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Molecular Characterization of Coagulase Negative Nosocomial *Staphylococcus* sp. by RFLP Method

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ABSTRACT

Coagulase negative nosocomial *Staphylococcus* spp. were collected from infected patient in and around Thanjavur. Colonies were analyzed by using morphological (staining, colony morphology and pigmentation) and biochemical (catalase, coagulase, acid production etc.) analyses. Twenty coagulase negative *Staphylococcus* spp. (CoNS) were identified by morphological and biochemical tests and submitted to molecular typing by plasmid DNA analysis, genomic DNA analysis, restriction endonuclease digestion pattern by using *EcoR I*, *Hind III*, and *Alu I* enzymes. 20 species of three different type of CoNS were identified, *viz.*, *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus*.

Key words : Coagulase negative *Staphylococcus* spp., Genomic DNA, Plasmid DNA

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Coagulase negative *Staphylococcus* (CoNS) are increasing in importance as causes of hospital acquired infection, particularly nasocomial bactermias (Christof von Eiff *et al*., 2001). Most of these Methicillin resistant CoNS are cause of nosocomial bactermias and the resistance of these pathogens to antimicrobial agents. Multiresistant CoNS also commonly colonize the skin of hospital of hospitalized patients and hospital personnel (Archer, 1991). *S. saprophyticus* appears to the predominant *Staphylococcus* sp. in acute urinary tract infections (Jordan, 1980).

The opportunistic pathogenicity is attributed to the factors like alteration of the integument of various parts of human body, which allows the bacteria to gain entry into the body. The pattern of antibiotic resistance in CoNS has been reported in a few earlier studies (Wallmark, 1978; Aber and Mackel, 1981; Marsik and Brake, 1983). Multiple drug resistance has been documented more often in disease causing strains of *S. epidermidis* than in skin colonizing strains (Archer, 1991).

Over the past several years microbiologist have searched for more rapid and efficient means of microbial identification. The identification and differentiation of microorganisms have principally relied on microbial morphology and growth variables. Advances in molecular biology over the past 10 years have opened new avenues for microbial identification characterization (Mullis and Falooma, 1987; Saiki *et al*., 1988). The study was undertaken for molecular characterization of CoNS from infected patients in and around Thanjavur hospitals by using RFLP method.

MATERIALS AND METHODS

The clinical specimens (pus, urine and others) were collected from infected patient in and around Thanjavur. Bacterial species were isolated by using Nutrient and Blood Agar medium. The organisms were then identified and observed by their morphological characteristic by using Gram's staining and biochemical characteristic by using Coagulase test, Carbohydrate fermentation test and Catalase test. Plasmid DNA and Genomic DNA were isolated by using Agarose Gel Electrophoresis method. Molecular typing of Genomic DNA of CoNS sp. was analyzed by RFLP method.

RESULTS AND DISCUSSION

The CoNS strains used in this study were isolated from pus smples (83%), urine samples (12.5%) and other sources (4.1%) (Table 1). The predominant isolates were Coagulase Positive *Staphylococcus* sp. (CoPS) followed by Gram negative organisms (Table 2). The type of pathogenic bacteria included CoNS, CoPS, *Staphylococcus* sp. and gram negative