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Full Length Research Paper

Species distribution and antibiotic sensitivity pattern of coagulase-negative *Staphylococci* other than *Staphylococcus epidermidis* isolated from various clinical specimens

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This study was undertaken to determine the species distribution and antibiotic resistance patterns of coagulase-negative *Staphylococci* (CoNS) other than *Staphylococcus epidermidis*. A total of 142 CoNS (except *S. epidermidis*) strains were isolated from a variety of clinical specimens in neutropenic patients at the Bone Marrow Transplant Centre of Tunisia between 2002 and 2004. All CoNS isolates were further identified by Api ID32 STAPH and ITS-PCR and antibiotic sensitivity was performed by disc diffusion method. *Staphylococcus haemolyticus* was the commonest species (38%) followed by *Staphylococcus hominis* (36%). All isolates were sensitive to vancomycin and 8 (6%) strains showed a reduced sensitivity to teicoplanin. Resistance to penicillin G and methicillin was 84 and 60%, respectively. Methicillin-resistant CoNS strains were determined to be more resistant to antibiotics than methicillin-susceptible CoNS strains. The *mecA* gene was detected by PCR in 65% (92/142) CoNS isolates. Out of 92 *mecA*-positive isolates, 90 were phenotypically methicillin-resistant and two were methicillin-susceptible. Phylogenetic analysis, carried out to study the evolution of *mecA* genes between different *Staphylococcal* species, revealed a high homology for such genes among *Staphylococci*.

Key words: Coagulase-negative *Staphylococci*, methicillin-resistance, *mecA* gene.

INTRODUCTION

Cancer patients are particularly susceptible to nosocomial infections because of their compromised immune system (Ashour et al., 2007). Over the two decades, neutropenic patients have been infected with changing spectrum of bacterial organisms (Zinner et al., 1999) and antimicrobial-resistant gram-positive strains are becoming increasingly frequent in these patients. CoNS have emerged as a major cause of infection in immuno-deficient compromised patients, especially in those with indwelling foreign bodies (von Eiff et al., 2001). Although *Staphylococcus epidermidis* causes most CoNS infections, many other species have been identified in association with human infections (Zinner et al., 1999). They have become a serious problem due to associated methicillin resistance, leading to significant limitations in therapeutic options (Abbassi et al., 2008). CoNS have historically been more resistant to antimicrobials, including the β-lactam antibiotics, than *Staphylococcus aureus* and some hospitals reveal rates of oxacillin resistance in CoNS approaching 90% (John et al., 2007; Martins et al., 2007). In reports from different parts of Europe, the oxacillin resistance in CoNS varies between 70 and 80% (Agvald-Öhman et al., 2004; Sader et al., 2004) and similar high rates of resistance are also reported from the United States, Canada and Latin America (Diekema et al., 2001; Vincent et al., 2000). In addition, cross resistance to non-β-lactam agents has been a recurrent theme over the past 40 years in the

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CoNS. Correct identification of CoNS species has become important in clinical laboratories, since several species have been recognized as potential pathogens, especially in a nosocomial setting (Layer et al., 2006). Hence, convenient, reliable and inexpensive identification methods are needed to identify most of the CoNS and discriminate between the species, commonly implicated in the majority of infections.

Methicillin-resistance is mediated by the *mecA* gene, which is carried by a mobile genomic element designated *Staphylococcal* cassette chromosome *mec* (SCC*mec*) (IWG-SCC, 2009). Several recent reports suggest that in CoNS, *mecA* gene is highly conserved (Ito et al., 2001; Rahimi et al., 2009) and have shown that this gene has been actively transmitted from one *Staphylococcal* species to another (Suzuki et al., 1992). CoNS were suggested to be active players in the horizontal transfer of *mecA* gene. Previous studies have shown that a *mecA* homologue ubiquitous in *Staphylococcus sciuri* may have been the evolutionary precursor of the structural gene of PBP2a (Couto et al., 1996).

Thus, the present study was undertaken with the primary aim of studying species distribution and antimicrobial resistance patterns of CoNS other than *S. epidermidis* isolated from a variety of clinical specimens in neutropenic patients at the Bone Marrow Transplant Centre of Tunisia with particular reference to phenotypic and genotypic expression of methicillin resistance. Also, we aimed to access the phylogeny and the evolution of *mecA* genes.

MATERIALS AND METHODS

Bacterial strains and identification

Organisms from clinical samples were cultured as per the routine procedures. A total of 142 consecutive non-repeat clinically significant CoNS strains belonging to different species, sampled between 2002 and 2004 from neutropenic patients hospitalized at the Bone Marrow Transplant Centre of Tunisia, were analyzed. Bacterial strains were recovered from different pathological specimens and were initially identified by conventional tests including: colony morphology (size and pigment), Gram staining, catalase test, coagulase tests, DNase tests and manitol fermenting. One CoNS isolate of each colony-morphology type, from each site and sampling occasion, was stored in glycerin-containing broth at -20°C until further analysis.

The identification at species level was carried out by Api ID32 STAPH (bioMérieux, Marcy l'Etoile, France) in accordance with the manufacturer's instructions. All isolates were confirmed for species identification by ITS-PCR, according to previously described methodology (Couto et al., 2001).

Susceptibility testing

Antibiotic susceptibility was determined using the Kirby Bauer disc diffusion method according to the recommendation of the French Society of Microbiology «Comité de l'Antibiogramme de la Société Française de Microbiologie» (CA-SFM) (http://www.sfm.asso.fr) on MH agar (Difco). Antimicrobial drugs tested included penicillin G (6 µg, 10 UI), oxacillin (5 µg), cefoxitin (30 µg), cotrimoxazole (1.25/23.75 µg), streptomycin (10 UI), gentamicin (15 µg), kanamycin (30 µg), tobramycin (10 µg), erythromycin (15 UI), pristinamycin (15 µg), lincomycin (15 µg), tetracycline (30 µg), chloramphenicol (30 µg), rifampicin (30 µg), ofloxacin (5 µg), vancomycin (30 µg), teicoplanin (30 µg), fosfomycin (50 µg) and fusidic acid (10 µg) (Sanofi Diagnostics Pasteur). The resistance phenotypes of oxacillin resistant isolates were determined by the double-disc test with oxacillin (5 µg), and cefoxitin (30 µg) after 24 h incubation at 37°C. The MICs of oxacillin were determined by Epsilometer test (E-test) method (AB-Biodisk) on MH agar (Difco) with an inoculum of 0.5 MC Farland standards as recommended. In accordance with the CA-SFM interpretive standards (micrograms per milliliter), CoNS strains for which the MIC was ≤0.25 µg/ml were considered oxacillin susceptible, whereas CoNS strains for which the MIC was >2 µg/ml were considered oxacillin resistant. *S. aureus* ATCC25923 was included to check the quality control of the antimicrobial susceptibility patterns. Multiresistance was defined as resistance to three or more antimicrobial classes.

DNA extraction

Chromosomal DNA was extracted from each CoNS strain by the small-scale phenol extraction method (Depardieu et al., 2004) with modifications: Cells from 1.5 ml of *Staphylococci* from an overnight shaken culture in brain heart infusion broth were harvested (15,000 × g, 5 min); suspended in 150 µl of a solution containing 10 mM Tris (pH 8.0), 1 mM EDTA, and lysostaphin (2 mg/ml; Sigma); and incubated at 37°C for 30 min. The resulting protoplasts were lysed with 3µl of proteinase K (200 µg/ml) and sodium dodecyl sulfate (10%) for 30 min at 55°C, and after two phenol-chloroform extractions, total DNA was recovered in the supernatant after centrifugation (15,000 × g, 5 min).

Detection of the *mecA* gene by PCR

In addition to the phenotypic determination of methicillin resistance, a simplex PCR assay which permits the detection of the *mecA* gene was performed as previously described (Frebourg et al., 2002) with modifications. The PCR primers for *mecA* were mecA-F, 5'-GGCTATCGTGTCACAATCGTT-3' and mecA-R, 5'-TCACCTTGTCCGTAACCTGA-3' containing a ClaI restriction site. The reaction mixture (50 µl total) containing 2 µl extracted DNA, 10 mM Tris/HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each dNTP, 1.25 U Taq DNA polymerase and 0.5 µM each primer was used for PCR to amplify the *mecA* gene in an automated thermal cycler (Promega). DNA amplification thermal cycling profile was as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of amplification (94°C for 1 min, 55°C for 1 min and 72°C for 2 min), ending with the 72°C for 5 min. The amplified product of 683 bp was detected by ethidium bromide staining following 1.5% agarose gel electrophoresis. The positive and negative control strains used in *mecA* detection were *S. epidermidis* RP62A and *S. aureus* ATCC25923, respectively.

Restriction analysis by PCR-RFLP

In order to confirm the presence of *mecA* gene, a PCR-RFLP was performed according to the manufacturer's recommendations (Promega, Madison, Wisconsin, USA). Fifteen (15) µl of each *mecA* amplified product were mixed and digested with 10U of ClaI restriction enzyme (Promega, USA), and incubated at 37°C for 3 h. The sizes of the restriction fragments were documented by electrophoresis, ehtidium bromide, UV transillumination and photography.

Table 1. Species distribution of CoNS isolated from a variety of clinical sources.

CoNS species	No. of isolates (%)	Clinical sources[a]				
		Catheter	Blood	Graft tissue	Pus	Other sources
S. haemolyticus	54 (38)	12	12	8	3	17
S. hominis	51 (36)	26	16	1	3	5
S. warneri	10 (7)	0	4	0	1	5
S. cohnii	6 (4)	0	4	0	1	1
S. xylosus	6 (4)	2	2	0	0	2
S. sciuri	5 (4)	1	1	0	0	3
S. simulans	3 (2)	1	0	0	0	2
S. lugdunensis	3 (2)	1	0	0	0	2
S. saprophyticus	2 (1.5)	1	0	0	0	1
S. capitis	2 (1.5)	0	0	0	0	2
Total	142 (100)	44 (31%)	41 (29%)	9 (6.5%)	8 (6%)	40 (28%)

[a]cerebrospinal fluid (1%), parenteral alimentation (2%), nose (2%), respiratory tract (4%), throat swab (3%), urine (1%), vaginal (1%), stool culture (1%).

DNA sequencing and phylogenic construction

DNA sequencing for the *mecA* gene was performed for six strains (3030 *Staphylococcus haemolyticus*, 2772 *Staphylococcus hominis*, 3447A *Staphylococcus warneri*, 5115 *Staphylococcus xylosus*, 4284 *Staphylococcus sciuri* and 6940 *Staphylococcus cohnii*) at the Institut Pasteur de Tunis. PCR products were purified with a PCR purification kit (QIAGEN, Hilden, Germany) and were sequenced by using an ABI Prism 377 DNA sequencer (Applied Biosystems/Perkin-Elmer). Sequence alignment was carried out using ClustalW (1.8). The phylogenetic tree was constructed using the MEGA4.0 program, the sequenced *mecA* gene's alignment was compared with *mecA* genes of different *Staphylococcus sp*. available in the GenBank database (http://www.ncbi.nlm;nih.gov).

Statistical analysis

Proportions were compared using the Chi-square test. The Chi-squared test was used to assess the statistical significance for a confidence level of 95% ($\alpha = 0.05$).

RESULTS

Based on our identification methods, we isolated a total of 142 coagulase-negative *Staphylococcal* isolates from different clinical specimens collected during the study period (2002-2004) from 142 neutropenic patients in the Bone Marrow Transplant Center of Tunisia. The species distribution of the isolates is shown in Table 1. *S. haemolyticus* was the commonest species (38%) followed by *S. hominis* (36%), *S. warneri* (7%), *S. sciuri* (4%), *S. cohnii* (4%), *S. xylosus* (4%), *S. simulans* (2%), *S. lugdunensis* (2%), *S. capitis* (1.5%) and *S. saprophyticus* (1.5%).The wards with the highest number of isolates included the hematological unit with 67 (47%) isolates and the graft unit with 42 (30%) isolates. The remaining 33 (23%) CoNS isolates were from outpatients. Patients from the graft (transplant) unit, haematological unit and outpatients were neutropenic or were receiving immunosuppressant drugs. Importantly, hospitalized patients had a central vein catheter. As illustrated in Table 1, the largest number of isolates was from catheter (31%) and blood cultures (29%). Only 6.5% were isolated from graft tissue and 6% from Pus. The remainder of CoNS (28%) was isolated from other sources such as cerebrospinal fluid, parenteral alimentation, nose, respiratory tract, throat, urine, genital and stool cultures.

Antimicrobial susceptibility testing revealed that all CoNS isolated from various clinical sources were sensitive to vancomycin, while 60% were resistant to methicillin. Methicillin-resistant strains showed MIC ≥256 µg/ml in 55% of cases. Sensitive strains showed MIC ranged from 0.016 to 0.25 µg/ml. Penicillin resistance was frequent (84%). Isolates showed also high rates of resistance to erythromycin (69%), cotrimoxazole (53%), gentamicin (50%), ofloxacine (47%), tetracycline (39%) and to fusidic acid (38%). Lower resistance rates were detected for rifampin (15%), fosfomycin (10%), chloramphenicol (6%) and pristinamycin (2%) Reduced susceptibility was observed for teicoplanin (6%). Resistance to antibiotics was seen more in the methicillin-resistant isolates compared with those that were methicillin sensitive. We also found that *S. haemolyticus* and *S. hominis* were the most resistant among the CoNS species other than *Staphylococcus epidermidis* studied (Table 2). The resistance to antibiotics in the methicillin resistant (MRCoNS) compared with those that were methicillin sensitive (MSCoNS) are displayed in Figure 1. All (100%) of the MRCoNS and MSCoNS were penicillin resistant. A coexisting resistance to a different antibiotic was significantly ($p<0.0001$) higher in MRCoNS compared with MSCoNS in the present study, with the exception of pristinamycin and fosfomycin.

Detection of the *mecA* gene was carried out in 92

Table 2. Antibiotic resistance profile of the two species most frequently isolated and the other CoNS.

Organism (no.)	Antibiotic resistance[a], no. (%)																	
	PN	OX	ST	GN	KN	TB	ER	PR	LN	TC	CH	RF	COT	OF	VA	TE[b]	FO	FU
All CoNS (142)	120 (84)	85 (60)	45 (32)	72 (50)	80 (56)	70 (49)	99 (69)	3 (2)	42 (29)	56 (39)	8 (6)	22 (15)	76(53)	67 (47)	0 (0,0)	8 (6,0)	14 (10	64(45)
S. haemolyticus (68)	51 (75)	43 (63)	33 (48)	43 (63)	43(63)	36 (53)	47 (69)	1 (1,5)	8 (12)	26 (38)	2 (3,0)	12 (18)	38 (56)	39 (57)	0 (0,0)	8 (12)	1(1,5)	20 (29)
S. hominis (65)	41 (63)	31 (48)	7 (11)	22 (34)	29 (45)	23 (16)	29 (35)	0 (0,0)	15 (23)	22 (34)	5 (8,0)	5 (8,0)	32 (49)	20 (31)	0 (0,0)	0 (0,0)	7 (11)	31 (48)
Other CoNS (59)	28 (47)	11 (19)	5 (8,0)	7 (12)	8 (14)	11 (19)	23 (39)	2 (3,0)	19 (32)	8 (14)	1 (2,7)	5 (8,0)	6 (10)	8 (14)	0 (0,0)	0 (0,0)	6 (10)	13 (22)

[a]PN: Penicillin G; OX: oxacillin; ST: streptomycin; GN: gentamicin; KN: kanamycin; TB: tobramycin; ER: erythromycin; PR: pristinamycin; LN: lincomycin; TC: tetracycline; CH: chloramphenicol ; RF: rifampin ; COT: cotrimoxazole ; OF: ofloxacin ; VA: vancomycin ; TE: teicoplanin ; FO: fosfomycin ; FU: fusidic acid. [b] reduced sensibility.

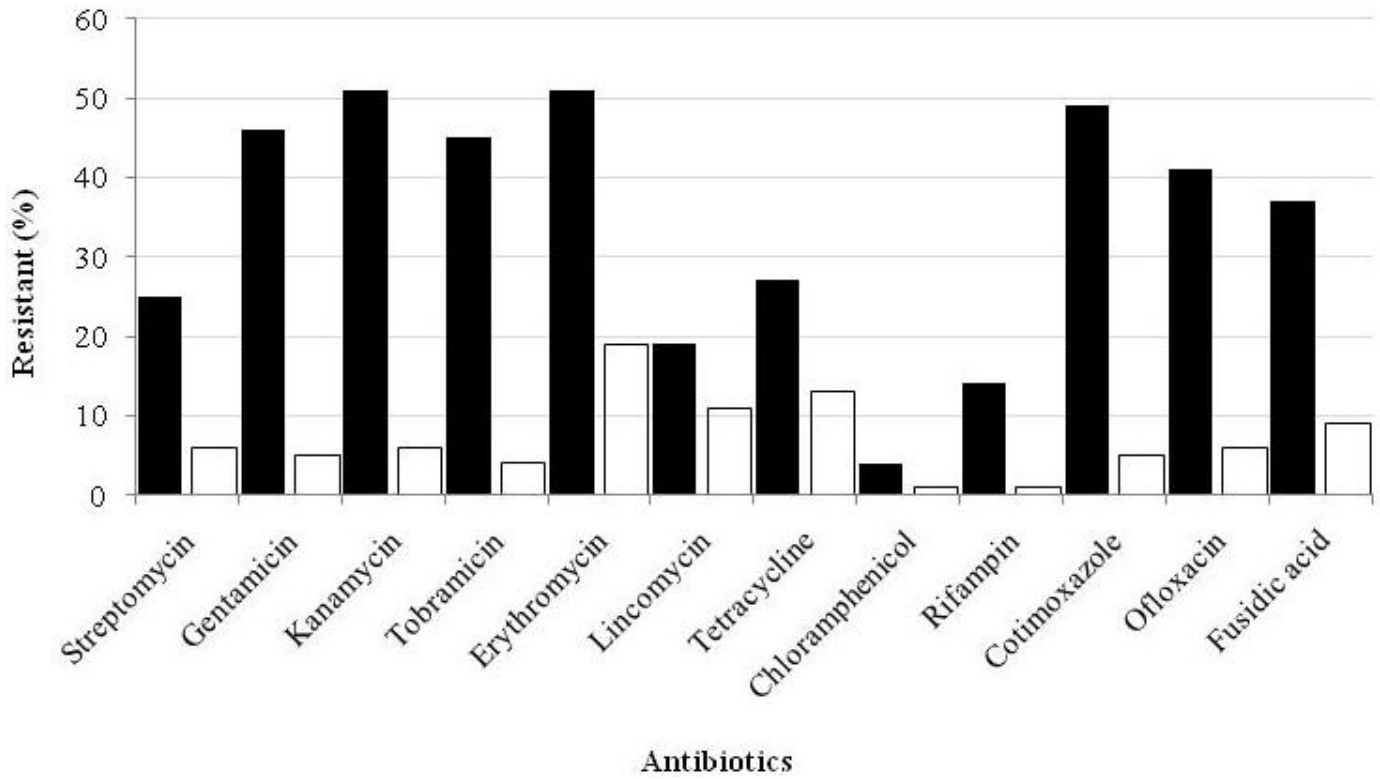

Figure 1. Antimicrobial resistance patterns in MRCoNS (filled bars) and MSCoNS (open bars) isolates.

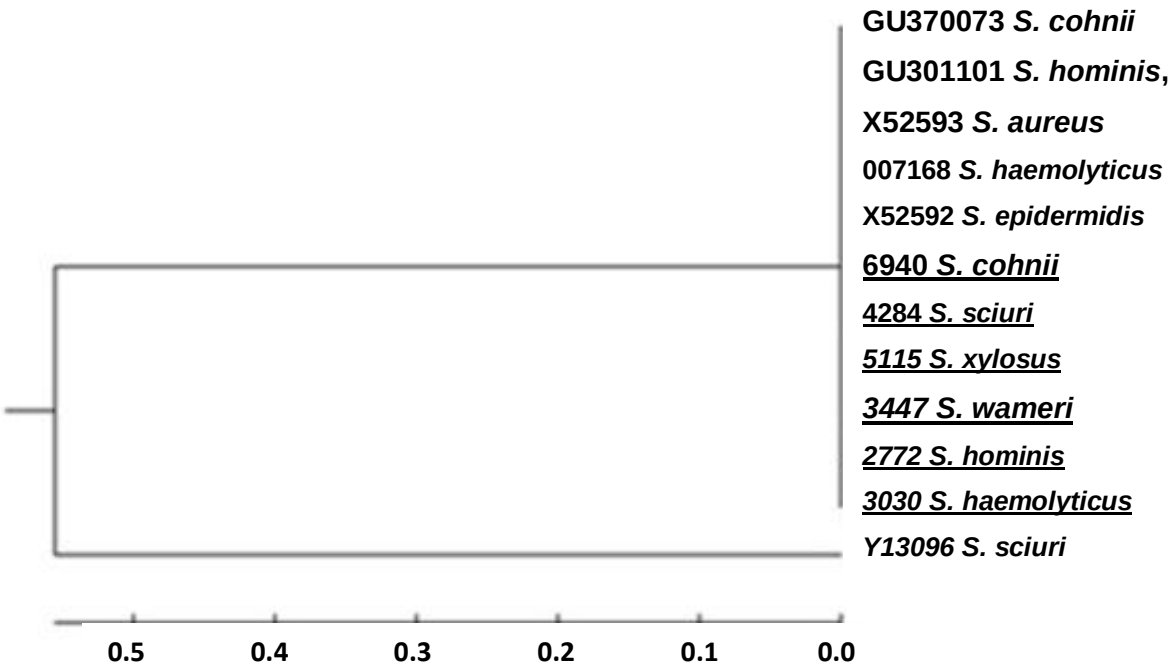

Figure 2. Phylogenetic tree for comparison of *mecA* gene sequences from CoNS isolates from our study (underlined) with sequences available on GenBank database. The tree was constructed by the Neighbour-Joining (NJ) method using MEGA version 4.0 (*Source*: Tamura et al., 2007).

(65%) CoNS isolates. The PCR-RFLP of *mecA* PCR product revealed the same restriction profile patterns in all *mecA*-positive strains. Knowing that the primers used for *mecA* gene amplification contained a restriction site, the *Cla*I digestion of *mecA* product of methicillin resistant CoNS confirmed the *mecA* presence. The percentage of *mecA*-positive strains was highest for *S. haemolyticus* (47%) and *S. hominis* (43%). According to PCR results, all phenotypically methicillin-resistant CoNS (90 isolates) showed the presence of the 683-bp fragment of the *mecA* gene, thereby confirming methicillin-resistance (the method's sensitivity = 100%). Besides, there were two *mecA*-positive methicillin-sensitive (MICs = 0.064-0.125 µg/ml) isolates, suggesting that the *mecA* gene is not consistently expressed. We designated these isolates as a silent *mec*A-carrying MRCoNS (pre-resistant strains). Of the 90 methicillin-resistant strains isolated in this study, 66 strains (73%) were found to be multidrug resistant, as shown in Table 2.

Sequencing of *mecA* gene was performed for six MRCoNS and the sequence alignment was carried out using ClustalW 1.8. BLAST search at the GenBank database with the *mecA* sequences for other species of CoNS displayed that CoNS isolates from our study were clearly closely related to GU301101 *S. hominis*, 007168 *S. haemolyticus*, GU370073 *S. cohnii* isolates and X52592 *S. epidermidis* (Ito et al., 2001; Takeuchi et al., 2005; Zong et al., 2010) in the database, with a nucleotide sequence identity of 100%. More interesting, a similar homology (100%) was found between *mecA* from the six CoNS isolates from our collection and the *mecA* from *S. aureus* (X52593 *S. aureus*) (Ryffel et al., 1990) (Figure 2). Likewise, all the examined isolates were related to Y13096 *S. sciuri* (Ito et al., 2001) with 85% identity. Phylogenic construction was carried out using MEGA 4.0 based on the nucleotide sequence of the *mecA* genes for all the tested isolates.

DISCUSSION

Despite the introduction of antimicrobial therapy and the recent improvements of medical services, CoNS are recognized as a major cause of nosocomial infections, especially in neutropenic patients. In our centre, the epidemiology of *S. epidermidis* has been extensively studied, but little known about that of the other CoNS (Abbassi et al., 2008; Bouchami et al., 2007). At present, resistance of *Staphylococcus* to methicillin is a problem of global proportions. This has underlined the need for species identification which is important in monitoring the reservoir and distribution of CoNS involved in infections and determining the etiological agent (Huebner et al., 1999). In this study, phenotypic and genotypic characteristics were used in the identification of the CoNS other than *S. epidermidis* isolates. Because of the similarity of the biochemical traits of the CoNS species, the correct identification of these species is not easy (Couto et al., 2001; Shittu et al., 2006). Molecular methods are highly desirable to permit a more precise

and full identification at species and subspecies level and must be used as valuable alternatives to commercial systems for identification of CoNS.

S. haemolyticus (38%) and *S. hominis* (36%) were the commonest species which is consistent with the reports of its isolation from clinical samples documented in various published studies (Cuevas et al., 2004; Secchi et al., 2008). However, the species of CoNS isolated in this study were slightly different from those isolated by Tan et al. (2006). It suggests that the distribution of CoNS species is variable and may differ from one country to another (Mohan et al., 2002).

The highest percentage of CoNS species was collected from catheter (31%) and blood (29%) samples mainly recovered from the haematological unit (30%). CoNS are the microorganisms most commonly isolated from blood and catheter in neutropenic patients (von Eiff et al., 2001). Several studies have found corresponding figures for the species distribution among clinical samples of CoNS (Singh et al., 2008). Infection with *S. epidermidis*, and less commonly with *S. haemolyticus* and *S. hominis*, usually involves implantation of medical devices (Akpaka et al., 2006).

Results of antibiotic susceptibility testing showed multidrug resistance and variability in sensitivity and resistance patterns, similar to the study of Mohan et al. (2002) and Pathak et al. (1994). In our study, maximum resistance was observed towards penicillin (84%) followed by erythromycin (69%), oxacillin (60%), cotrimoxazole (53%), gentamicin (50%), ofloxacine (47%), tetracycline (39%) and to fusidic acid (38%). These resistance rates were similar or even higher compared to previous reports for clinical isolates (Mohan et al., 2002). All isolates were sensitive to vancomycin, which is included in empirical therapy for neutropenic patients, 6% *S. haemolyticus* isolates showed a reduced susceptibility to teicoplanin. Reduced susceptibility to teicoplanin is observed in about 30% of *S. haemolyticus* and more rarely in *S. epidermidis* (Achour et al., 2008). It was widely accepted that *S. haemolyticus* is uniquely predisposed among CoNS to develop glycopeptides resistance as this was the first CoNS species in which vancomycin and teicoplanin resistance was identified (Schwalbe et al., 1987). It is noteworthy that 73% of the MRCoNS isolates were resistant to more than four antibiotics which confirm the large spread of multidrug-resistant CoNS isolated from clinical samples as previously reported (Santos et al., 2000; Diekema et al., 2001; Koksal et al., 2007). The heavy use of several antibiotics in certain hospital facilities may select for multiple-resistant commensal organisms including MRCoNS. *S. haemolyticus* and *S. hominis* were slightly more resistant to the antibiotic agents other than β-lactams than were the other CoNS species other than *S. epidermidis* studied. This correlated well with the study conducted by Minto et al. (1999) who reported a greater resistance in *S. haemolyticus* and *S. hominis* than in *S. epidermidis*. Interestingly, the high oxacillin resistance rate in our study (60%) was associated with a high resistance level (MIC ≥256 µg/ml in 55% of cases) similarly to what was observed in other studies (Cuevas et al., 2004; Perez et al., 2008) where methicillin resistance rates were high and increased even progressively. In addition, a coexisting resistance to a different antibiotic was significantly higher in methicillin-resistant CoNS compared with methicillin-sensitive CoNS.

More than half (65%) of the isolates were *mecA*-positive with highest percentages in *S. haemolyticus* and *S. hominis* (47 and 43%, respectively). This wide distribution of the *mecA* gene in isolates belonging to nine CoNS species has been also previously demonstrated (Secchi et al., 2008). Such a wide distribution of the *mecA* gene seems to be explained by the following two hypotheses (i) the *mecA* gene was carried by a common ancestor cell of both *S. aureus* and CoNS species and the gene has been inherited by all *Staphylococcal* species of the present day (ii) the *mecA* gene has been actively transmitted from one *Staphylococcal* species to another (Suzuki et al., 1992).

In the present study, there were two *mecA*-positive methicillin-sensitive (MICs = 0.064-0.125 µg/ml) isolates. This trend has been observed in CoNS and *S. aureus* isolates. The discordance of PCR and disc diffusion method could be caused by the heteroresistant nature of the *Staphylococci* or by absence of *mecA* gene expression on phenotype level (Martineau et al., 2000).

Comparative analysis of nucleotide sequences of *mecA* genes showed that CoNS from our study shared 100% sequence identity with GU301101 *S. hominis*, 007168 *S. haemolyticus* and GU370073 *S. cohnii* isolates, X52592 *S. epidermidis* and X52593 *S. aureus*, and 85% identity with Y13096 *S. sciuri*. Altogether, the results suggest that the *mecA* gene was very well conserved among *Staphylococcal* species. Similar results were reported that *mecA* gene is highly conserved among *Staphylococcal* species (Petinaki et al., 2001; Ito et al., 2001; Rahimi et al., 2009).The *mecA* gene is considered to have originated in some coagulase-negative *Staphylococcus* species (Wu et al., 1996) and was then transferred into *S. aureus* to generate methicillin-resistant *S. aureus* MRSA (Suzuki et al., 1993; Musser et al., 1992). In human medicine, it has been demonstrated that horizontal transfer from a primitive *Staphylococci* species, *S. sciuri*, into an *S. aureus* chromosome may be occurred (Wu et al., 1996). It is likely that the SCC*mec* serves as the carrier of the *mecA* gene moving across *Staphylococcal* species (Ito et al., 2001; Noto et al., 2006).

Conclusion

This study showed variability in species distributions and

in the antibiotic susceptibility pattern of CoNS other than *S. epidermidis*. The determination of species of CoNS could help in determining the contribution of each species to antibiotic resistance in the hospital and help in designing effective surveillance and control strategies. In addition to the disk susceptibility tests which are widely available, PCR can ensure results to properly guide antimicrobial therapy. The demonstration that the *mecA* gene is highly conserved in *Staphylococci* species may aid in the understanding and management of methicillin-resistant among *Staphylococci*.

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