Identification and molecular characterization of mannitol salt positive, coagulase-negative staphylococci from nasal samples of medical personnel and students

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The identification of mannitol salt positive, coagulase-negative staphylococci (CNS) is often disregarded when *Staphylococcus aureus* is screened in clinical samples using mannitol salt agar. However, the emergence of CNS as important human pathogens has indicated that reliable methods for the identification of clinically significant CNS are of great importance in understanding the epidemiology of infections caused by them. The identification and molecular characterization of mannitol salt positive CNS from nasal samples of medical personnel and students is reported here. A total of 84 mannitol salt positive staphylococcal isolates were obtained from 240 nasal samples, of which 15 were CNS. The API STAPH system classified the CNS isolates into six species, and one-third of the isolates were identified with confidence levels of <80%. 16S–23S rRNA intergenic spacer length polymorphism analysis (ITS-PCR) identified only two species (*Staphylococcus haemolyticus* and *Staphylococcus saprophyticus*). This identification was confirmed by antibiotyping, species-specific PCR and PFGE. The results from this study indicate that ITS-PCR is a potentially useful and reliable tool, enabling hospital laboratories to obtain rapid, full and accurate identification of CNS at the species level.

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INTRODUCTION

The identification of bacterial pathogens in human infection plays a key role in the management of patients in health care institutions. In many clinical laboratories, *Staphylococcus aureus* is usually isolated on nonspecific media (e.g. blood agar) and then presumptively identified before definitive overnight characterization (Kloos & Bannerman, 1995). In an attempt to achieve presumptive isolation in a single step, mannitol salt agar (MSA) was developed in 1945 for the selective isolation of pathogenic staphylococci in the clinical microbiology laboratory (Chapman, 1945; Blair *et al.*, 1967). The growth and production of yellow colonies, due to the high salt content of the medium and fermentation of mannitol, is regarded as a presumptive tool in the identification of *S. aureus*. It is also described as a characteristic for the differentiation of coagulase-positive staphylococci from coagulase-negative staphylococci (CNS) (Duguid, 1989). However, there are reports that some CNS can also produce

yellow colonies on MSA (Martinez *et al.*, 1992; Merlino *et al.*, 1996; Mir *et al.*, 1998; Jayaratne & Rutherford, 1999; Simor *et al.*, 2001; Zadik *et al.*, 2001). Mannitol salt positive CNS impede and delay the isolation and subsequent identification of *S. aureus* in clinical samples on the primary plate. Nevertheless, they have attracted very little attention and in most cases are not identified to species level.

As a group, the CNS are among the most frequently isolated bacteria in the clinical microbiology laboratory. They are becoming increasingly important as causative agents of hospital-acquired bacteraemia, with the increasing use of prosthetic devices and other invasive technologies in medical institutions (von Eiff *et al.*, 2002). Furthermore, CNS are nosocomial pathogens associated with multiple antimicrobial-resistance mechanisms including, in particular, methicillin resistance (Hanberger *et al.*, 2001). Therefore, the full and accurate identification of CNS species, which is still difficult in most clinical laboratories, is of diagnostic value, and of clinical and epidemiological importance (Varaldo & Biavasco, 1997; Couto *et al.*, 2001; De Paulis *et al.*, 2003).

Abbreviations: CNS, coagulase-negative staphylococci; ITS-PCR, 16S–23S rRNA intergenic spacer length polymorphism analysis; MSA, mannitol salt agar.

The aim of this study was to identify mannitol salt positive CNS to the species level, using a commercial identification kit (API STAPH) and 16S–23S rRNA intergenic spacer length polymorphism analysis (ITS-PCR), a PCR-based method. Characterization of the isolates based on biotyping, antibiotyping and PFGE was also investigated.

METHODS

This investigation was part of a surveillance programme on staphylococcal nasal carriage among medical personnel and students in a referral hospital in Nigeria. The surveillance programme was conducted from January to February 2001. A total of 60 individuals (20 medical doctors, 20 preclinical students and 20 clinical students) at the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria, were included in the study after obtaining permission from the Hospital Authority and informed consent from the volunteers. This hospital is a major referral centre for more than one million people living within a 40 km radius from Ile-Ife.

The medical personnel were new graduates of the medical profession, and the preclinical group were first and third year medical students. Furthermore, the clinical cohort that participated in this study were fourth and final year students.

Nasal swabs were taken from each individual every week, for a period of 4 weeks. The swabs were immediately streaked on MSA (Biolife) and incubated at 37 °C for 48 h. Thereafter, distinct well-separated yellow colonies on MSA were noted and used for further characterization. Staphylococci were identified based on Gram reaction (Gram-positive cocci in clumps), catalase, coagulase (tube method using rabbit plasma) and DNase tests. Biotyping was based on the hydrolysis of urea, Tween 80 and pigmentation on Isosensitest agar (Coia *et al.*, 1990). Pigmentation was classified as yellow (Y), cream (C) or white (W). The biotyping tests were incubated at 35–37 °C and read after 48 h. Coagulase and DNase-negative staphylococcal isolates were identified by the API STAPH system (bioMérieux) according to the manufacturer's instructions. Identification profiles using the API STAPH system were categorized based on the following confidence levels: 'excellent', 90–99%; 'very good', 80–90%; 'good', 70–80%; 'not definitive', 50–70%.

Antibiotyping of mannitol salt positive CNS. Susceptibility testing was performed using the agar disc diffusion technique following the recommendations given by the National Committee for Clinical Laboratory Standards (NCCLS, 2000). The antibiotics (Mast Diagnostics) included penicillin (10 U), oxacillin (1 µg), streptomycin (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), tobramycin (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), trimethoprim (5 µg), mupirocin (200 µg), novobiocin (5 µg) and fusidic acid (10 µg). Isolates with zone diameters ≤16 mm for novobiocin and ≤18 mm for fusidic acid (antibiotics not included in the NCCLS guidelines) were adjudged resistant to these compounds (Goldstein *et al.*, 1983; Skov *et al.*, 2001).

Molecular identification of mannitol salt positive CNS. A multiplex PCR assay was conducted to confirm phenotypic identification of the isolates as CNS and oxacillin resistant. Primers *nuc*-1 (5'-GCGATTGATGGTGATACGGTT-3') and *nuc*-2 (5'-AGCCAAGCCTTGACGAACATAAGC-3'), and *mecA*1 (5'-CTCAGGTACTGC-TATCCACC-3') and *mecA*-2 (5'-CACTTGGTATATCTTCACC-3'), which amplified a 280 bp segment of the *S. aureus* species-specific *nuc* gene and the 449 bp *mecA* gene, were employed (MacKenzie *et al.*, 2002).

Molecular identification was performed by ITS-PCR (Couto *et al.*, 2001; Lee & Park, 2001) using the primers described by Jensen *et al.* (1993). Three to five colonies were resuspended in NET buffer (10 mM Tris, 1 mM EDTA, 10 mM NaCl) containing 10 U achromopeptidase (Sigma), and incubated at 50 °C for 10–15 min. Extracted DNA (2 µl) was added to 12.5 µl Reddy Load (Abgene) PCR mix (0.2 mM dATP, dCTP, dGTP and dTTP, 3 mM MgCl₂, 0.625 U *Taq* polymerase, 20 mM Tris base, 50 mM KCl) (Abgene) and 25 pmol primers (MWG-Biotech) G1 (5'-GAAGTCGTAACAAGG-3') and L1 (5'-CAAGGCATCCACCGT-3'). Tissue culture-grade water (Sigma) was added to give a final volume of 25 µl. Cycling conditions consisted of 34 cycles of 95 °C, 55 °C and 72 °C for 1 min each, followed by a final cycle of 72 °C for 7 min. PCR products (6 µl) were loaded into 1.5% Mastgel GP agarose (Mast Diagnostics) gels. Electrophoresis was performed in 0.5 × TBE buffer pH 8 (45 mM Tris-borate, 5 mM EDTA) at 100 V for 10.5 h. The patterns of the PCR products were visually compared with previously characterized clinical isolates obtained in the health facility, and NCTC (National Collection of Type Cultures) reference strains. A representative of each pattern by ITS-PCR was further tested by appropriate species-specific primers as described by Gribaldo *et al.* (1997).

PFGE typing. PFGE typing of *Sma*I (Fermentas) digested DNA was carried out by a modification of the protocol described by Bannerman *et al.* (1995). A colony was inoculated in brain heart infusion broth and incubated overnight at 37 °C without agitation. The pellet from 0.4 ml culture was washed in 0.8 ml NET buffer (10 mM Tris, 1 mM EDTA, 10 mM NaCl) and resuspended in 0.2 ml NET buffer. An aliquot of 50 µl lysozyme (500 µg ml⁻¹) (Sigma), 50 µl lysostaphin (400 µg ml⁻¹) (Sigma) and 100 µl 2% agarose (Bio-Rad), at 50 °C, was then added. The cell/agarose suspension was loaded into block moulds (Bio-Rad) and allowed to solidify at 4 °C. Cells were lysed by incubation at 37 °C overnight in lysis buffer (6 mM Tris base, 100 mM EDTA, 1 M NaCl, 0.5% Brij 58, 0.2% sodium deoxycholate, 0.5% lauroylsarcosine). This was followed by a second overnight incubation at 50 °C in 1 ml proteolysis buffer (1% lauroylsarcosine, 75 µg Proteinase K, 0.5 M EDTA). The blocks were washed three times, with shaking, at 37 °C for 30 min in 1 ml TE buffer (10 mM Tris base, 1 mM EDTA). Thereafter, one quarter of each agarose block was washed four times at 37 °C for 30 min in 1 ml sterile distilled water. The agarose blocks were then digested with 30 U *Sma*I for 3 h, according to the manufacturer's instructions, and loaded into the wells of a 1% PFGE certified agarose gel (Bio-Rad). Electrophoresis was performed in 0.5 × TBE buffer (pH 8) (Invitrogen) by the contour-clamped homogeneous electric field method with a CHEF system (Bio-Rad). The fragments were separated with a linear ramped pulse time of 6.8–63.8 s over a period of 23 h at 14 °C. The agarose gel was stained in 1 µg ethidium bromide ml⁻¹ solution (Sigma) for 1 h, visualized under UV and photographed (SynGene bioimaging system). The banding patterns of the isolates were compared and analysed based on the classification of CNS species by Snopkova *et al.* (1994).

RESULTS

A total of 84 mannitol salt positive staphylococcal isolates were recovered from 240 nasal samples obtained in this study. A total of 69 isolates (82%) were confirmed as *S. aureus* based on positive coagulase and DNase tests. The remaining 15 (18%) were coagulase and DNase negative. The absence of the *S. aureus* species-specific *nuc* gene product further confirmed phenotypic identification as CNS. The mannitol salt positive CNS were assigned by the API STAPH system into six species: *S. haemolyticus* (7

isolates), *Staphylococcus epidermidis* (2), *Staphylococcus hominis* (2), *Staphylococcus saprophyticus* (2), *Staphylococcus cohnii* (1) and *Staphylococcus warneri* (1) (Table 1). On the basis of the API STAPH system confidence level categories, eight isolates gave excellent identification profiles, two very good, and five with either good or not definitive identification profiles. Interestingly, three isolates (S13-4, M1B4 and M3T4), which produced yellow colonies on MSA, did not utilize mannitol according to the API STAPH system.

Identification by ITS-PCR classified the mannitol salt positive CNS isolates into only two species: *S. haemolyticus* (11) and *S. saprophyticus* (4) (Table 1, Fig. 1). The banding patterns of three isolates (M3T3, M3T4 and M4Y3) differed at one or two loci from the NCTC type strains for *S. haemolyticus* and *S. saprophyticus*, respectively. These isolates and representatives of isolates with typical patterns were tested with *S. haemolyticus* and *S. saprophyticus* species-specific primers, which confirmed the ITS-PCR identification (Fig. 2). The API STAPH system and ITS-PCR agreed in the identification of nine isolates at the species level (Table 1). However, this agreement did not correlate with the confidence level categories of the API STAPH system. Three isolates with excellent identification and two isolates with not definitive identification by the API STAPH system did not correspond with the results obtained by ITS-PCR.

Antibiograms classified the mannitol salt positive CNS isolates into two main groups (Table 1). All the isolates identified as *S. haemolyticus* by ITS-PCR were susceptible to fusidic acid and novobiocin, and isolates classified as *S. saprophyticus* were resistant to these antibiotics. One of the isolates, S11 (4), identified as a novobiocin-susceptible CNS (*S. hominis*) by the API STAPH system, was resistant to novobiocin. The five *S. haemolyticus* isolates, which exhibited resistance to oxacillin by the disc diffusion method, were *mecA* positive by PCR. However, oxacillin-resistant *S. saprophyticus* isolates were *mecA* negative.

There was excellent correlation between the PFGE patterns and ITS-PCR (Fig. 3). In particular, a single large fragment (>674 kb) distinguished the banding patterns of the *S. saprophyticus* and *S. haemolyticus* isolates.

DISCUSSION

The ability of various media to isolate *S. aureus*, differentiate coagulase-positive and -negative staphylococci or inhibit CNS has been the basis of a number of studies. These media include mannitol salt oxacillin tellurite medium (Martinez *et al.*, 1992), lipovitellin salt mannitol agar (Merlino *et al.*, 1996), soft salt mannitol agar-cloxacillin (Mir *et al.*, 1998), methicillin aztreonam MSA (Perry *et al.*, 1998), mannitol salt oxacillin agar (Jayaratne & Rutherford, 1999),

Table 1. Identification of nasal mannitol salt positive CNS isolates based on the API STAPH system and ITS-PCR, and characterization by antibiotyping, biotyping and *mecA* gene detection

Isolate	Source of specimen*	API code	API STAPH (confidence level)	ITS-PCR	Antibiotyping†	Biotype‡	<i>mecA</i> gene by PCR
S8	Medical doctor	2622051	<i>S. haemolyticus</i> (99.8%)	<i>S. haemolyticus</i>	PnOxTe	NNW	+
S3 (2)	Medical doctor	6632051	<i>S. haemolyticus</i> (93.3%)	<i>S. haemolyticus</i>	PnOxErGnCpTrTeKmSt	NNW	+
S5 (3)	Medical doctor	2622051	<i>S. haemolyticus</i> (99.8%)	<i>S. haemolyticus</i>	TeKmSt	NNW	–
S13 (4)	Medical doctor	2614051	<i>S. haemolyticus</i> (93.2%)	<i>S. haemolyticus</i>	PnOxGnTrTeKm	NNW	+
S10 (3)§	Medical doctor	6636071	<i>S. haemolyticus</i> (89.2%)	<i>S. haemolyticus</i>	PnChCpTrTe	NNW	–
S10 (4)§	Medical doctor	6636171	<i>S. haemolyticus</i> (83.3%)	<i>S. haemolyticus</i>	PnOxChTrTe	NNW	+
M3T3	Medical student (pre)	6622051	<i>S. haemolyticus</i> (96.2%)	<i>S. haemolyticus</i>	PnOxErGnTrTeKmTb	NNW	+
M6O2	Medical student (clin)	6626013	<i>S. epidermidis</i> ¶ (66.1%)	<i>S. haemolyticus</i>	Fully susceptible	NNW	–
M3T4	Medical student (pre)	6606113	<i>S. epidermidis</i> ¶ (97.8%)	<i>S. haemolyticus</i>	PnGnTeKm	NNW	–
M1B4	Medical student (pre)	6216153	<i>S. hominis</i> ¶ (92.4%)	<i>S. haemolyticus</i>	Tr	NNW	–
S2 (3)	Medical doctor	6334111	<i>S. warneri</i> ¶ (78.3%)	<i>S. haemolyticus</i>	TeKmSt	NNW	–
S9 (3)	Medical doctor	6374140	<i>S. cohnii</i> ¶ (99.8%)	<i>S. saprophyticus</i>	PnOxCpFdTrTeNv	NNW	–
S1 (4)	Medical doctor	6634112	<i>S. saprophyticus</i> (71.4%)	<i>S. saprophyticus</i>	FdNv	PNY	–
S11 (4)	Medical doctor	6232153	<i>S. hominis</i> ¶ (52.1%)	<i>S. saprophyticus</i>	FdNv	NNC	–
M4Y3	Medical student (clin)	6276152	<i>S. saprophyticus</i> (74.3%)	<i>S. saprophyticus</i>	FdOxNv	PNW	–

*Pre, preclinical student; clin, clinical student.

†Pn, Penicillin; Ox, oxacillin; Er, erythromycin; Gn, gentamicin; Cp, ciprofloxacin; Tr, trimethoprim; Te, tetracycline; St, streptomycin; Km, kanamycin; Tb, tobramycin; Fd, fusidic acid; Ch, chloramphenicol; Nv, novobiocin. All the isolates were sensitive to mupirocin.

‡Biotype shows urease hydrolysis, Tween 80 hydrolysis and pigment results, in that order – N, negative; P, positive; Y, yellow; W, white; C, cream.

§Isolates S10 (3), S10 (4) were obtained from the same individual.

||Isolates M3T3 and M3T4 were obtained from the same individual.

¶Isolates misidentified by the API STAPH system. All the unmarked ones were identified unequivocally by both methods.

Fig. 1. ITS-PCR analysis. Agarose gel lane 1, *S. aureus* NCTC 10442; lane 2, S8; lane 3, S3 (2); lane 4, clinical *S. haemolyticus* strain; lane 5, S5 (3); lane 6, S13 (4); lane 7, S10 (4); lane 8, clinical *S. haemolyticus* strain; lane 9, S10 (3); lane 10, M3T3; lane 11, clinical *S. haemolyticus* strain; lane 12, clinical *S. saprophyticus* strain; lane 13, S9 (3); lane 14, M6O2; lanes 15 & 16, *S. aureus* epidemic clone EMRSA 15 & 16, respectively; lane 17, M3T4; lane 18, S1 (4); lane 19, M1B4; lane 20, S11 (4); lane 21, M4Y3; lane 22, S2 (3).

desferrioxamine oxacillin tellurite egg-yolk MSA (Zadik *et al.*, 2001), selective mannitol broth (Gurran *et al.*, 2002) and Columbia agar supplemented with desferrioxamine, amphotericin B, polymyxin B, and oxacillin or methicillin (Monsen *et al.*, 2003). They also include CHROMagar Staph. aureus (Gaillot *et al.*, 2000; Carricajo *et al.*, 2001), *S. aureus* ID (Perry *et al.*, 2003), CHROMagar MRSA (Diederer *et al.*, 2005) and oxacillin resistance screening agar (Simor *et al.*, 2001; Becker *et al.*, 2002; Blanc *et al.*, 2003). Although recommendations of these media for the presumptive identification of *S. aureus* in clinical samples have been indicated, in most cases, mannitol salt positive CNS are often regarded as unwanted contaminants, and therefore are not further characterized. However, the emergence of CNS as human pathogens and reservoirs of antimicrobial resistance has indicated that a reliable method for the identification of clinically significant CNS at the species

level is needed to provide a better understanding of the pathogenic potential of various CNS species (Heikens *et al.*, 2005).

In a surveillance programme on staphylococcal nasal carriage among medical personnel and students in a referral hospital in Nigeria, 15 (18%) out of a total of 84 mannitol salt positive (yellow) staphylococcal isolates were confirmed as CNS by the absence of the *S. aureus* species-specific *nuc* gene product. Previous investigations have indicated that 40–50% of mannitol salt positive isolates on oxacillin resistance screening agar were in fact CNS (Becker *et al.*, 2002; Blanc *et al.*, 2003). In our study, a total of 11 isolates of *S. haemolyticus* and 4 of *S. saprophyticus* utilized mannitol, producing yellow colonies on MSA. Surprisingly, three of the isolates (S13-4, M1B4 and M3T4) did not utilize mannitol in the API STAPH system. We have also observed atypical biochemical profiles for some *S. aureus* isolates in which mannitol-negative methicillin-resistant *S. aureus* isolates (on MSA) produced acid from mannitol in the API STAPH system (Shittu, 2005). The reason for this finding is unclear and warrants further investigation. Several studies have reported mannitol fermentation on various media by *S. haemolyticus* (Martinez *et al.*, 1992; Mir *et al.*, 1998; Becker *et al.*, 2002; Blanc *et al.*, 2003) and several CNS species, including *S. epidermidis* (Jayaratne & Rutherford, 1999; Blanc *et al.*, 2003), *S. saprophyticus* (Mir *et al.*, 1998), *S. warneri*, *S. hominis*, *S. cohnii* (Becker *et al.*, 2002; Blanc *et al.*, 2003), *Staphylococcus simulans*, *Staphylococcus chromogenes* (Becker *et al.*, 2002) and *Staphylococcus sciuri* (Becker *et al.*, 2002; Shittu *et al.*, 2004).

Fig. 2. Identification of typical and atypical ITS-PCR patterns of *S. haemolyticus* and *S. saprophyticus* using species-specific primers. Agarose gel lane 1, 100 bp ladder; lane 2, M3T3 – *S. haemolyticus*; lane 3, S10 (3) – *S. haemolyticus*; lane 4, M4Y3 – *S. saprophyticus*; lane 5, S11 (4) – *S. saprophyticus*; lane 6, *S. haemolyticus* reference strain NCTC 11042; lane 7, *S. saprophyticus* reference strain NCTC 7292.

A variety of methods have been proposed for the identification of CNS. These include conventional laboratory techniques, commercial identification systems and molecular biology-based methods (Lee & Park, 2001). Commercial identification kits are used in most clinical laboratories to identify CNS. However, these diagnostic systems appear not to be able to make a reliable distinction

Fig. 3. PFGE of *Sma*I digest of chromosomal DNA. Lane 1, S8; lane 2, S3 (2); lane 3, S5 (3); lane 4, S13 (4); lane 5, S10 (3); lane 6, S10 (4); lane 7, M3T3; lane 8, M6O2; lane 9, M3T4; lane 10, M1B4; lane 11, S2 (3); lane 12, S9 (3); lane 13, S1 (4); lane 14, S11 (4); lane 15, M4Y3; lane 16, *S. aureus* NCTC 8325.

between the different species of CNS because of the variable expression of phenotypic characters (Birnbaum *et al.*, 1991; Bannerman *et al.*, 1993; Grant *et al.*, 1994; Ieven *et al.*, 1995; Rennerberg *et al.*, 1995; Calvo *et al.*, 2000). Misidentification of CNS species using commercial identification systems, in comparison with the reference (conventional) (De Paulis *et al.*, 2003; Cunha *et al.*, 2004) and molecular methods (Couto *et al.*, 2001; Lee & Park, 2001; Becker *et al.*, 2004; Sivadon *et al.*, 2004; Ben-Ami *et al.*, 2005; Carretto *et al.*, 2005; Fujita *et al.*, 2005; Heikens *et al.*, 2005; Skow *et al.*, 2005), have been reported. In this study, the API STAPH system classified the 15 CNS isolates into 6 species, whereas the ITS-PCR identified only 2 species. The overall agreement of the API STAPH system and ITS-PCR was 60 % (9 out of 15 isolates). However, four isolates identified as *S. haemolyticus* by ITS-PCR were classified by the API STAPH system as *S. epidermidis* (2), *S. hominis* (1) and *S. warneri* (1). Similarly, two *S. saprophyticus* isolates (ITS-PCR identification) were grouped as *S. cohnii* and *S. hominis* by the API STAPH system (Table 1). Several investigators have assessed the ability of various molecular methods to identify CNS species (Mendoza *et al.*, 1998; Martineau *et al.*, 2001; Poyart *et al.*, 2001; Yugueres *et al.*, 2001; Drancourt & Raoult, 2002; Becker *et al.*, 2004; Carretto *et al.*, 2005; Skow *et al.*, 2005). ITS-PCR is a recognized method for typing and identification of various bacterial species, including the staphylococci,

and has been applied successfully to identify CNS to the species and subspecies level (Gurtler & Stanisich, 1996; Couto *et al.*, 2001; Lee & Park, 2001; Shittu *et al.*, 2004; Fujita *et al.*, 2005; Stepanovic *et al.*, 2005). We confirmed the reliability of the ITS-PCR method by testing representatives of each pattern with species-specific primers as described by Gribaldo *et al.* (1997). PCR assays using species-specific primers confirmed the ITS-PCR species identification of three isolates that produced DNA patterns with minor differences from characterized NCTC strains. Snopkova *et al.* (1994) described the classification of CNS species based on the overall similarity of their *Sma*I PFGE restriction patterns. The PFGE patterns observed in this study correlated well with the ITS-PCR species identification. Antibiotograms based on the susceptibility of CNS to novobiocin have been an important diagnostic tool in differentiating species of CNS (Goldstein *et al.*, 1983; Freney *et al.*, 1999; Marsou *et al.*, 2001). The 15 CNS in this study were divided into two main groups based on susceptibility to fusidic acid and novobiocin, supporting the ITS-PCR identification.

Resistance to oxacillin in CNS has become a problem, as CNS express resistance to all β -lactam antibiotics, and leads to a significant limitation in therapeutic options (York *et al.*, 1996). In this study, five oxacillin-resistant *S. haemolyticus* isolates were *mecA* positive, while the two *S. saprophyticus*

isolates, which exhibited resistance to oxacillin using the disc diffusion method, lacked the *mecA* gene. York *et al.* (1996) and Bogado *et al.* (2001) have observed this trend, and suggested that a mechanism other than the production of PBP2a may be involved in methicillin resistance in *S. saprophyticus*. It also reaffirms the problematic nature in screening for methicillin resistance and the need for reliable methods for its detection in this species. The hyper-production of β -lactamase has been suggested as a major factor in false-positive detection of oxacillin resistance in CNS (Ghoshal *et al.*, 2004).

This investigation, along with earlier studies, confirms that additional tests are needed to differentiate between *S. aureus* and CNS mannitol-fermenting (yellow) colonies on MSA (Simor *et al.*, 2001; Becker *et al.*, 2002). Molecular methods, such as ITS-PCR, are more reliable for identification of CNS than the API STAPH system, and ITS-PCR is a potentially useful tool enabling hospital laboratories to obtain full and accurate identification of CNS.

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