in their expression of resistance to β -lactam agents, in that large differences in the degree of resistance were seen among the individual cells in a population.² It was further reported that varying test conditions had major effects on the detection of resistance.³⁻⁶ The consequent problems with methicillin susceptibility testing have been the subject of many subsequent publications and there are many recommendations, some conflicting, regarding the most reliable methods for routine use. This confusion is partly because there are marked differences between strains,⁷ and different populations of strains have been included in different studies. Also, there may be a variable interaction between the various factors affecting the expression of resistance; thus, for example, the effect of modifying the NaCl concentration may depend on the medium and the temperature of incubation.

The basis of most methicillin resistance is the production of an additional penicillin-binding protein, PBP2' or PBP2a,^{8,9} mediated by the *mecA* gene.¹⁰ *mecA* gene is an additional gene found in methicillin-resistant staphylococci and with no allelic equivalent in methicillin-susceptible staphylococci. There are several additional genes that affect the expression of methicillin resistance in *S. aureus*,^{11,12} but these genes are found in susceptible as well as resistant strains.

Low-level resistance has also been described in strains with alterations to existing PBPs.¹³⁻¹⁵ The clinical importance of this phenomenon is unknown but it appears that such strains are not commonly isolated at present. Some strains that hyperproduce penicillinase may also appear to have low-level resistance under some conditions.^{16,17} There are no reports of failure of treatment with penicillinase-resistant penicillins in infections with methicillin-susceptible, penicillinase-hyperproducing *S. aureus*. In addition, in an experimental model of endocarditis in rats, oxacillin was shown to be equally effective in treatment of infections with fully susceptible and penicillinase-hyperproducing strains.¹⁸ The clinical significance of this latter behaviour is therefore doubtful.

Thus, strains termed "borderline" in susceptibility to methicillin and oxacillin may (i) have altered PBPs, (ii) be penicillinase hyperproducers or (iii) carry the *mecA* gene but be highly heterogeneous in the expression of resistance, with only a small proportion of the population of cells expressing higher levels of resistance. The conditions used for susceptibility testing may be modified to increase the proportion of cells expressing higher levels of resistance in strains with the *mecA* gene, but distinguishing penicillinase-hyperproducing strains from truly resistant (i.e. *mecA*-positive) strains remains problematic.

Although some commercial systems for the detection of methicillin resistance are available, most laboratories use diffusion methods for routine tests. The "gold standard" for antimicrobial susceptibility testing has been the MIC determined by a dilution method. However, MIC methods are affected as much as diffusion tests by variation in test conditions and some "false resistant" reports in diffusion tests are probably actually related to errors in the reference MIC tests. In recent years MIC methods have been replaced by molecular methods that detect the *mecA* gene as the "gold standard" for defining classical methicillin resistance in *S. aureus*. These molecular methods will not detect resistance unrelated to *mecA*.

Factors affecting the detection of methicillin resistance

The expression of resistance is markedly affected by test conditions although the UK "epidemic" strains of MRSA appear less affected than some others. Optimal conditions for phenotypic susceptibility testing vary between strains and no single test will detect all resistant strains.

Agent tested

With MRSA there is cross-resistance between methicillin and other β -lactam agents. Resistance is most reliably detected in tests with methicillin or oxacillin and tests with either of these agents should be taken as representative of all β -lactam agents.^{19,20} The situation is less clear with coagulase-negative staphylococci (CNS) but until further information is available it is prudent also to assume cross-resistance between methicillin/oxacillin and other β -lactams. Oxacillin in discs is reported to be less labile than methicillin on storage²¹ but oxacillin is less resistant to hydrolysis by staphylococcal β -lactamases so problems with hyperproducers of penicillinase are reduced with methicillin. However, methicillin may cease to be available in the near future as it is not currently being manufactured.

Culture medium

Mueller-Hinton and Columbia agars give better discrimination between susceptible strains and MRSA than IsoSensitest and DST agars.²²⁻²⁶ Similar differences have been noted with CNS, with which there is evidence also that Columbia agar is superior to Mueller-Hinton agar.²⁵⁻²⁷ Variation in the performance of Mueller-Hinton medium from different manufacturers^{28,29} and between different batches of Mueller-Hinton

medium from a single manufacturer has been reported.³⁰ Possible differences between batches of Columbia agar from different manufacturers have yet to be investigated. There are few data on the performance of different liquid media, as reports on media other than Mueller-Hinton broth are rare.

NaCl concentration

The addition of up to 5% NaCl to the test medium for MRSA has been widely used but the benefit is dependent on the NaCl concentration, the basal medium, the temperature of incubation, the inoculum and the test format. Addition of NaCl to Mueller-Hinton, Columbia and DST media improves the detection of methicillin resistance.^{23,25,26} The effect on Iso-Sensitest agar is less clearly beneficial for many strains, and is detrimental for some.²³ The growth of a few strains is adversely affected by 5% NaCl in all media, resulting in false susceptible reports. This problem is particularly evident with CNS. A concentration of 2% NaCl is as effective as 5% NaCl in enhancing the expression of resistance but is markedly less inhibitory to those strains that will not tolerate 5% NaCl.³¹

Inoculum

With strains of MRSA and methicillin-resistant CNS that are particularly heterogeneous, increasing the inoculum increases the chances of detecting the minority of cells comprising the resistant sub-population, although the size of the sub-population expressing resistance will still depend on test conditions other than inoculum. If the other conditions are favourable for expression of resistance, increasing the inoculum may have little effect. A high inoculum together with higher concentrations of NaCl make discrimination between resistant and susceptible populations difficult, and MIC endpoints for susceptible strains in dilution methods may be very difficult to read. In disc diffusion tests with oxacillin in particular, the use of a larger inoculum together with an increased concentration of NaCl may result in increased numbers of false resistant reports.³¹

Incubation conditions

Reducing the temperature of incubation of tests generally improves the detection of resistance,⁴ and incubation at 30°C gives better discrimination of susceptible and resistant populations than incubation at 35°C.²² A few strains of *S. aureus* and, more commonly, CNS do not grow well at 30°C, particularly in the presence of 5% NaCl, and this may lead to false susceptible results. With some strains, resistant sub-populations grow slowly and detection of resistance may be improved by extending incubation to 48h, although again this depends on other test conditions.

Reading endpoints

For many isolates, heteroresistance results in fading or trailing endpoints in agar dilution MIC tests so that the accurate reading of MICs is difficult. In disc diffusion tests, resistance is commonly indicated by reduced zone sizes, but some strains, under some conditions, may present zones containing scattered growth ranging from a few small colonies to large numbers of different sized colonies, zones with growth fading towards the disc, or rings of inhibition and growth around the disc.

Test methods

MIC by agar dilution

MICs for some resistant strains can be changed dramatically by altering the test conditions. The MICs of susceptible strains are affected less by the conditions. Hyperproducers of penicillinase are more likely to be reported resistant as the inoculum and NaCl concentration increase. Two per cent NaCl in Mueller-Hinton or Columbia agars and an inoculum of 10⁴ cfu/mL will clearly distinguish most resistant and susceptible strains.^{31,32}

Medium. Mueller-Hinton or Columbia agar with 2% NaCl. Prepare the medium according to the manufacturer's instructions and add 2% NaCl. After autoclaving, mix the medium well and prepare plates containing antibiotics as described by Andrews.³³ The plates should be used within 24h of preparation.

Inoculum. 10⁴ cfu/inoculum spot. Touch at least four representative colonies and inoculate them into Mueller-Hinton or IsoSensitest broth. Grow organisms overnight at 37°C until turbid. Alternatively, suspend colonies to an even turbidity in broth or water. In either case, then adjust the density of the suspension so that on inoculation of plates with a multipoint inoculator the inoculum is 10⁴ cfu/inoculum spot.

Incubation. Twenty-four hours at 30°C.

Reading. Read the MIC as the point of complete inhibition of growth. Trailing endpoints or reduced

numbers of colonies other than a single colony in the spot should **not** be ignored.

Interpretation. *S. aureus*: methicillin S ≤ 4 mg/L, R ≥ 8 mg/L; oxacillin S ≤ 2 mg/L, R ≥ 4 mg/L. In the case of CNS there is currently considerable debate about appropriate breakpoints, and reports of correlation between *mecA* status and methicillin or oxacillin MIC are conflicting. The United States National Committee for Clinical Laboratory Standards (NCCLS)³⁴ has reduced the oxacillin breakpoint to S ≤ 0.25 mg/L, R ≥ 0.5 mg/L. While there is support for reduction in the breakpoint for susceptible strains to ≤ 0.5 mg/L or ≤ 1 mg/L,³⁵ other reports suggest that there may be differences between species so that a breakpoint for susceptible strains of ≤ 1 mg/L is appropriate for *Staphylococcus epidermidis* and ≤ 2 mg/L for other species.³⁶ The discrepancies between recommendations are likely to be a combination of differences in MIC methods used and differences between species. It is also possible that use of *mecA* as the gold standard requires caution, as other significant (but unelucidated) mechanisms of resistance may be present in some CNS strains. The problem remains to be satisfactorily resolved and tentative oxacillin breakpoints of S ≤ 1 mg/L, R ≥ 2 mg/L are suggested here.

Control strains. Include a susceptible control *S. aureus* strain (ATCC 29213 or NCTC 6571) with each batch of tests. *S. aureus* NCTC 12493 is a methicillin-resistant strain that can be used to check that conditions are favourable for the detection of resistance. MICs for control strains should be within one two-fold dilution step of the following values:

ATCC 29213: methicillin 1 mg/L; oxacillin 0.25 mg/L; NCTC 6571: methicillin 1 mg/L, oxacillin 0.25 mg/L.

MICs by broth microdilution

The microdilution method recommended by NCCLS is the only widely used broth dilution method. The method includes the use of Mueller-Hinton broth supplemented with 2% NaCl, an inoculum of 5×10^5 cfu/mL prepared by suspension of colonies, and incubation at 35°C for 24 h.^{34,37}

MICs by Etest

The Etest has the advantage that it is as easy to set up as a disc diffusion test but gives a result in the form of an MIC. Etest MICs are affected by variation in test conditions in a similar way to agar dilution tests. There are several reports comparing the Etest with dilution and PCR methods with generally favourable results, depending on the particular combination of medium and incubation conditions used.^{31,38-40} The manufacturer (AB Biodisk, Solna, Sweden) recommends the use of Mueller-Hinton agar with 2% NaCl, an inoculum adjusted to the density in the range of 0.5-1 McFarland standard, inoculation with a swab, and incubation at 35°C for 24 h (48 h for CNS).

Agar breakpoint and screening methods

Breakpoint methods are essentially MIC methods that test only the breakpoint concentrations. Hence the conditions and breakpoint concentrations described for agar dilution tests may be used in breakpoint tests. An agar screen method has been recommended by the NCCLS.³⁷ The method involves inoculation of selective medium with a spot or a streak of the test strain on a swab dipped into a colony suspension adjusted to the density of a 0.5 McFarland standard. The medium used is Mueller-Hinton agar supplemented with 4% NaCl and 6 mg/L oxacillin, and plates are incubated at 35°C for 24 h (48 h for CNS). Isolates that are borderline resistant may not be detected by this method.

Disc diffusion:

As already emphasized, the results of disc diffusion tests are markedly affected by test conditions. The following are recommended.

Medium. Columbia agar with 2% NaCl. Mueller-Hinton with 2% NaCl may be used for tests on *S. aureus* but Columbia agar with 2% NaCl is superior for CNS. The method has been tested with Columbia agar from Oxoid (Basingstoke, UK). The performance of Columbia agar from other manufacturers may differ, and equivalent performance of media from different manufacturers needs to be confirmed. Prepare the medium according to the manufacturer's instructions and add 2% NaCl. After autoclaving mix the medium well and pour plates to a depth of 3-4 mm (22.5 mL \pm 2.5 mL in a 90 mm Petri dish). Dry the plates and store them as described for the BSAC standardized disc diffusion method.⁴¹

Inoculum. Semi-confluent growth. Touch at least four representative colonies and inoculate them into Mueller-Hinton or IsoSensitest broth. Grow organisms for 4 h at 37°C. Alternatively, make a suspension of the organisms in water. Adjust the density of the inoculum so that semi-confluent growth is obtained after incubation, as described for the BSAC standardized disc diffusion method.⁴¹

Control strains. Include a sensitive control *S. aureus* strain (ATCC 25923 or NCTC 6571) with each batch of tests. *S. aureus* NCTC 12493 is a methicillin-resistant strain that can be used to check that the conditions are favourable for the detection of resistance.

Inoculation. Use a sterile swab to inoculate the surface of the medium with the standardized culture; this can be achieved by streaking in three directions or by the use of a rotary plater.

Antimicrobial discs. Methicillin 5 µg or oxacillin 1µg. The discs should be stored and handled as described for the BSAC standardized disc diffusion method.⁴¹ Place a disc on the surface of the medium, ensuring that there is close contact with the medium.

Incubation. Twenty-four hours at 30°C in air.

Reading. Measure the zones to the nearest millimetre. Examine the zones carefully in good light to detect colonies within the zone; some of these may be very small. Any colonies within zones may be indicative of heteroresistance, but to exclude the possibility of a mixed culture, should be identified and retested.

Zone diameters of control strains should be within the tentative ranges shown in the Table. NCTC 12493 should show no zones of inhibition with either disc.

Interpretation. *S. aureus* and CNS : methicillin S ≥15 mm, R ≤14 mm; oxacillin S ≥15 mm, R ≤14mm

Limitations. Some hyperproducers of penicillinase may produce small zones of inhibition, <15 mm in diameter, whereas the greater majority of true methicillin/oxacillin-resistant strains produce no zones of inhibition. If in doubt, isolates should be tested by a PCR or probe method to detect the *mecA* gene. An increase in methicillin/oxacillin zone size in the presence of clavulanic acid is not a reliable test for hyperproducers of penicillinase as zones of inhibition of some MRSA also increase in the presence of clavulanic acid. Rarely, hyper-producers of penicillinase give no zone in this test and will therefore not be distinguished from MRSA strains, except by seeking *mecA*.

The present breakpoints for CNS are supported by one study,³⁶ but experience is limited and these breakpoints should be regarded as tentative.

PCR methods:

There are many reports of PCR methods for detecting *mecA* gene.^{15,42} The PCR methods are now regarded as the gold standard for detection of methicillin resistance. Borderline resistance which is not mediated by *mecA* will not be detected,^{15,43} but such resistance is currently uncommon and has yet to be shown to be clinically significant. However, low-cost kits based on PCR methods are not yet available and the method is currently most likely to be used as a reference technique.

Automated/rapid methods

The Vitek, Rapid ATB Staph and Microscan methods are generally reported to be reliable for testing methicillin susceptibility of *S. aureus* although problems have been reported with some MRSA strains and with CNS.⁴⁴⁻⁴⁸ Rapid results may be provided by Crystal MRSA method, but this method still requires several hours of incubation and is not reliable for CNS.⁴⁹ A slide latex agglutination test has also been developed.⁵⁰ In this method, latex particles coated with monoclonal antibodies are used to detect PBP2a, which is extracted from test colonies. The method is rapid (10 min for a single test) and is highly sensitive and specific for *S. aureus* grown on blood agar,⁵¹⁻⁵³ but may not be reliable for CNS or for colonies grown on screening media containing NaCl.⁵⁴

References

1. Jevons, M.P. (1961). "Celbenin"-resistant staphylococci. *British Medical Journal* **i**, 124-5.
2. Knox, R. (1961). "Celbenin"-resistant staphylococci. *British Medical Journal* **i**, 126.
3. Barber, M. (1964). Naturally occurring methicillin-resistant staphylococci. *Journal of General Microbiology* **35**, 183-90.
4. Annear, D.I. (1968). The effect of temperature on resistance of *Staphylococcus aureus* to methicillin and some other antibiotics. *Medical Journal of Australia* **i**, 444-6.
5. Sutherland, R. and Rolinson, G.N. (1964) Characteristics of methicillin-resistant staphylococci. *Journal of Bacteriology* **87**, 887-99.
6. Hewitt, J., Coe, A.W. and Parker, M.T. (1969). The detection of methicillin resistance in *Staphylococcus aureus*. *Journal of Medical Microbiology* **2**, 443-56.
7. Tomasz, A., Nachman, S. and Leaf, H. (1991). Stable classes of phenotypic expression in methicillin-resistant clinical isolates of staphylococci. *Antimicrobial Agents and Chemotherapy* **35**, 124-9.
8. Hartman, B.J. and Tomasz, A. (1984). Low-affinity penicillin-binding protein associated with β -lactam resistance in *Staphylococcus aureus*. *Journal of Bacteriology* **158**, 513-6.
9. Reynolds, P.E. and Brown, D.F.J. (1985). Penicillin-binding proteins of β -lactam-resistant strains of *Staphylococcus aureus*. Effect of growth conditions. *FEBS Letters* **192**, 28-32.
10. Matsuhashi, M., Song, M.D., Ishino, F., Wachi, M., Doi, M., Inoue, M. *et al.* (1986). Molecular cloning of the gene of a penicillin-binding protein supposed to cause high resistance to beta-lactam antibiotics in *Staphylococcus aureus*. *Journal of Bacteriology* **167**, 975-80.
11. de Lencastre, H. and Tomasz, A. (1994). Reassessment of the number of auxillary genes essential for expression of high-level methicillin resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **38**, 2590-8.
12. Labischinski, H., Ehler, K. and Berger-Bächi, B. (1998). The targeting of factors necessary for expression of methicillin resistance in staphylococci. *Journal of Antimicrobial Chemotherapy* **41**, 581-4.
13. de Lencastre, H., Sa Figueiredo, A.M., Urban, C., Rahal, J. and Tomasz, A. (1991). Multiple mechanisms of methicillin resistance and improved methods for detection in clinical isolates of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **35**, 632-9.
14. Montanari, M.P., Tonin, E., Biavasco, F. and Varaldo, P.E. (1990). Further characterization of borderline methicillin-resistant *Staphylococcus aureus* and analysis of penicillin-binding proteins. *Antimicrobial Agents and Chemotherapy* **34**, 911-3.
15. Bignardi, G.E., Woodford, N., Chapman, A., Johnson, A.P. and Speller, D.C.E. (1996). Detection of the *mec-A* gene and phenotypic detection of resistance in *Staphylococcus aureus* isolates with borderline or low-level methicillin resistance. *Journal of Antimicrobial Chemotherapy* **37**, 53-63.
16. Chambers, H.F., Archer, G. and Matsuhashi, M. (1989). Low-level methicillin resistance in strains of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **33**, 424-8.
17. McDougal, L. and Thornsberry, C. (1986). The role of β -lactamase in staphylococcal resistance to penicillinase-resistant penicillins and cephalosporins. *Journal of Clinical Microbiology* **23**, 832-9.
18. Thauvin-Eliopoulos, C., Rice, L.B., Elioupolos, G.M. and Moellering, R.C. (1990). Efficacy of oxacillin and ampicillin-sulbactam combination in experimental endocarditis caused by β -lactamase-hyperproducing *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **34**, 728-32.

19. Acar, J.F., Courvalin, P. and Chabbert, Y.A. (1970). Methicillin-resistant staphylococemia: bacteriological failure of treatment with cephalosporins. *Antimicrobial Agents and Chemotherapy*, **10**, 280-5.
20. Chambers, H.F., Hackbarth, C.J., Drake, T.A., Rusnack, M.G. and Sande, M.A. (1984). Endocarditis due to methicillin-resistant *Staphylococcus aureus* in rabbits: expression of resistance to beta-lactam antibiotics in vitro and in vivo. *Journal of Infectious Diseases* **149**, 894-903.
21. Drew, W.L., Barry, A.L., O'Toole, R. and Sherris, J.C. (1972). Reliability of the Kirby-Bauer disc diffusion method for detecting methicillin-resistant strains of *Staphylococcus aureus*. *Applied Microbiology* **24**, 240-7.
22. Brown, D.F.J. and Kothari, D. (1974). The reliability if methicillin sensitivity tests on four culture media. *Journal of Clinical Pathology* **27**, 420-6.
23. Brown, D.F.J. and Yates, V.S. (1986). Methicillin susceptibility testing of *Staphylococcus aureus* on media containing five percent sodium chloride. *European Journal of Clinical Microbiology and Infectious Diseases* **5**, 726-8.
24. French, G.L., Ling, J., Hui, Y.W. and Oo, H.K.T. (1987). Determination of methicillin-resistance in *Staphylococcus aureus* by agar dilution and disc diffusion methods. *Journal of Antimicrobial Chemotherapy* **20**, 599-608.
25. Milne, L.M., Curtis, G.D.W., Crow, M., Kraak, W.A.G. and Selkon, J.B. (1987). Comparison of culture media for detecting methicillin resistance in *Staphylococcus aureus* and coagulase negative staphylococci. *Journal of Clinical Pathology* **40**, 1178-81.
26. Milne, L.M., Crow, M., Emptage, A. G. & Selkon, J.B. (1993). Effects of culture media on detection of methicillin resistance in *Staphylococcus aureus* and coagulase negative staphylococci by disc diffusion methods. *Journal of Clinical Pathology* **46**, 394-7.
27. Law, D., Megson, D.M., Keaney, M.G.L. and Ganguli, L.A. (1992). The influence of salt concentration on the detection of methicillin resistance in coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy* **30**, 603-14.
28. Hindler, J.A. and Inderlied, C.B. (1985). Effect of the source of Mueller-Hinton agar and resistance frequency on the detection of methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* **21**, 205-10.
29. Hindler, J.A. and Warner, N.L. (1987). Effect of the source of Mueller-Hinton agar on the detection of oxacillin resistance in *Staphylococcus aureus* using a screening methodology. *Journal of Clinical Microbiology* **25**, 734-5.
30. Coombs, G.W., Pearman, J.W., Khinsoe, C.H. and Boehm, J.D. (1996). Problems in detecting low-expression-class methicillin resistance in *Staphylococcus aureus* with batches of Oxoid Mueller-Hinton agar. *Journal of Antimicrobial Chemotherapy* **38**, 551-3.
31. Huang, M.B., Gay, T.E., Baker, C.N., Banerjee, S.N. and Tenover, F.C. (1993). Two percent sodium chloride is required for susceptibility testing of staphylococci with oxacillin when using agar-based dilution methods. *Journal of Clinical Microbiology* **31**, 2683-8.
32. Watson, A.P., Brown, D.F.J. (1998). Methods for determining susceptibility of *Staphylococcus aureus* to methicillin and oxacillin. In *Abstracts of the Thirty-eighth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, CA, 1988*. Abstract D-53, p. 144. American Society for Microbiology, Washington, DC.
33. Andrews, J.M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* **48**, Suppl. 1, 5-16.
34. National Committee for Clinical Laboratory Standards (1999). *Performance standards for antimicrobial susceptibility testing. Supplement M100-S9*. NCCLS, Wayne, PA.

35. Kohner, P., Uhl, J., Kolbert, C., Persing, D. and Cockerill, F. (1999). Comparison of susceptibility testing methods with *mecA* gene analysis for determining oxacillin (methicillin) resistance in clinical isolates of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. *Journal of Clinical Microbiology* **37**, 2952-61.
36. Andrews, J.M., Boswell, F.J. and Wise, R. (2000). Establishing MIC breakpoints for coagulase-negative staphylococci to oxacillin. *Journal of Antimicrobial Chemotherapy* **45**, 259-61.
37. National Committee for Clinical Laboratory Standards. (1997). *Methods For Dilution Antimicrobial Susceptibility Tests For Bacteria that Grow Aerobically. - Fourth Edition: Approved Standard M7-A4*. NCCLS, Wayne, PA.
38. Novak, S.M., Hindler, J. & Bruckner, D.A. (1993). Reliability of two novel methods, Alamar and E Test, for detection of methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology* **31**, 3056-7.
39. Petersson, A.C., Miörner, H. and Kamme, C. (1996). Identification of *mecA*-related oxacillin resistance in staphylococci by the Etest and the broth microdilution method. *Journal of Antimicrobial Chemotherapy* **37**, 445-56.
40. Weller, T.M.A., Crook, D.W., Crow, M.R., Ibrahim, W., Pennington, T.H. and Selkon, J.B. (1997). Methicillin susceptibility testing of staphylococci by Etest and comparison with agar dilution and *mecA* detection. *Journal of Antimicrobial Chemotherapy* **39**, 251-3.
41. Andrews, J.M. for the BSAC Working Party Report on Susceptibility Testing. (2001). BSAC standardized disc susceptibility testing method. *Journal of Antimicrobial Chemotherapy* **48**, Suppl. 1, 43-57.
42. Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H. and Watanabe, S. (1991). Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *Journal of Clinical Microbiology* **29**, 2240-4.
43. Araj, G.F., Talhouk, R.S., Simaan, C.J. and Maasad, M.J. (1999). Discrepancies between *mecA* PCR and conventional tests used for detection of methicillin resistant *Staphylococcus aureus*. *International Journal of Antimicrobial Agents* **11**, 47-52.
44. Frebourg, N.B., Nouet, D., Lemee, L., Martin, E. and Lemeland, J.F. (1998). Comparison of ATB staph, rapid ATB staph, Vitek, and E-test methods for detection of oxacillin heteroresistance in staphylococci possessing *mecA*. *Journal of Clinical Microbiology* **36**, 52-7.
45. Woods, G.L., LaTemple, D. and Cruz, C. (1994). Evaluation of MicroScan rapid gram-positive panels for detection of oxacillin-resistant staphylococci. *Journal of Clinical Microbiology* **32**, 1058-9.
46. Wallet, F., Roussel-Delvallez, M. and Courcol, R.J. (1996). Choice of a routine method for detecting methicillin-resistance in staphylococci. *Journal of Antimicrobial Chemotherapy* **37**, 901-9.
47. Hussain, Z., Stoakes, L., Lannigan, R., Longo, S. and Nancekivell, B. (1998). Evaluation of screening and commercial methods for detection of methicillin resistance in coagulase negative staphylococci. *Journal of Clinical Microbiology* **36**, 273-4.
48. Ribeiro, J., Vieira, F.D., King, T., D'Arezzo, J.B. and Boyce, J.M. (1999). Misclassification of susceptible strains of *Staphylococcus aureus* as methicillin-resistant *S. aureus* by a rapid automated susceptibility testing system. *Journal of Clinical Microbiology*, **37**, 1619-20.
49. Knapp, C.C., Ludwig, M.D. and Washington, J. (1994). Evaluation of BBL Crystal MRSA ID system. *Journal of Clinical Microbiology*, **32**, 2588-9.
50. Nakatomi, Y. and Sugiyama, J. (1998). A rapid latex agglutination assay for the detection of penicillin-binding protein 2'. *Microbiology and Immunology* **42**, 739-43.

51. Van Griethuysen, A., Pouw, M., van Leeuwen, N., Heck, M., Willemse, P., Buiting, A. *et al.* (1999). Rapid slide latex agglutination test for detection of methicillin resistance in *Staphylococcus aureus*. *Journal of Clinical Microbiology* **37**, 2789-92.
52. Vuopio-Varkila, J., Swenson, J., Killgore, G., Hill, B., McAllister, S. and Tenover, F.C. (1999). Evaluation of MRSA-Screen™ to detect methicillin-resistant staphylococci. In *Abstracts of the Thirty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, CA, 1999. Abstract 874, p. 207. American Society for Microbiology, Washington, DC.
53. Willey, B.M., Tennant, B., Moore, T.C., Pearce, L., McGeer, A., Low, D.E. *et al.* (1999). Evaluation of a simple, rapid methicillin-resistant *Staphylococcus aureus* identification protocol. In *Abstracts of the Thirty-ninth Interscience Conference on Antimicrobial Agents and Chemotherapy*, San Francisco, CA, 1999. Abstract 871, p. 206. American Society for Microbiology, Washington, DC.
54. Brown, D.F.J. & Walpole, E. (2001). Evaluation of the Mastalex latex agglutination test for methicillin resistance in *Staphylococcus aureus* grown on different screening media. *Journal of Antimicrobial Chemotherapy* **47**, 187-9.