A STRATEGY FOR RAPID IDENTIFICATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL CARRIER STATUS

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ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a multi drug resistant organism responsible for severe outbreaks of life threatening infections in hospitals which are difficult to treat. They are spread by nasal carriage among the hospitalised patients, staff and visitors. Mannitol cloxacillin salt agar (MCSA) is a single tube method to identify MRSA. However, tubes showing growth and change in colour on biochemical characterisation often do not prove to be MRSA. In this study we have combined two strategies for the rapid identification and isolation of MRSA by culture in MCSA and multiplex PCR for *mecA* and *femB* genes. Anterior nasal swabs obtained from nursing staff and patients admitted to a large referral hospital, were inoculated into MCSA. Of the 100 tubes inoculated, 8 tubes showed change in colour and growth. On conventional testing 4 were MRSA, 3 were methicillin sensitive *S aureus* (MSSA) and 1 was Methicillin Sensitive Coagulase Negative *S aureus* (MSCNS). Genotyping by multiplex PCR revealed 5 MRSA, 2 MSSA and 1 MRCNS. The Multiplex PCR technique to rapidly identify presence of *mecA* and *femB* genes showed presence of both *mecA* and *femB* bands in all MRSA. The methicillin sensitive organisms showed absence of *mecA* gene while coagulase negative organisms showed absence of the *femB* gene. Combining MCSA with multiplex PCR for *mecA* and *femB* genes made the test both rapid and specific. Use of this strategy would enable rapid screening of nasal carriers and early implementation of hospital infection control measures.

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KEY WORDS: Mannitol cloxacillin salt agar; Methicillin Resistant *S aureus*; Methicillin Sensitive Coagulase Negative *S aureus*; Methicillin Sensitive *S aureus*.

Introduction

MRSA cause life-threatening infections, but they are no more pathogenic than methicillin-sensitive strains. Difficulties occur because of incorrect or missed identification of MRSA, and hence inappropriate or ineffective treatment of infections. However, new methods of detection and new agents for treatment are being developed for MRSA [1].

MRSA was initially detected in Europe in the 1960s, soon after the introduction of methicillin. Naturally-resistant strains were isolated before the use of methicillin or related agents. After a decline in the 1970s, new epidemic strains that differed from the original MRSA emerged in Europe and USA and have now reached global proportions. Most strains are highly resistant to antibiotics and are only sensitive to vancomycin or teicoplanin. MRSA with reduced susceptibility to vancomycin have also emerged [2]. On the basis of evidence from countries where MRSA is not a problem, it has been suggested that early detection, effective infection control measures, and rational antibiotic use will limit the transmission of these organisms; however, spread is still increasing in many countries [3].

In order to avoid sudden out breaks caused by MRSA, regular screening of patients, hospital staff and newly transferred-in patients from other hospitals needs to be carried out. The conventional microbiological techniques lack the sensitivity, accuracy and rapidity needed to correctly identify and prevent a probable out break caused by MRSA. MCSA is a single tube method commonly used to identify MRSA [4]. Coagulase positive *Staphylococci* ferment mannitol and are able to grow in the presence of high salt concentrations. Addition of cloxacillin makes it selective for MRSA. However, tubes showing growth and change in colour on biochemical characterisation often do not prove to be MRSA. MRSA are characterised by the presence of *mecA* and *femB* genes which can be detected by a multiplex PCR [5]. In this study we have combined the two methods, culture in MCSA for the rapid identification and isolation of MRSA and multiplex-PCR. This has been used to rapidly identify the carriage of MRSA in hospitalised patients.

Material and Methods

100 anterior nasal swabs were collected from patients hospitalised for more than 2 weeks and nursing staff at a large referral hospital in September 1999. Informed consent was obtained prior to sample collection. The swabs were directly inoculated at bed side into tubes containing 10 ml of MCSA (1%), NaCl (10%),

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TABLE 1
Genotyping and Phenotyping characteristics of isolated staphylococci

	Tube numbers							
	6	15	42	69	73	78	86	87
Catalase	+	+	+	+	+	+	+	+
Slide coagulase	+	-	+	+	+	+	+	+
Tube coagulase	+	+	-	-	+	+	+	+
ABST								
Ampicillin	R	S	S	S	R	R	S	R
Cefotaxime	R	R	R	S	R	R	S	R
Netilmicin	R	R	R	S	R	R	S	R
Gentamicin	R	R	S	R	R	R	S	R
Clindamycin	R	S	S	R	R	S	R	S
Augmentin	R	S	S	S	MS	R	S	S
Erythromycin	R	R	R	R	R	R	R	R
Trimethoprim	R	R	R	R	R	R	R	R
Sulpha	R	R	R	R	R	MS	R	R
Chloramphenicol	S	S	S	S	S	S	R	S
Vancomycin	S	S	S	S	S	S	S	S
Diagnosis of conventional ABST	MRSA	MSSA	MSCNS	MSSA	MRSA	MRSA	MSSA	MRSA
<i>mecA</i> Gene	+	+	+	-	+	+	-	+
<i>femB</i> Gene	+	+	-	+	+	+	+	+
Diagnosis following genotyping	MRSA	MRSA	MRCNS	MSSA	MRSA	MRSA	MSSA	MRSA

Bactopeptone (1%), Beef extract (0.1%), agar (0.8%) and cloxacillin (6 µg/ml), Phenol Red pH indicator, pH (7.4) [4]. Tubes were incubated for 24 hours at room temperature. Growth in the tubes was noted. All the isolates which were Gram positive cocci (GPC) in clusters were tested for catalase, slide coagulase and tube coagulase test by conventional methods. The colonies were subjected to slide coagulase test, tube coagulase test and antimicrobial susceptibility (ABST) by the comparative Stokes method. Sensitivity testing to methicillin was carried out using a disc diffusion technique. Known control strains of MSSA (NCTC 11561) and known MRSA were plated in two quadrants of a Mueller Hinton agar and two test strains were plated on the other two quadrants. A 5 µg disc of methicillin was placed in the center and incubation at 30°C was carried out for 24 hours. Results were read against known controls.

For PCR about 10 colonies on each plate were lightly touched using a straight wire loop. The colonies were emulsified in 2 µL of Tris EDTA Buffer (pH 7.4) deposited in a microcentrifuge tube. Bacteria were then lysed by irradiation in a BPL microwave oven using seven pulses of 60 seconds each with a 60 second interval in between. No further DNA extraction was carried out. After irradiation 25 µL of premixed multiplex PCR mix was added. Each 25 µL reaction mix contained : 10 X PCR Buffer 2.5 µL, GATC Mix 1.5 µL, distilled water 19.5 µL, Primer mix (2.5 pM each) 1 µL, Taq Polymerase 0.25 µL (The PCR core Kit, Bangalore Genie). Primers concentration was 2.5 pM per 25 µL reaction mix. Their sequences were as previously described [5-8].

mecA1-5': GTA GAA ATG ACT GAA CGT CCG ATA A,

mecA2-5': CCA ATT CCA CAT TGT TTC GGT CTA A,

femB1-5': TTA CAG AGT TAA CTG TTA CC

femB2-5': ATA CAA ATC CAG CAC GCT CT.

PCR was carried out in a thermal cycler (Hybaid Omni E). Cycling parameters were: a hot start of 94°C for 4 min was fol-

lowed by 30 cycles of melting at 94°C for 45 sec, annealing at 50°C for 45 sec and extension at 72°C for 60 sec. Electrophoresis was carried out using 10 µL of product in a 2% agarose gel containing ethidium bromide at 50V for 2 hours. A 100 bp ladder was simultaneously loaded as molecular weight marker. The results were read in a UV transilluminator and photographed using a Wratten No 9 filter.

Results

Of the 100 swabs taken, 8 tubes showed growth on MCSA (tubes 6,15,42,69,73,78, 86 and 87). The biochemical characterisation and results of ABST are shown in Table-1. Using conventional biochemical testing, of the 8 organisms isolated, 4 were shown to be MRSA (tubes 6,73,78 and 87), 3 were MSSA (tubes 15,69 and 86) and 1 was seen to be MSCNS (tube 42). On PCR, the *mecA* gene gave a 310 bp fragment, *femB* gene gave a 651 bp fragment. The Multiplex PCR technique was able to identify the presence of *mecA* and *femB* genes and showed presence of both the bands in the 5 organisms (tubes 6,15,73,78 and 87). The 2 MSSA organisms showed presence of *femB* gene only (tube 69 and 86) and absence of *mecA* gene. MRCNS showed the presence of *mecA* band only (tube 42). The results of the multiplex PCR are shown in Fig-1.

Discussion

In healthy subjects, over time, three patterns of *S aureus* carriage can be distinguished : about 20% of people are persistent carriers, 60% are intermittent carriers and approximately 20% almost never carry *S aureus*. Elimination of carriage status appears to be an attractive preventive strategy in patients at risk [6]. Airborne dispersal of *S aureus* by shedders in association with a viral URI may be an important mechanism

Fig. 1 : Photograph of PCR products show *mec A* and *fem B* bands in MRSA (isolates 6,15,73,78, 87), only the *fem B* band in MSSA (isolates 69,86) and only *mec A* band in MRCNS (tubes 42). The first lane shows the molecular weight marker of 100 bp ladder. relevant controls are shown in lanes 2 (MRCNS), lane 3 (MSSA) and lane 4 (MRSA).

of transmission of MRSA in hospitals [9]. MRSA from a patient can be transferred to other patients on medical equipment or on the hands of staff who do not adhere strictly to infection control measures [10]. In children throat and perianal site screenings have a higher sensitivity than nasal swabs in identifying carrier status [11]. Studies have shown that MRSA can persist in the oral cavities for more than five years, and that oral cavity can serve as a reservoir for MRSA with the potential to cause nosocomial infections [12]. Studies show that carriers harbour MRSA for more than three years and that, culture of the anterior nasal swab alone is a valid and efficient method for the detection of persistent MRSA carriage [13]. The present study used anterior nasal swabs to identify carriage status of MRSA.

MRSA carriage on admission to the hospital may be an increasing and underestimated problem. Troillet et al [14] have shown the prevalence of MRSA nasal carriage to be 2.6% on admission to hospital. In our study, the MRSA colonisation of anterior nares was found in 5% of the anterior nasal swabs. They were resistant to penicillin, sulphamethoxazole, trimethoprim, tetracycline and gentamicin. In a Japanese study 9% nurses and 5% doctors were found to be nasal MRSA carriers. The isolates were resistant to multiple antibiotics but they remained sensitive to vancomycin [15]. Auto-infection from nasal carriage or cross-infection, probably via staff hands seemed to be the

most common mode of acquisition of MRSA infections [16]. A study from Pakistan has shown MRSA in 1.78% hospital personnel [17]. In the present study MRSA has been studied in hospital staff and patients admitted more than two weeks in the hospital and showed a 5% carriage rate. Mir et al [4] used the soft MCSA test, a highly specific bedside test for detection of colonisation with MRSA. Our study combines the use of MCSA with PCR for the rapid diagnosis of MRSA.

Identification of MRSA by drug-susceptibility tests alone poses a serious problem, because a considerable number of clinical *S aureus* isolates are borderline resistant to methicillin. Hence, a quick and sensitive method of PCR based amplification for the detection of the *mecA* gene is necessary. The *mecA* gene codes for the drug resistant polypeptides called penicillin-binding protein 2a (PBP2a) or 2'(PB2'), and mediates the clinically relevant resistance to all beta-lactam antibiotics [18]. For appropriate treatment of MRSA infection, rapid detection of *mecA* is extremely important. However, identical *mecA* genes have been found in both coagulase-positive and coagulase-negative methicillin resistant *Staphylococcus*. A second gene related to the expression of methicillin-resistance has been called *femA*. Oshima et al [7] amplified both *mecA* and *femA* genes by PCR and found the *mec A* gene was positive in all MRSA strains and 6% MSSA strains. The *femA* gene was positive in all MRSA and MSSA strains. On the other hand the *femA* gene was absent from coagulase-negative *Staphylococcus* strains with the methicillin resistant phenotype. Towner et al [8] used the *mecA* and *femB* gene to detect MRSA. The *femB* gene is involved in pentaglycine side chain formation and inter peptide bridge as well as expression of methicillin resistance. Cotter et al [19] used a one-tube triplex PCR wherein three genes, *mecA*, *femA* and the extracellular thermonuclease gene (*nuc*), were simultaneously amplified. MSSA and coagulase-negative *Staphylococci* were also tested and the assay was found to be MRSA specific. Our study studied the *mecA* and *femB* genes in 5 of the isolates rendering them to be classified as MRSA. The PCR assay is a sensitive and reliable procedure for the rapid diagnosis of MRSA infection, even in cases in which the conventional MIC assay failed to detect MRSA [20]. In our study, 5 MRSA showed the presence of the *mecA* and *femB* genes.

This study compared and combined culture of anterior nasal swabs in MCSA with a multiplex PCR as a strategy for early detection of MRSA. Accurate and rapid results could be available in as short as 24 hours

following this method. This strategy can be used to rapidly detect and prevent outbreaks of MRSA infection in hospital wards. Thus, culture on MSCA and combination with multiplex PCR for *mecA* and *femB* made the test both rapid and specific.

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