PREVALENCE, SPECIATION AND ANTIBIOTIC RESISTANCE PROFILES OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATES FROM CLINICAL SAMPLES

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ABSTRACT

BACKGROUND
Coagulase-Negative Staphylococci (CONS) are opportunistic pathogens and have become major cause of nosocomial infections. The present study was undertaken to speciate the clinical isolates of CONS and study their antibiotic sensitivity patterns also showed a change and increased degree of antibiotic resistance is being documented. As there are a number of species of coagulase negative staphylococci after their isolation from various specimens received, they were speciated and their antibiotic was studied. Since most of the infections caused by coagulase negative staphylococci are hospital associated, it would serve as a useful guide to the healthcare providers. Hence, the study attempted to speciate the clinical isolates of coagulase negative staphylococci and to study their antibiotic sensitivity patterns.

MATERIALS AND METHODS
Samples were collected from various sources under aseptic conditions based on standard guidelines from patients of surgical, medical, orthopaedic wards, MGM Hospital, Warangal and its satellite hospitals. Total 210 samples were collected and the prevalence of CONS by cultural and biochemical methods was studied. Antibiotic susceptibility patterns were determined by Kirby-Bauer disc diffusion method as per CLSI guidelines. Speciation of CONS was done by slide using tube coagulase test, ornithine decarboxylase test, urease activity, trehalose fermentation, mannitol fermentation and novobiocin resistance test.

RESULTS
Out of 210 samples tested, 100 (47.61%) were CONS and majority of the CONS isolates were hospital acquired infections (68%; n = 68). Highest number of CONS isolates were observed in catheters (32/42) followed by wound (30/60), blood samples (14/39), urine (15/55) and CSF (1/6). Among the 100 CONS isolates, S. epidermidis was the commonest species isolated found in 48% (n = 48), followed by S. saprophyticus (20%; n = 20), S. haemolyticus (15%; n = 15), S. hominis (14%; n = 14) and S. caprae (3%; n = 3). Antibiotic susceptibility testing showed the maximum number of isolates was resistant to amoxicillin (70%), cotrimoxazole (65%), ciprofloxacin (61%) and penicillin (53%), while majority of the CONS isolates were methicillin sensitive (81%) and vancomycin sensitive (69%).

CONCLUSION
The results of the present study reiterate the need for proper species identification and antibiotic susceptibility patterns for proper management of nosocomial infections caused by CONS and also for epidemiological purpose.

KEYWORDS
Coagulase-Negative Staphylococci, Speciation, Antibiogram, Antibiotic Susceptibility.

ulcers, pyoderma and impetigo, patients with intravenous canulæ were chosen as subjects of study after obtaining approval from the Institutional Ethics Committee and written informed consent was taken from the patients. The comorbid, immunosuppressed patients, patients with Diabetes mellitus, patients with Malnutrition and on Steroid therapy, patients with Hypoprothrombinemia, patients with Malignancies and on Antimalignancy drugs was completely excluded.

Serous, serosanguinous or purulent discharge from the ulcers or wounds was collected with sterile swabs from the base of the lesions without touching the surrounding area of skin. In case of spreading lesions of skin and subcutaneous tissue (such as progressive gangrene), the material was collected from the active margins of the lesions rather than from central portion. When the exudate was minimal, gentle pressure was applied at the base of the lesion and the expressed discharge was collected with the swab. Care was taken to avoid topical application of any antibiotics at least 24 hrs. before collection of the sample. Two swabs were collected from each patient, one for making smears and another for culture. Swabs sterilised by autoclaving were used to collect specimens in preference to swabs sterilised by hot air oven.

The samples were processed as per the standard reference procedures (Bailey and Scott 2007). Gram positive cluster forming staphylococci which are catalase positive, oxidase negative, bacitracin resistant, fuzarolodone sensitive and fermentative by the oxidation fermentation test were identified as staphylococcal. The staphylococci strains were subjected to slide and tube coagulase test and those strains which were negative by both methods were identified as Coagulase Negative Staphylococci (CONS). The identification of all the species of CONS isolated was done by using various standard biochemical tests. Antibiotic of isolates was performed using Kirby-Bauer method (Clinical and Laboratory Standards Institute (CLSI) guidelines).

RESULTS

Table 1. Number and Percentage of Coagulase Negative Staphylococci Isolates in the Present Study

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Penicillin</td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td>2.</td>
<td>Oxacillin</td>
<td>23</td>
<td>45</td>
</tr>
<tr>
<td>3.</td>
<td>Ciprofloxacin</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>Vancomycin</td>
<td>16</td>
<td>52</td>
</tr>
<tr>
<td>5.</td>
<td>Cotrimoxazole</td>
<td>59</td>
<td>9</td>
</tr>
<tr>
<td>6.</td>
<td>Amoxicillin</td>
<td>56</td>
<td>12</td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study (Table 1), 100 CONS isolates were obtained by processing 210 clinical samples (47.61%). These results indicate the prominent place occupied by CONS in infection and more so in the hospital infections.

The percentage isolation of CONS from clinical samples is not available in similar studies; Shoba et al 2005 collected 205 swabs from the various places in the hospital and from healthy hospital staff. They obtained a percentage of 31.7%; surprisingly, all the staphylococcal isolates in their study were CONS. This study represents the magnitude of the hospital source, from which infection can be transmitted to vulnerable patients through various invasive procedures if proper care and aseptic precautions are not taken.

The predominant species of CONS in present study was S. epidermidis (Table 2); 48 strains of S. epidermidis were isolated. S. epidermidis was the most commonly encountered species among the CONS. Its prevalence as nosocomial pathogen was very much related to medical procedures and practices than the capacity of the organism to establish infection (Kloos WE and Bannerman TL). Carlos et al identified an endemic strain of S. epidermidis in the hospital producing bacteraemia in the neonatal intensive care unit. S. epidermidis is a notorious slime producer and easily establishes biofilm on polymers within which the organism can limit the effectiveness of antibiotic therapy and multiplies further as reported by Shoba et al 2005. Staphylococcus epidermidis is prevalent in 49.23% of hospital sites including the skin of the healthcare providers; 14% of these strains were oxacillin resistant, thus S. epidermidis can be termed as an important hospital pathogen and hospital infection control programs should include eradication of this organism from the hospital sites.

20% of CONS isolates were identified as S. saprophyticus (Table 2). This organism unlike S. epidermidis was more a member of the community. It is a common organism isolated from urine from community acquired urinary infections in young sexually active females. Establishment of S. saprophyticus as pathogen requires repeated careful processing and quantitative urine cultures as the organism inhabits the normal urethra and perineal skin, it is likely to contaminate urine samples during collection. S. saprophyticus has high capacity to adhere and colonise on surfaces, but unlike S. epidermidis it is a poor producer of slime (Kleeman KT et al).

In the present study, 15% of isolates were identified as S. haemolyticus (Table 2). S. haemolyticus is also part of the

Table 2. Number and Percentage of Coagulase Negative Staphylococci Species found in the 100 Isolates

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name of the Species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>S. epidermidis</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>2.</td>
<td>S. saprophyticus</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>S. haemolyticus</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>4.</td>
<td>S. hominis</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>5.</td>
<td>S. caprae</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>


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human normal skin flora. It has been documented as a cause of nosocomial bacteremias. Vancomycin resistance has been reported in this organism as well as multidrug resistance. The presence of multiple antibiotic resistant S. haemolyticus in the hospital environment and transmission of resistant clones through the hands of healthcare workers have been documented by several investigators using molecular methods (Kloos WE and George CG).\textsuperscript{7} Compared to S. epidermidis this organism colonises in much fewer numbers and less commonly incorporated in clinical illness.

S. hominis isolates were 14\% among the total CONS isolated in the present study. Among the 14 strains 9 were from wounds, 2 strains each from blood and urine and 1 from catheter. This species is a comensal of the skin of humans and has occasionally been isolated from infections as a low-grade pathogen; however, under antibiotic pressure it is known to develop resistance more easily and readily (Kloos WE and Musselwhite MS).\textsuperscript{8}

S. caprae strains (3\%) occupied least importance among the total CONS isolates in the present study (Table 2); among them 2 are from wound and 1 from urine. S. caprae is rarely cultured from clinical specimen when compared to S. epidermidis. It has been reported in association with bone and joint infection (Banerjee SN et al and Richards MJ et al).\textsuperscript{9,10} Strains of S. caprae isolated from humans are known to contain a 5 gene ica operon that code for the gene products in biofilm formation. The gene products exhibit subtotal aminoacid identity with those of S. epidermidis.

The antibiogram of Coagulase negative staphylococci (Table 3) under study shows that out of the 68 isolates of CONS 26 (38.23\%) were sensitive to Penicillin and 42 (61.76\%) were resistant; 45 (66.17\%) were sensitive to Oxacillin and 23 (33.82\%) were resistant; 20 (29.41\%) were sensitive to Ciprofloxacin and 48 (70.58\%) were resistant; 52 (76.47\%) were sensitive to Vancomycin and 16 (23.52\%) were resistant; 9 (13.23\%) were sensitive to Cotrimoxazole and 59 (86.76\%) were resistant; 12 (17.64\%) were sensitive to Amoxicillin and 56 (82.35\%) were resistant.

CONCLUSION

In the present study, Coagulase negative Staphylococci were the most predominant organisms (210/100; 47.61\%) in various clinical samples and thus Coagulase Negative Staphylococci have been increasingly gaining importance in hospital infections compared to E. coli, Klebsiella, MRSA and Pseudomonas. Majority of the isolates were from IV catheters (76.1\%) stressing the need for more aseptic precautions in ICU settings. Most predominant species of CONS in this study was S. epidermidis followed by S. saprophyticus. Among the 68 isolates of CONS Penicillin resistance is 61.76\%, Oxacillin resistance is 33.82\%, Ciprofloxacin resistance is 70.58\%, Vancomycin resistance is 23.52\%, Cotrimoxazole resistance is 86.72\% and Amoxicillin resistance is 82.35\%. Hence, Vancomycin is the drug of choice for the treatment of nosocomial infections caused by CONS and restricted and choicest use of this antibiotic can greatly reduce the risk of acquisition of resistance by the organism.

REFERENCES