CHAPTER 1

INTRODUCTION

Human beings, like other animals harbour a wide array of microorganisms either on or in their bodies. The normal microbial flora are more or less constant for each species and are broadly divided into residents and transients. Members of the normal flora form part and parcel of the host and play an important role in the body economy. They can become pathogenic when host defense falter or they interfere with invasion or colonization of the body by pathogens. Even they can cause confusion in diagnosis due to their ubiquitous presence in the body and their resemblance to some of the pathogens. On the contrary, certain opportunistic pathogens among the normal microbial flora cause disease when the body’s defense mechanisms fail. A shift in the normal flora of the individuals can occur in environments laden with pathogens (Murray, 1998).

The term nosocomial infection is applied to infections developing in the hospitalized patients, not present or in incubation at the time of their admission. Such infection may become evident during their stay in hospital or sometimes only after their discharge. Several factors contribute to occurrence and severity of hospital infections. Many patients in hospital have impaired defense mechanisms due to their disease or therapy administered. They are therefore, highly susceptible to infections. The hospital environment is highly laden with a wide variety of pathogens. Patients shed them from their bodies; hospital personal spread them through hands, clothes, hospital dust and air. Hospital infections are in a sense; diseases of
medical progress. Advances in treatment of cancer, organ transplantation, implanted prostheses and other sophisticated medical technologies enhance the risk of infection to patients. (Bennet and Brachman, 1996).

The most frequent nosocomial infections are infections of surgical wounds, urinary tract infections and lower respiratory tract infections. The WHO study and others have also shown that the highest prevalence of nosocomial infections occurs in intensive care units and in acute surgical and orthopedic wards. Infection rates are higher among patients with increased susceptibility because of old age, underlying disease or chemotherapy (Ducell et al., 2002). Almost any pathogen can on occasion cause hospital infection but those that are able to survive in the hospital environment for long periods and develop resistance to antibiotics and disinfectants are particularly important in this respect. The most common nosocomial pathogens are Staphylococcus, Pseudomonas aeruginosa, Enterococcus, Bacillus, Klebsiella pneumonia, E.coli and Acinetobacter sps. (Arun et al., 2000). The staphylococci are the most frequently isolated species from clinical specimens in the Microbiology laboratory with the exception of the Enterobacteriaceae.

The staphylococci are important pathogenic bacteria responsible for a variety of diseases in humans and other animals. They are the most common cause of hospital acquired infections and antibiotic resistant strains have become endemic in hospitals in most countries causing major public health issues. In addition; the incidence of new strains that cause severe community acquired infections in healthy people is increasing and antibiotic resistant strains are emerging in agriculture and domestic animals. Staphylococci are widespread in nature although they are mainly found living on the skin, skin glands and mucous membranes of mammals and birds. They may be found
in the mouth, blood, mammary glands and intestinal, genitourinary and upper respiratory tracts of the hosts. Staphylococci generally have a benign or symbiotic relationship with their host; however they may develop the lifestyle of a pathogen if they gain entry into the host tissue through trauma of the cutaneous barrier, inoculation by needles, or direct implantation of medical devices or foreign bodies. Infected tissues of the host may support large populations of staphylococci and in some situations; they may persist for long periods. On the other hand some of the human staphylococcal species are transient or temporary residents on domestic animals (Devriese, 1984).

Staphylococci are usually divided into two groups based on the ability to produce coagulase, an enzyme that clots blood plasma. The coagulase positive group that includes *Staphylococcus aureus* which is an important human pathogen. Coagulase negative group is a large and heterogenous group with a diverse natural habitat that includes humans, birds, fishes and other animals. The species most frequently isolated from human material is *S. epidermidis*. CNS formerly regarded as harmless inhabitants of the skin are often found as contaminants in clinical specimens. They are now regarded as major cause of significant clinical infections and are also involved in animal diseases. Several studies have shown that they are more often associated with bovine subclinical mastitis. Two main reasons for increasing rate of CNS infections are the spreading antibiotic resistance among CNS and increasing use of medical devices in recent years. It is however difficult to distinguish between pathogenic CNS and non pathogenic resident flora, since their virulence factors have not yet been well defined.

The virulence factors of CNS include surface proteins that promote colonization of host tissues, invasions that promote bacterial spread in tissues
(leukocidin, hyaluronidase), surface factors that inhibit phagocytic engulfment (capsule), biochemical properties that enhance their survival in phagocytes (catalase production) and membrane damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin). CNS also produce several enzymes that could contribute to virulence such as lipase, esterase, DNase and TNase. However, the role of these enzymes on pathogenesis of CNS remain unclear (Otto, 2004). There can also be differences among isolates from different geographical areas with respect to their virulence properties. Hence it is important to analyze the species distribution and virulence properties of CNS isolated from human and animal sources in Kerala.

In recent decades CNS have emerged as important nosocomial pathogens due to the progress that has been made in the field of medicine. CNS are associated with bacteremia, wound related infections, intravascular catheter-related infections and a variety of postoperative infections. They have the ability to develop resistance to all hitherto described antibiotics and cause diseases and show wide variability in their pattern of resistance to antimicrobial agents. Multidrug- resistant CNS are currently a common finding among hospitalized patients. The emergence of multidrug resistant CNS has limited the therapeutic arsenal, thus increasing the risk of treatment failure and costs (Monsen et al., 2005). Antibiotic resistance in CNS strains may be unstable. Penicillin resistance is developed by the production of beta lactamase (penicillinase) which inactivates penicillin by splitting the beta lactam ring. β-lactamase is encoded in staphylococci by the blaZ gene, which is located on a large plasmid. Methicillin resistant S.epidermidis (MRSE) is recognized as pathogens that can cause serious nosocomial infection that contribute to mortality and morbidity in hospitals around the world (Maple et al., 1989). Resistance to methicillin in staphylococci is primarily mediated
Introduction

by penicillin binding protein (PBP-2a) which is a unique cell wall synthesizing enzyme.

With the increase in methicillin resistance in staphylococcal species, other antibiotics have been used in the treatment of serious infections caused by this group of bacteria. Aminoglycosides are a group of antibiotics with a complex mechanism of activity. Tobramycin and gentamicin are possible to use for treatment of staphylococcal infections. To avoid development of resistance to aminoglycosides, combination therapy has been suggested; combining aminoglycoside with a β-lactam antibiotic, vancomycin, or (when treating a biofilm-related infection) rifampicin (Miranda et al., 2006). Over the past several years, resistance of the CNS to many classes of antimicrobial agents has emerged. The glycopeptide vancomycin has been regarded as one of the last resorts for treatment of infections due to MRSA and MR-CNS. Increasing use of vancomycin has led to the emergence of CNS with decreased susceptibility to vancomycin. The major CNS are generally more resistant to antimicrobial agents than is S.aureus (John et al., 1978). The information on clinically significant CNS from India especially Kerala is very limited. Thus this study is of great significance.

In addition to the use of antibiotics in numerous infectious diseases, a wide variety of biocides are used to control infections in the form of antiseptics, disinfectants and non antibiotic drugs. S.epidermidis and other CNS produces biofilm that makes the eradication of microorganisms more difficult. Salicylic acid (SAL), N-acetyl cysteine (NAC) and certain other non steroidal anti inflammatory drugs decrease the production of slime and therefore prevent the formation of biofilms and the adherence of S.epidermidis to medical polymers (Faber et al., 1992). So a comparative study on the effects of these agents will be useful.
Although CNS are part of commensal organisms *S. epidermidis* is becoming a frequent cause of infection associated with catheters and other indwelling medical devices. These organisms when attached to the surfaces of foreign bodies may produce an extracellular slime that prevents the access of effective antimicrobial agents to the cell surface thereby allowing the persistence of CNS on catheters. CNS can initiate infection on biomaterials by attaching directly to polymer. The ability to form the polysaccharide slime or biofilm in CNS are the most important virulence factor by which they adhere to and colonize artificial materials. Various methods have been employed to modify polymer surfaces and load antimicrobial agents into medical devices, thereby produce bacteria-inhibitory and bactericidal surfaces. These significantly reduce bacterial colonization and adherence. Therefore a study on the effect of antibiotic(s) on surface colonization by CNS will be relevant.

The biofilm formation in *S. epidermidis* the major CNS responsible for the nosocomial infection occurs as a result of different biosynthetic processes. The heterogeneity in the biofilm structure and composition makes this an important property to be investigated to develop measures for the prevention and treatment of infection. The presence of antibiotic resistance genes along with the biofilm forming characters makes *S. epidermidis* and other CNS as potentially harmful microbes to be screened regularly. Most of the studies on this area were studied by different investigators all over the world, but a study on biofilm formation by CNS from India is very limited. Understanding the role and interaction of all potential biofilm factors with each other is necessary to identify new targets and possibility to develop new therapeutic approaches for treating device-related, clinically significant infections involving *S. epidermidis*. 
Recent advances in the knowledge of the molecular mechanism of biofilm formation and the production and regulation of virulence factors in S. epidermidis and other CNS species have opened the way to the development of number of therapies that are ‘antibiofilm’ in nature. Therapeutic strategies are aimed at the disintegration of biofilms or the use of enzymes to dissolve the biofilm matrix. Prophylactic measures include the development of effective immunotherapy and vaccination.

The purpose of the present study was to provide a current summary of the microbiological, clinical and epidemiological aspects of coagulase negative staphylococci. In recent decades CNS have emerged as important nosocomial pathogens. They are reported as etiological agents of infections associated with catheters and other indwelling medical devices. Due to the presence of some virulence factors and multiple drug resistance, these organisms are occupying the status as emerging pathogens in Clinical Microbiology. In view of the importance of these bacteria as emerging pathogens and the scarcity of reports of study from southern part of India, a study was carried out with the following objectives.
OBJECTIVES

- To isolate and identify coagulase negative staphylococci from different sources.
- To study the virulence properties in the identified species of CNS.
- To analyse antibiotic sensitivity pattern and plasmid profile of the isolated CNS species.
- To assess the adherence of CNS to different smooth surfaces.
- To test the methods to reduce or inhibit biofilm formation and surface colonization by the CNS isolates.
- To explore the genetic basis of biofilm formation.