ORIGINAL ARTICLE

Detection of methicillin and mupirocin resistance in *Staphylococcus aureus* isolates using conventional and molecular methods: a descriptive study from a burns unit with high prevalence of MRSA

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Aims: To compare conventional phenotypic methods for the detection of methicillin and mupirocin resistance in *Staphylococcus aureus* in routine laboratory practice with reference to an established molecular method.

Methods: This study was conducted on a selection of 65 isolates of methicillin resistant *Staphylococcus aureus* (MRSA) from a burns unit in India which is endemic for MRSA. The Kirby–Bauer and modified Stokes disc diffusion tests and the Vitek™ breakpoint minimum inhibitory concentration (MIC) were performed on all isolates using the presence of the mecA gene as the reference standard. Gel based and colorimetric polymerase chain reaction (PCR) assays were evaluated as molecular methods for the diagnosis of MRSA. A commercial latex agglutination test, the Mastalex™, was assessed for the detection of penicillin binding protein 2a (PBP2a), the mecA gene product. Conventional disc diffusion and molecular methods were investigated for the detection of mupirocin resistance.

Results: Fifty one of 65 isolates were positive for the mecA gene. All three phenotypic methods showed high sensitivity (> 96.2%), whereas the specificity varied: 50% for Kirby–Bauer, 87.5% for modified Stokes, and 93.3% for Vitek. The colorimetric PCR was less cumbersome than the gel based PCR; there was complete concordance between both systems. The Mastalex™ kit showed good correlation with PCR. One isolate was found to be mupirocin resistant and harboured the mupA gene.

Conclusions: The specificity of routine laboratory tests for MRSA detection was variable. mecA gene detection, the "gold standard" to confirm ambiguous results, is difficult to perform in routine diagnostic laboratories. The Mastalex™ kit for the detection of PBP2a is an alternative that could be used in most laboratories. High level mupirocin resistance can be confirmed with genotypic methods.

Since first reported by Jevons in 1961,[1] methicillin resistant *Staphylococcus aureus* (MRSA) has been recognised as an important and universal hospital acquired pathogen causing endemic and epidemic infections in health care centres world wide.[2] Health care workers and infection control personnel depend on the laboratory for the reliable detection of MRSA in clinical specimens. This has implications for the treatment of invasive infections, perioperative prophylaxis, and infection control procedures. Surveillance of MRSA locally, nationally, and globally is also dependent on accurate laboratory reporting.

Nasal mupirocin has an important role to play in the eradication of MRSA carriage.[3] Both low and high level resistance have been reported during treatment with nasal mupirocin.[4] However, the emergence of a high degree of resistance to this drug has threatened its value as a therapeutic agent.[3 4] Therefore, the accurate detection of mupirocin resistance in MRSA is clinically relevant.

> "Both low and high level resistance have been reported during treatment with nasal mupirocin"

The purpose of our study was: (1) to compare several phenotypic methods, including a commercial latex agglutination kit that detects the mecA gene product (penicillin binding protein 2a; PBP2a), for the detection of methicillin resistance in *S aureus* with reference to the presence of the mecA gene as the standard and (2) to detect high level mupirocin resistance by identification of the mupA gene in a selection of isolates from an endemic situation.

MATERIALS AND METHODS

We selected 65 isolates of MRSA from patients and staff at the burns unit, St John's Medical College Hospital (SJMCH), Bangalore, India. Within an endemic situation in the burns unit from 1994 to 1998, these isolates represented clustering of MRSA infections. Sixty two of these isolates were from patients (58 from skin swabs and four from blood) and three from staff nasal carriers.

Comparison of phenotypic methods for the routine detection of MRSA

All MRSA isolates were identified and susceptibility tested at the department of microbiology, SJMCH, Bangalore using the Kirby–Bauer technique with oxacillin discs (1 μg) and interpreted according to 1992 National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[5]

Abbreviations: BSAC, British Society for Antimicrobial Chemotherapy; ELONA, enzyme linked oligonucleotide assay; HRP, horseradish peroxidase; MIC, minimum inhibitory concentration; MRSA, methicillin resistant *Staphylococcus aureus*; NCCLS, National Committee for Clinical Laboratory Standards; PBP2a, penicillin binding protein 2a; PCR, polymerase chain reaction; SJMCH, St John's Medical College Hospital; UCLH, University College London Hospitals; UCLMS, University College London Medical School

Table 1 Comparison of conventional and molecular methods for the detection of methicillin resistance in *Staphylococcus aureus* isolates (n=65)

Test method	mecA positive (n=51)	mecA negative (n=14)	Sensitivity (%)	Specificity (%)
Kirby Bauer				
Resistant	51	14	100	50
Sensitive	0	0		
Modified Stokes				
Resistant	51	12	100	87.5
Sensitive	0	2		
Vitek™				
Resistant	49	1	96.2	93.3
Sensitive	2	13		

Table 2 Identification of MRSA: comparison of PBP2a production with mecA detection (n=50)

PBP2a detection	mecA positive (n=39)	mecA negative (n=11)	Sensitivity (%)	Specificity (%)
Positive	38	0	97.5	100
Negative	1	11		

Susceptibility to oxacillin and methicillin in all 65 strains was repeated at University College London Hospitals (UCLH) using the modified Stokes method.[6] In addition, breakpoint minimum inhibitory concentrations (MICs) for oxacillin were done on the Vitek™ analyser. *Staphylococcus aureus* ATCC 25923 was used as the control for the Kirby–Bauer and Vitek™ analyser methods, whereas *S aureus* NCTC 6571 was used in the modified Stokes method.

Detection of the mecA gene using the minigel and colour based PCR

DNA was extracted from all isolates and stored at –20°C. Detection of the mecA gene was performed using a gel based polymerase chain reaction (PCR) standardised at the department of microbiology, UCLH, using published primers.[7] Six controls were included in each run, two positives of a known mecA positive isolate of MRSA and four negatives of PCR grade sterile distilled water.

A colour based polymerase chain reaction enzyme linked oligonucleotide assay (PCR-ELONA), standardised in the department of virology, University College London Medical School (UCLMS) for the multiplex PCR detection of herpes viral DNA (M Howard, unpublished data, 1998) was modified, standardised, and validated for the detection of mecA in all isolates (L Emery *et al.* Development of a novel "PCR-ELONA" for the detection of *Staphylococcus aureus mec*A DNA. Abstract from the Fourth International Conference of the Hospital Infection Society, Edinburgh, September, 1998).

Briefly, the method of PCR-ELONA was as follows: the primer sequences chosen for the PCR were screened on an international database to show specificity for the mecA locus. One of these primers was biotinylated and an internal probe to the mecA locus was labelled with horseradish peroxidase (HRP; Perkin-Elmer, UK). A standard PCR reaction using the two primers followed by agarose gel electrophoresis was carried out to determine the titre of mecA DNA in a known positive clinical isolate by end point titration. This isolate was then used as a positive control in all subsequent assays.

In the ELONA system the products of PCR amplification were visualised by hybridisation with an HRP–oligonucleotide probe and the subsequent reaction with a colorimetric substrate. The assay was performed in a standard serological microtitre plate coated with streptavidin. The sensitivity and specificity of the PCR-ELONA was evaluated against the gel based PCR.

Detection of PBP2a using a commercial latex agglutination kit

The mecA product (PBP2a) was detected using the Mastalex™ MRSA kit. This is a commercial kit that detects the PBP2a present in MRSA. A boiled, centrifuged extract of a suspected colony of MRSA is mixed with latex particles sensitised with monoclonal antibody directed against PBP2a; a suspension of unsensitised latex particles is used as the control.

Detection of resistance to mupirocin

All isolates were tested for mupirocin resistance using 5 μg discs at UCLH. A selection of these isolates (n = 25) was tested for the mupA gene as a marker for high level mupirocin resistance by PCR. This investigation was performed in the Laboratory of Hospital Infection, Central Public Health Laboratory, Colindale, London. Controls for this experiment included four strains of *S aureus* that were mecA positive mupA positive; mecA negative mupA negative; mecA positive mupA negative; and mecA negative mupA positive, respectively.[4]

RESULTS

Comparison of phenotypic methods for the routine detection of MRSA

Table 1 shows the results of the comparison of conventional phenotypic testing methods for MRSA using the mecA gene detection test as a reference standard.

Detection of mecA gene amplification PCR products using the minigel and ELONA systems

Of the 65 isolates tested, there was complete concordance for detection of the mecA gene between the minigel PCR and the PCR-ELONA. The same 51 isolates were positive for the mecA gene when the two different methods for the detection of PCR products were used. Fourteen isolates were negative for the mecA gene.

Detection of PBP2a

Fifty isolates were tested for PBP2a detection using a commercial latex agglutination test—the Mastalex™ MRSA

kit. The production of PBP2a and concomitant mecA gene detection were compared in the same cohort of strains (table 2).

Detection of mupirocin resistance

Of the 65 isolates tested for mupirocin using the 5 μg disc, one was found to be resistant. The same isolate was found to harbour the mupA gene when a selection of 25 isolates was tested using a multiplex PCR.

DISCUSSION

The accurate diagnosis of MRSA in the laboratory is vital for patient management. It is also essential for the meaningful interpretation of surveillance data. Currently, surveillance data are difficult to interpret because there is no uniformity of testing methods for the detection of MRSA, and laboratories vary in their standard operating procedures and interpretation of breakpoint MIC values.[5 6 8] In addition, breakpoint values for defining methicillin resistance vary between recommendations issued by the British Society for Antimicrobial Chemotherapy (BSAC) and the NCCLS.[5 9] This problem is compounded in strains of MRSA that show heterogeneous resistance to methicillin. In our study the specificity of the Kirby–Bauer method was the lowest (50%) among the phenotypic methods studied. Other studies have reported a specificity averaging 80%,[10] and in a recent comparative study[11] the specificity was shown to range between 41.7% and 58.3%. Recent studies have compared different phenotypic methods and have shown that, with modifications of test conditions and alteration of breakpoint MICs,[12] phenotypic methods, including the Kirby–Bauer method, can reliably detect oxacillin resistance in *S aureus.*[11] The BSAC has issued new guidelines[13] for the detection of methicillin resistance in staphylococci, which adds another dimension to the laboratory diagnosis of MRSA.

> "Detection of the mecA product, PBP2a, was a highly sensitive and specific technique for the detection of methicillin resistance in *Staphylococcus aureus*"

Numerous studies have shown that the phenomenon of heterogeneous resistance is an inherent limitation to the accuracy of susceptibility testing for methicillin in *S aureus*.[10 15 16] Tests based on the detection of the mecA gene using PCR or DNA hybridisation will correctly identify even the most heterogeneous and borderline strains, and mecA detection is considered the "gold standard" for methicillin resistance.[7 11 17] This has also been corroborated in a previous study from our centre (G Hill *et al.* Comparison of laboratory methods for the detection of methicillin resistance in *Staphylococcus aureus.* Abstract from the Fourth International Conference of the Hospital Infection Society, Edinburgh, September, 1998). Conventional PCR methodology is not always suitable for busy diagnostic laboratories; the introduction of the colour based PCR-ELONA simplifies the procedure and the use of an HRP conjugated oligonucleotide probe internal to the primers creates an extra level of specificity.

Detection of the mecA product, PBP2a, was a highly sensitive and specific technique for the detection of methicillin resistance in *S aureus*. The discrepancy in the one isolate that harboured the mecA gene but was negative for PBP2a might have resulted from failure to express the gene phenotypically.[18] Several workers have corroborated the high sensitivity and specificity of MRSA detection with this method, even in strains with ambiguous and borderline oxacillin resistance.[18–20]

Low level resistance to mupirocin in MRSA as detected by using the 5 μg screening disc has no clinical relevance.[4 10] High level resistant strains can be identified using the E-test method, agar dilution technique, or possibly a 25 μg disc screen, and confirmed with the detection of the mupA gene.[4] This form of resistance is being increasingly encountered in the UK, particularly in the southeast of England.[4] At the SJMCH laboratory, resistance to mupirocin ranged between 8.3% and 10% over a 10 year period when a 5 μg screen test was used on selected isolates (PU Krishnan *et al*. Changing patterns of methicillin resistant *Staphylococcus aureus* in a burns unit over a 10 year period. Abstract from the Fourth International Conference of the Hospital Infection Society, Edinburgh, September, 1998). Interestingly, some of these resistant isolates appeared before mupirocin was available for patient use in India. Routine testing for high level mupirocin resistance should form part of the antibiotic susceptibility testing of all isolates of MRSA.

Enhancing the specificity of the routine laboratory identification of MRSA is important in hospitals with a high prevalence of this organism. A cost effective option in most laboratories would be to adopt a well standardised phenotypic technique with stringent quality control measures for day to day testing, and to retest ambiguous results with a second conventional phenotypic method. Isolates that give inconsistent results with two different conventional tests could then be tested with the Mastalex™ kit and sent to a reference laboratory for mecA detection. Screening for high level mupirocin resistance should be confirmed with a genotypic method. Carefully planned long term studies will be required to assess whether the savings made by using the relatively less expensive disc tests with low specificity outweighs infection control and treatment costs incurred with a single false positive report.

Take home messages

- mecA gene detection is the "gold standard" for methicillin resistant *Staphylococcus aureus* (MRSA) testing but it is difficult to perform in routine diagnostic laboratories
- A cost effective option would be to adopt a well standardised phenotypic technique with stringent quality control measures and to retest ambiguous results with a second conventional phenotypic method
- Isolates that give inconsistent results with two different conventional tests could then be tested with the Mastalex™ kit and sent to a reference laboratory for mecA detection
- Screening for high level mupirocin resistance should be confirmed with a genotypic method

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