SUMMARY AND CONCLUSION

Coagulase negative staphylococci are often found among the normal flora of human and animal skin. They have been regarded as harmless skin commensals and dismissed as culture contaminants. In recent years their role as pathogens and their increasing incidence have been recognized. In view of the potential role of coagulase negative staphylococci as pathogens a study was carried with the objective of characterization of isolates from various sources.

Coagulase-negative staphylococci (CNS) from different sources i.e. human healthy skin, clinical samples and domestic animals were identified and incidences of virulence properties were studied. The predominant CNS species isolated was *S. epidermidis* in both healthy skin and clinical samples. It comprised 30.6% and 35.75% of the CNS isolates in human healthy skin samples and clinical samples respectively. In domestic animals the predominant species was *S. hyicus*, which comprised about 36.32% of the CNS isolates. The CNS isolated from clinical samples, healthy skin and domestic animal samples differed in species distribution.

The analysis of virulence properties revealed the production of capsule in 33.85%, slime in 41.62%, biofilm in 36.61%, siderophore in 27.46% and synthesis of enzymes including esterase in 20.38%, DNase in 13.3% and TNase in 8.98% of the CNS isolates. The presence of TNase in the clinical samples while absent in healthy samples to be investigated. The
rate of incidence of all these virulence factors was higher for clinical samples (52.23%) and domestic animal samples (32.04%) compared to the human healthy skin (15.73%). These virulence studies revealed that domestic animals may serve as a source of pathogenic CNS. These virulence factors are significant and having important role in the pathogenesis of infections. Studies on these virulence properties may help in the development of strategies to prevent or treat infections caused by CNS species.

The antimicrobial susceptibility test and plasmid pattern analysis were performed with the strains. Incidence of antibiotic resistance was highest in the isolates of *S. epidermidis* compared to the isolates of other species. Antibiotic susceptibility test showed that CNS isolated from human healthy skin was completely susceptible to vancomycin (100%). Multiple antibiotic resistance was frequent among the isolates from all the three sources. Majority of CNS species in clinical samples were resistant to most of the antibiotics except vancomycin and rifampicin. 10% clinical sample isolates of *S. epidermidis, S. saprophyticus and S. haemolyticus* were resistant to vancomycin. Antibiotic resistances of CNS from three different sources showed significant differences. 7% *S. epidermidis*, 6% *S. hyicus* and 5% *S. chromogenes* from domestic animal samples showed resistance to vancomycin. 84% of the penicillin resistant CNS strains were positive for β-lactamase test.

Plasmid pattern analysis is a valuable tool for epidemiologic studies of CNS. 8 out of 20 strains of CNS tested showed plasmids by agarose gel electrophoresis. Phenotypically similar plasmids were found in the strains CNS E-13 and CNS E-20 by the *Eco*RI digestion. Chromosomal encoded resistance towards penicillin, methicillin, rifampicin and ciprofloxacin was also observed. A comparison of results of the present study with the earlier
reports shows an increase in multiresistance (including rifampicin and vancomycin) among the CNS isolates. Due to this reason it may be difficult to treat infections by CNS in the near future. Hence effective measures to postpone resistance development or to resolve resistance problem are to be developed. It is high time to implement effective surveillance and control strategies to solve this problem.

The qualitative and quantitative assessment of slime production and adherence to surfaces made up of different materials were evaluated. In the tube method 18 (72%) isolates showed slime formation in glass test tubes. The slime production on PVC catheter by SEM method and photomicrowgraph of glass slide also showed bacterial cells with slime layer surrounding them. Adherence on microtitre plates using TSB with and without glucose by spectrophotometric methods revealed that 15(60%) isolates were strongly adherent in TSB with glucose whereas in TSB without glucose 13 (52%) isolates showed were so. This indicated that glucose can induce biofilm polysaccharide PIA by inducing ica gene, encoded by the ica operon.

Comparison of influence of Salicylic acid (SAL) and N- acetyl cysteine (NAC) on biofilm formation by fifteen strains of S. epidermidis isolated from clinical samples was done. At a concentration of 0.06mg/ml of SAL, 6 strains turned weakly adherent (OD ≤ 0.240) where as 0.5 mg/ml of NAC was required to get the same effect. Adherence value of S. epidermidis in the presence of different concentration of SAL and NAC showed statistically significant difference. Thus the influence of SAL and NAC on biofilm formation can be applied as a simple and straightforward strategy to reduce the chances of bacterial colonization and subsequent biofilm formation.
A technique of incorporating rifampicin into a silicon polymer in preventing surface colonization was evaluated by scanning electron microscopy (SEM) and culture methods. The impregnated polymer showed good antimicrobial activity up to 3h. At the same time rifampicin-impregnated catheter incubated for 12h and 24 h showed an increased number of bacteria surrounded by biofilm. This indicates the necessity for higher antibiotic loading or the use of combination with another antibiotic to exclude the possibility of the development of resistance. Further studies are required to investigate the feasibility of incorporation of a combination of rifampicin with other antibiotics into silicone polymer to minimize the risk of surface colonization.

Among *S. epidermidis* isolates from human clinical samples, methicillin resistant *S. epidermidis* (MRSE) produced biofilm more intensively (83%) than the sensitive strains (45%). The prevalence of biofilm forming (*icaAB*) and methicillin resistance (*mecA*) genes among the isolates of *S. epidermidis* was studied and their sequence were analysed. The PCR amplification of all the twenty tested strains showed positive result, except one strain (CNS E - 87) which was biofilm positive by spectrophotometric methods, suggesting that *ica* independent and possibly protein dependent pathways can replace PIA synthesis in the biofilm accumulation phase. This signifies the requirement of further studies to investigate the mechanism by which specific proteins can form heterogenous biofilm in *S. epidermidis* and the role of *agr* quorum sensing in biofilm production.

The sequence data of *icaAB* gene showed 98% similarity to *Staphylococcus epidermidis* intercellular adhesion operon (Acc no. gb/AY 138959.1). The *mecA* gene sequence showed 99% similarity to *Staphylococcus*
epidermidis strain BCB-F63 SCCmec type IVA element (Acc no. gb/GU 451307.1). This confirms the result at the sequence level.

In conclusion, the present study indicates differences in phenotypic properties exhibited by CNS isolates from different sources. The incidence of virulence factors and the multiple drug resistance among CNS isolates suggest that proper monitoring measures are to be initiated in nosocomial infections caused by these bacteria. Further detailed studies on molecular mechanism behind biofilm formation can be helpful in designing effective surveillance and strategies to control CNS infections.