SPECIATION AND ANTIBIOGRAM OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM CLINICAL SPECIMENS WITH SPECIAL REFERENCE TO BIOFILM PRODUCTION

By

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Dissertation submitted to

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requirements for the degree of

DOCTOR OF MEDICINE
In
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Date: **Dr. Raj Mohammed D Malled**

Place: Bijapur.

LIST OF ABBREVIATIONS

Agr	:	Accessory gene regulator
Aap	:	Accumulation associated protein
Bap	:	Biofilm associated protein
CoNS	:	Coagulase Negative Staphylococci
CVCs	:	Central venous catheters
ESS	:	Extracellular slime substance
FAME	:	Fatty acid Modifying enzyme
GGI	:	Gonococcal growth inhibitor
ica	:	intercellular adhesion
MR	:	Methicillin Resistance
MR-CoNS	:	Methicillin Resistant Coagulase Negative Staphylococci
MS-CoNS	:	Methicillin Sensitive Coagulase Negative Staphylococci
MLEE	:	Multilocus enzyme electrophoresis
MSCRAMMs	:	Microbial surface components recognizing adhesive matrix molecules
NICU	:	Neonatal intensive care unit
NVE	:	Native valve endocarditis
PSA	:	Polysaccharide adhesion
PIA	:	Polysaccharide intercellular adhesion
PSMs	:	Phenol soluble modulins
PBP	:	Penicillin binding proteins

PNAG	:	Poly N-acetyl glucosamine
PCR	:	Polymerase chain reaction
Slush	:	Staphylococcus lugdunensis synergistic hemolysins
SCC	:	Staphylococcal chromosomal cassette
TCA	:	Tricarboxylic acid cycle
UTI	:	Urinary tract infection

ABSTRACT

SPECIATION AND ANTIBIOGRAM OF COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATED FROM CLINICAL SPECIMENS WITH SPECIAL REFERENCE TO BIOFILM PRODUCTION

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INTRODUCTION

Coagulase Negative Staphylococci(CoNS) are among the most frequent isolated bacterial species, being regarded as nonpathogenic are now been recognized as relevant opportunistic pathogens. Biofilms produced by them is considered to be a virulent marker, responsible for poor therapeutic response. Widespread occurrence of Methicillin resistance poses a therapeutic problem pressing need for newer antimicrobial agents.

OBJECTIVES

To isolate and speciate Coagulase Negative Staphylococci and evaluate biofilm production. To determine antimicrobial susceptibility pattern with special reference to Methicillin resistance.

MATERIALS AND METHODS

130 Coagulase Negative Staphylococci were studied from various clinical specimens. Speciation was done by the method of five simple test scheme. Drug susceptibility was performed by Kirby Bauer's disk diffusion method and Oxacillin was used for determining Methicillin Resistance. Biofilm formation was detected by Christensen's Tube Method, and Congo Red Agar method.

RESULTS

S.epidermidis was the most frequent isolate, 77 (59.23%), followed by S. saprophyticus 16.1%, S.haemolyticus 6.9%, and others. Biofilm formation was detected in 72 (55.38%). Methicillin Resistance was seen in 68 (52.3%).

CONCLUSION

This study shows that Coagulase Negative Staphylococci which were considered non pathogenic are implicated in many infections. They are becoming more virulent and more resistant to commonly used antibiotics.

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INTRODUCTION

Coagulase Negative Staphylococci are among the most frequent isolated bacterial species in clinical microbiology. They colonize the skin, throat, nose, and represent a major part of the normal bacterial flora of healthy people. Although CoNS have long being regarded as nonpathogenic, they have now been recognized as relevant opportunistic pathogens[1]. The isolation of CoNS from non sterile sites is usually ignored or reported without further species level identification[2].

The range of infections believed to be caused by CoNS are wide and include bacteremia, especially in preterm low birth weight infants, immunocompromised individuals native valve endocarditis (NVE) and prosthetic valve endocarditis, osteomyelitis, pyoarthritis, peritonitis during continuous ambulatory dialysis, mediastinitis, prostatitis, infections of permanent pacemakers, vascular grafts and intravascular catheter, CSF shunts, and prosthetic joints and a variety of orthopedic devices and UTI[3,4,5].

CoNS are the most common of nosocomial primary bloodstream infections, accounting for about 31% of cases[6].

CoNS species comprises of *S.epidermidis, S.saprophyticus, S.haemolyticus, S.auricularis, S.capitis* subsp. *capitis*, *S.capitis* subsp. *urealyticus*, *S.caprae, S.hominis, S.lugdunensis, S.pasteuri, S.schleiferi, S.simulans, S.warneri, S.cohnii subspecies cohnii, S.sciuri* and *S.xylosus.*

S.epidermidis is by far the most frequently recovered organism accounting to about 50-80%. Almost all infections caused by S.epidermidis are nosocomially acquired and is implicated mostly in infections associated with indwelling catheters. It is also documented as a pathogen in bacteremia, endocarditis, UTIs, surgical wound

infections, infections of various prosthetic devices, CSF shunt infections, peritoneal dialysis related infections and ophthalmic infections[7].

Various strains of S.epidermidis are capable of forming biofilms and that is a major concern for people with catheters, heart valves and other implants[8].

In biofilms produced by S.epidermidis capsular polysaccharide adhesion (PSA) plays an essential role in initial bacterial adherence and polysaccharide intercellular adhesin (PIA) in the aggregation of cells, both of which are encoded by the ica (*intercellular adhesin*) operon[9]. Biofilm production is considered to be a virulence marker of S. epidermidis[8]. It is also responsible for poor therapeutic response and relapse[10].

The second most frequently encountered CONS species is *S.haemolyticus*. It has been implicated in native-valve endocarditis, septicemia, peritonitis, bones and joints and wound infections. *S.saprophyticus* causes urinary tract infection in young healthy sexually active women. *S.lugdunensis* has been implicated in endocarditis with massive valve destruction[7].

CoNS have historically been more resistant to antimicrobials, including the β-lactam antibiotics with 66% isolates resistant to methicillin[11,12]. The widespread occurence of methicillin resistance among them poses a therapeutic problem and the possibility of genetic exchange of the resistance between CoNS and S.aureus[13]. Cross resistance to other non- β-lactam antibiotics has been reported for the last 4 decades. Thus there is a pressing need for newer antimicrobial agents with good antistaphylococcal activity[11].

AIMS AND OBJECTIVES

1. To isolate and speciate Coagulase – negative Staphylococci
2. To evaluate biofilm production
3. To determine antimicrobial susceptibility pattern with special reference to methicillin resistance.

REVIEW OF LITERATURE

History

About 150 years ago, cocci were first observed in diseased tissues and in pus obtained from human abscesses.

In 1871, Von Recklinghausen named these organisms as “micrococci”.

In 1880, Pasteur and Ogston described the small spherical bacteria isolated from the pus of furuncles and abscesses and called them micrococci. Pasteur thought these bacteria were pathogenic, but it was Ogston who demonstrated in a convincing manner that they produced inflammation and suppuration[14].

In 1883, Sir Alexander Ogston gave the name Staphylococcus (Staphyle, in Greek, meaning ‘bunch of grapes’; kokkos, meaning a berry)[14].

Although Ogston was the first to recognize the importance of staphylococci in disease and to name them, Rosenbach got the credit for the generic name Staphylococcus and the species name aureus. In 1884, he was the first to isolate the organism, grow it in pure culture, and study its laboratory characteristics. He named the orange and white colony producers as Staphylococcus pyogenes aureus and Staphylococcus pyogenes albus, respectively[14].

In 1885, Passet described Staphylococus pyogenes citreus[14].

In 1891, the Staphylococcus epidermidis albus was proposed as an attenuated form of Staphylococcus pyogenes albus[14].

Unfortunately the name Staphylococcus was not accepted by others and most early classifications included these organisms in the genus Micrococcus, which had first been used in 1875 by Cohn[14].

In 1903, Loeb demonstrated S. pyogenes aureus was capable of coagulating goose plasma. This property of coagulation was initially ignored and it did not become a key character until 1957[14].

In 1905-1906, Andrews and Gordon proposed a classification of human staphylococci based on pigmentation and pathogenicity for guinea pigs; four species were recognized: S. pyogenes (orange, pale yellow, or white, highly pathogenic); S. epidermidis albus (white, feebly pathogenic); and two other species which were both white and nonpathogenic[14].

In Bergey's Manual of Determinative Bacteriology, from the first edition (1923) till the sixth edition (1948), the number of species listed under the genus Staphylococcus varied from none to nine. In the sixth edition, the Staphylococcus genus was deleted and all staphylococci were relegated to the genus Micrococcus. In the seventh edition (1957), the genus Staphylococcus was reintroduced. In addition, two species, S.aureus and S.epidermidis, were recognized on the basis of anaerobic utilization of mannitol and the production of coagulase by the former. In the eighth edition (1974), a third species S.saprophyticus was recognized. This coagulase negative organism was originally classified as Micrococcus[14].

According to Bergey's manual of systematic bacteriology 1986 edition, the family Micrococcaccae includes four genera, *Planococcus*, *Micrococcus*, *Stomatococcus*, and *Staphylococcus*. Subsequent genetic studies and chemotaxonomic analysis documented the diversity of these microorganisms and

established that these four genera should not be combined into a single family. Planococci and Staphylococci belong to the bacillus/lactobacillus, while Micrococci/Stomotococci are related to an assortment of amycelial actinomycetes[7].

According to the new edition of Bergey's manual of systemic bacteriology, the staphylococci are in the phylum Firmicutes and comprise of genus I in family V (Staphylococcacea) order I (Bacillales) in class III Bacilli[7].

The genus *Staphylococcus*, of the family Micrococcacae is separated into several species based on genotypic, biochemical and pathogenic characteristics. The exact criteria for dividing Staphylococci into species are continually evolving, and various investigators have applied the same species names to organisms with different characteristics. This redundant naming of distinct Staphylococcal species has been confusing for example, *S.epidermidis* in the broad sense originally referred to any of the coagulase negative staphylococci; currently the term *S.epidermidis* is more strictly defined to include only one of the biochemically distinct species of the coagulase negative Staphylococci[7].

When only two species, S.aureus and S.epidermidis were clearly defined, in the Bergey's seventh edition, it placed all CoNS into a single species which consisted of organisms with considerable heterogenicity[14].

The first to recognize specific types within the heterogenous group of CoNS was Biard-Parker. In 1963, he proposed a classification system for the Micrococci, dividing the catalase positive, Gram positive cocci into three genera: *Staphylococcus, Micrococcus*, and *Sarina*. The Staphylococci were divided into six sub groups based on biochemical characters like coagulase reaction, acetoin production, mannitol fermentation with acid production both aerobically and anerobically and phosphatase activity. This classification by Bergey's Manual and Biard-Parker into subgroups and

biotypes had further provided the basis for many other studies in the classification of CoNS[14].

In 1975, Kloos and Schleifer subdivided CoNS into nine different species on the basis of extensive morphological, physiological and biochemical characters and cell wall peptidoglycan and teichoic acids[14].

In 1982 six more species were identified bringing the number of CoNS to 15 as proposed by Kloos and Schleifer[14].

By 1985, there were 19 recognized species of CoNS, 8 of which exhibited a possible association with human infection[15].

By 2002, there were 32 CoNS species and 15 subspecies[16].

By 2005, the genus *Staphylococcus* was divided into 38 species and 17 subspecies, half which are indigenous to humans. Those that are most pathogenic in humans are S.aureus, S.epidermidis, S.haemolyticus, S.saprophyticus, S.lugdunensis and S.schleiferi[17].

By 2006, Coagulase Negative Staphylocci include 41 types/subtypes[18].

Currently several commercial kits are available for the identification of CoNS. The API Staph IDENT product uses a battery of 10 miniaturized tests that are inoculated with a heavy suspension of the organism to be identified and the result read in 5 hrs. The API Staph IDENT has a high degree of congruence (>90%) when compared with the conventional method of Kloos and Schleifer. The API ID32 Staph is a 24-hr strip system consisting of a panel of 32 cupulae and currently has 26 biochemical tests. The strip can be used with the automated bioMerieux ATB system, which includes a densitometer, an inoculator, a reader, a microcomputer and a

printer[7,14]. The Microbial Identification System (MIS) automates identification by combining cellular fatty acid analysis with computerized high resolution gas chromatography and the fully automated Riboprinter Microbial Characterization System based on ribotype pattern analysis. With these products, identification of most species and subspecies can be made with an accuracy of 70-90%[3].

Coagulase Negative Staphylococci may be sub classified by several techniques currently under investigation, including plasmid pattern analysis, slime production, phage typing, DNA homology, cell wall analysis and adherence to plastic. Of these methods, plasmid analysis appears to be the most promising for clinical use. Plasmid DNA profiles are determined by agarose gel electrophoresis and can be used as an epidemiologic marker for clinically important isolates. Production of slime, an extracellular polysaccharide, has also been shown to be associated with strains of CONS causing disease. Antibiotic resistance patterns are not a reliable means of sub classifying CONS, since these patterns may be unstable[15].

Epidemiology:

- In 1958, Smith and co-workers, noted the potential pathogenicy of CoNS by collecting data from patients with septicemia[5].
- In 1962, Pereira reported that a certain group of CoNS (now known as S.saprophyticus) caused UTI's[5].
- In 1965, Wilson and Stuart reported that CoNS were found in pure culture in 53 of 1,200 (4.4%) cases of wound infections[5].
- In 1971, Pulverer and Pallich found that in about 50% of all pyogenic lesions observed in hospital patients, CoNS were present in pure cultures[5].

- In the 1980s, the range of infections believed to be caused by CoNS, especially by S.epidermidis, was quite wide[5].
- In the US, bacteremia due to unspeciated CoNS have increased from 9% to 27% from 1980 to 1989[11].
- In 1970s, S.epidermidis accounted for less than 4% of pathogens recovered from nosocomial Infections, and by 1990s, the relative frequency with which CoNS were recovered from nosocomial infections had tripled to 11%[4].
- CoNS are now the most common cause of nosocomial primary bloodstream infections, accounting for about 30% of cases and are the second most common nosocomial pathogens recovered from surgical site infections, exceeded only by S.aureus[4].
- Since the development of prosthetic heart valves, CoNS have become one of the most common causes of endocarditis following cardiac valve surgery accounting for 30-67% of cases that present within 2 months of surgery[4].

Morphology :

Staphylococci are 0.5 – 1.5 μm, Gram positive cocci, arranged in clusters, aggregates of pairs, tetrads, and short chains; however when examined in pathological specimens or liquid culture, they may occur as single cells or pairs of cells. They are non motile and non spore forming. Capsule or slime layer (diffuse capsule) may be present more commonly in vivo – especially in colonization by S. epidermidis[3,7].

Species of CONS (Found in humans)[7].

S. Epidermidis, S.saprophyticus, S.haemolyticus, S.hominis, S.sciuri, S.xylosus, S.cohnii subspecies cohnii, S.auricularis, S.capitis subspecies capitis, S.capitis subspecies, urealyticus, S.caprae, S.lugdunensis, S.schleiferi, S.pasteuri, S.warneri, S.saccharolyticus.

The above mentioned species are pathogenic to human beings.

Species of CoNS and their usual Habitat:[15,16,19]

ORGANISM	**HABITAT**
S.epidermidis	Scalp of pre-adolescents, skin of face, axillae & groin
S.haemolyticus	Scalp of preadolescents
S.saprophyticus	Human skin, usually in perineal and inguinal areas
S.lugdunensis	Below the belt colonizer, especially inguinal and perineal
S.capitis	Head of adults, external auditory canal
S.hominis	Scalp of pre adolescents, axillae and groin
S.auricularis	Human external auditory canal
S.simulans	Scalp of preadolescents
S.warneri	Human Skin
S.scurii	Farm animals, rodents, marine animals and human skin
S.xylosus	Rodents, human skin, goat milk and cheese
S.cohnii	Human skin and non primates
S.intermedius	Dogs, minks, horses, cats
S.lutrae	Sea otters
S.hyicus	Cattle and cow's milk, pigs
S.casseolyticus	Cows milk

S.caprae	Human skin and urine
S.schleiferi	Human skin
S.pasteuri	Human skin
S.lentus	Sheeps and goats
S.saccharolyticus	Human mucous membranes

Cultural characteristics:[7,16]

Staphylococci grow readily on the media like blood agar, nutrient agar, tryptic soy agar, brain heart infusion agar, etc. Growth occurs within a temperature range of 10 - 42^{0}C, the optimum being 37^{0}C, and within pH 7.4 – 7.6. They are aerobes and facultative anaerobes.

- In liquid media, uniform turbidity is produced
- On Mac Conkey's medium, small pink colonies are seen
- On Blood agar, hemolytic as well as non hemolytic colonies are produced
- On Nutrient agar, tryptic soy agar, and brain heart infusion agar, colonies are large, convex, smooth, shiny, opaque, and easily emulsifiable.
- *S.epidermidis* colonies are small to medium,1 – 2 mm in diameter, translucent grey white, non haemolytic and the slime producing strains produce sticky colonies.
- *S.saprophyticus* colonies are large, entire, very glossy, smooth, opaque, butyrous, more convex, non haemolytic and half of the strains are pigmented.
- *S.haemolyticus* colonies are smooth, butyrous, white, opaque, β haemolytic and may be non pigmented or cream to orange in color.

- *S.lugdunensis* colonies are larger, smooth, glossly, non haemolytic cream to orange in color or non-pigmented, entire-edged and their centres are slightly domed.
- *S.hominis* colonies mimic the characteristics of *S.epidermidis* colonies.
- *S.warneri* mimics the cultural characteristics of *S. lugdunensis* colonies.
- *S. schleiferi* colonies are non-pigmented, non haemolytic, smooth, glossy, and slightly convex with entire edges.
- *S. simulans* colonies are large raised circular, non pigmented with entire edge, smooth slightly glistening, non-haemolytic or haemolytic colonies.
- *S. capitis* colonies are small to medium smooth, slightly convex opaque glistening with entire edge and non haemolytic colonies.
- *S. cohnii* colonies are small to large convex with entire edge, circular smooth glistening opaque pigmented or unpigmented, some show haemolysis.
- *S. xylosus* colonies are large raised to slightly convex circular smooth to rough, opaque, dull to glistening. Some colonies show yellow pigmentation, non haemolytic colonies.
- *S. sciuri* colonies are medium to large, raised, smooth glistening circular opaque haemolytic or non-haemolytic colonies.

Biochemical reactions:[7]

- *S.epidermidis:* is Catalase positive, modified oxidase negative, slide and tube coagulase negative, urea is hydrolyzed, acetoin is produced, alkaline phosphatase is produced, ornithine decarboxylase variable, nitrates reduced to nitrites, resistant to polymyxin B, sensitive to novobiocin, ferments glucose, maltose, sucrose, fructose, lactose and mannose.

- *S. saprophyticus*: Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, does not reduce nitrate to nitrites, does not produce alkaline phosphatase, produces acetoin, hydrolysis urea, ornithine is not decarboxylated, polymixin B sensitive, novobiocin resistant, ferments glucose, maltose, lactose, mannitol (variable), sucrose, trehalose and fructose.
- *S. haemolyticus*: Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, urea is not hydrolysed, acetoin is produced, nitrates are reduced, alkaline phosphatase is not produced, ornithine not decarboxylated, polymixin B sensitive, novobiocin sensitive, ferments glucose, maltose, sucrose, trehalose, fructose, and mannitol (variable).
- *S.lugdunensis*: Catalase-positive, modified oxidase-negative, slide coagulase-positive, Tube coagulase negative, ornithine decarboxylase positive, pyrolidonyl arylamidose activity present, urea hydrolysis variable, acetoin produced, alkaline phosphatase not produced, nitrates reduced, sensitive to polymyxin B and novobiocin. Ferments glucose, maltose, sucrose mannose and trehalose.
- *S. warneri*: Catalase positive, modified oxidase negative, slide and tube coagulase negative, urea hydrolysed, acetoin produced, alkaline phosphatase not produced, ornithine not decarboxylated, sensitive to polymyxin B & novobiocin, does not reduce nitrate to nitrites ferments glucose, fructose, maltose, sucrose, trehalose, mannitol (variable).
- *S. hominis*: Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrate reduction test variable, alkaline phosphatase not produced, urea hydrolysed, acetoin produced, sensitive to novobiocin and

polymixin B, ferments glucose, maltose, sucrose, fructose, trehalose and lactose (variable).

- *S.simulans*: Catalase-positive, modified oxidase-negative, nitrates are reduced to nitrites, urea hydrolysed, alkaline phosphatase variable, acetoin variable, slide and tube coagulase-negative, sensitive to novobiocin and polymyxin B, ferments fructose, mannose (variable), maltose (variable), lactose, sucrose, trehalose and mannitol.
- *S.schleiferi*: Catalase-positive, modified oxidase-negative, slide coagulase-negative, tube-coagulase positive (subspecies coagulans), acetoin produced, nitrates are reducted to nitrites, Pyrrolidonyl arylamidase activity present, urea not hydrolysed, alkaline phosphatase produced, sensitive to polymixin B and novobiocin, ferments glucose and mannose.
- *S. capitis*: Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrates are reduced to nitrites, alkaline phosphatase not produced, acetoin production variable, urease hydrolysis variable, sensitive to polmyxin B and novobiocin, ferments glucose, fructose, mannose, sucrose and mannitol.
- *S.cohnii*: Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrates are not reduced to nitrites, alkaline phosphatase not produced, urea not hydrolysed but *S.cohnii* subsp. *urealyticum* hydrolyses urea; sensitive to polymixin B, resistant to novobiocin, acetoin not produced, ferments fructose, mannose, maltose, trehalose and mannitol.
- *S. xylosus*: Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrates are reduced to nitrites, akalaine phosphatase produced, acetoin produced, urea hydrolysed, sensitive to polymixin B,

resistant to novobiocin, ferments fructose, mannose, xylose, arabinose, maltose, sucrose, trehalose and mannitol.

- *S. sciuri*: Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrates are reduced to nitrites, urea hydrolysed, resistant to novobiocin, sensitive to polymixin B, acetoin not produced, ferments glucose, sucrose, mannitol and trehalose.

Pathogenesis :

The most frequently encountered CONS species associated with human infections is *Staphylococcus epidermidis*, in particular in association with intravascular catheters. In addition, *S. epidermidis* is the predominant agent of nosocomial bacteremia, prosthetic-valve endocarditis, surgical wounds, central nervous system shunt infections, intravascular catheter- related infections, peritoneal dialysis-related infections, and infections of prosthetic joints[7].

Nearly 80% of the S.epidermidis contributes to the formation of Biofilms. They produce cell-surface and extracellular macromolecules that initiate and subsequently enhance bacterial adhesion to the plastic surfaces of foreign bodies to form a biofilm. Initial specific adherence to the plastic surfaces is mediated by capsular polysaccharide-adhesin called PS/A encoded by the *ica* locus of the S.epidermis genome[8,9].

Heilmann and co workers identified an autolysin-adhesin coded for by a gene called *altE* that works in concert with PS/A. Following initial adhesion to biomaterials, intercellular adhesion mediated by PIA is necessary for formation of

polysaccharide matrix biofilm. Like PS/A, the PIA structural genes also reside within the four gene chromosomal operon[8,9].

Some strains of *S.epidermidis* produce a fatty acid–modifying enzyme (FAME) that inactivates bactericidal fatty acids by esterifying them to cholesterol and inactivating fatty acids and allowing *S.epidermidis* to live on and in the skin for long period. Some strains produce lipases which help in skin colonization, biofilm formation and initiation of cutaneous infections. Another attachment mechanism used by *S.epidermidis* involves specific interactions with various serum, plasma and tissue components of the host, including connective tissue proteins like collagen and laminin and serum derived proteins like fibronectin and vitronectin. Such interactions may constitute the initial steps in tissue colonization and establishment of infections in the absence of foreign bodies such as catheters or shunts[18].

S. saprophyticus is regarded as a more important opportunistic pathogen in human UTIs, especially in young, sexually active females. It was considered to be the second most common cause of acute cystitis or pyelonephritis (10-11%) in these patients after *Escherichia coli* (80%)[7,14]. It accounts for 41-86% of upper urinary tract infection. In urine specimens from these patients, the organism is frequently present in quantities ≤1,00,000 CFU/ml, but will be detected in sequential specimens.

It is implicated in acute urethral syndrome, catheter associated urinary tract infections, prostatis in elderly men and rarely bacteremia, sepsis and endocarditis. In 1999, Hell and colleagues reported the isolation of *S.saprophyticus* as a cause of nosocomically acquired pneumonia[7].

S. saprophyticus also displays a tissue trophism specificity, i.e., for uroepithelial cells of the urogenital tract. The receptor-mediated adherence is believed to be the first major step in the development of UTI.

S. saprophyticus have the ability to produce Extracellular slime substance (ESS). Urea is essential for ESS production by *S. saprophyticus*. ESS of *S. saprophyticus* may be a risk factor for the development of urinary stones, especially in urine in which the urea concentration is high.

The *S.saprophyticus* virulence factors participating in the biofilm formation include, Autolysin Ass, which mediates adhesion to fibronectin on uroepithelial surfaces and haemagglutination. The surface fibrillary protein Ssp, functions as intercellular adhesion in formation of multilayered clusters.

Other virulence factors include urease which causes destruction of bladder tissue and bacterial invasion, lipase, elastase and FAME[5].

S. haemolyticus is the second most frequently encountered CoNS species in the clinical laboratory. This species has been implicated in NVE, septicemia, peritonitis, wound, bone, and joint infections, and UTIs[5].

These strains have relatively high MICs (from 2 to >8 mg/ml) for vancomycin, suggesting selection of resistant clones arising from previously susceptible organism populations. Strains of *S.haemolyticus* that are resistant to both glycopeptides (vancomycin and teicoplanin) have been reported. Glycopeptide resistance in *S.haemolyticus* is usually expressed heterogeneously with both susceptible and resistant sub-populations existing within a single culture. The reduced susceptibility of *S.haemolyticus* to teicoplanin is due to the substitution of glycine with other amino acids in peptidoglycan. The emergence of antibiotic resistance has underscored the

importance of correct identification and susceptibility testing of these isolates and monitoring of their spread within the hospital environment[7].

S. lugdunensis was first described in 1988, by Freney et al. in Lyon, France[20]. It has rapidly established itself as a significant human pathogen. It colonizes the human inguinal area and is predominantly involved in abscesses in the pelvic girdle region.

The infections caused by *S.lugdunensis* are more serious. It is implicated in native valve, prosthetic valve and pacemaker-associated endocarditis, meningitis, skin and soft tissue abscesses, cellulitis, peritonitis, infected hip prosthesis, osteomyelitis, vertebral diskitis, vascular line infections, oral infections, septic arthritis following arthroscopic surgery, UTIs, ventriculo-peritoneal shunt infections[7].

Oxacillin resistant strains of *S.lugdunensis* carrying the *mec*A gene have been reported and characterized.

S.lugdunensis produces a variety of virulence factors like α- and β-hemolysins, lipases and esterases[7]. It does not bind to fibrinogen like other CoNS; instead in produces peptides called S.lugdunensis Synergistic hemolysins (Slush)[18].

Staphylococcus warneri was first described in 1975 by Kloos and Schleifer. This species represents about 1% of the Staphylococci, normally found on human skin. It has been reported to cause bacteremia, infective endocarditis, cerebrospinal fluid shunt infection, subdural empyema, vertebral osteomyelitis, and UTI[21]. These strains produce an extracellular enzyme that has both lipase and phospholipase activity[7].

S. hominis is found on the skin of humans. It has been reported to cause endocarditis, catheter related sepsis in immune compromised hosts[7].

S.simulans is found on the skin and in the urethra of healthy women. It causes septicemia, osteomyelitis, native valve endocarditis, septic arthritis, vertebral osteomyelitis, and prosthetic joint infection. *S.simulans* possesses a capsule that inhibits phagocytosis and contributes to virulence[7].

S.schleiferi causes wound infections, bacteremia, indwelling catheter infection, nosocomial urinary tract infection, prosthetic valve endocarditis, and osteomyelitis. Virulence factors like glycocalyx, lipases, esterases, haemolysins are produced by this species which contribute to the pathogenicity[7].

S.capitis species is a part of the normal human flora and found surrounding the sebaceous gland on the scalp and forehead. In 1992 this species has been reported to cause native and prosthetic valve endocarditis and sepsis in critically ill premature infants[7].

S.cohnii is the normal flora on the skin. It is an emergent opportunistic agent causing community acquired pneumonia, catheter related sepsis in immuno compromised patients, chorioamniotis and neonatal sepsis with meningitis[7].

S.xylosus has been reported as a cause of upper and lower urinary tract infections, endocarditis with intravenous drug use[7].

S. sciuri is known to cause wound, soft tissue infections, endocarditis, UTI, septic shock, endophthalmitis, peritonitis. S.sciuri produces a variety of putative virulence factors like biofilm, exotoxins, DNAase and proteolytic activity[7].

SLIME

Slime production has been shown to play a major role in the pathogenesis of infections caused by CoNS. According to Koneman et al[7], CoNS also produce other virulence factors such as hemolysin, lipase, protease, urease, and DNAse.

Slime is the extracellular matrix of biofilm forming staphylococci, It is composed of deacetylated polymer of beta-1-6-linked *N*-acetylglucosamine called as polysaccharide intercellular adhesion (PIA), together with other polymers such as teichoic acids and proteins.

PIA biosynthesis is accomplished by the products of the ica gene locus, which comprises of an N-acetylglucosamine transferase (icaA and icaD), a PIA deacetylase (icaB), a putative PIA exporter (ica C), and a regulatory gene (ica R).

Slime plays an important role in non specific mechanism of antibiotic resistance[22]. Staphylococci encased in a matrix of slime are resistant to the action of antimicrobial agents.

In 1972, Bayston and Penny first observed that many strains of CoNS formed mucoid deposits on the cerebrospinal fluid shunts and speculated that mucoid growth is important in the pathogenesis of shunt infections. Later studies concluded that CoNS have a propensity to infect medical appliances and produce foreign body infections because they preferentially adhere to the surface of the appliances[23].

Slime is a marker of clinically relevant infections[24].

Factors affecting Slime production

Media containing glucose, casein (tryptone) and oleic acid enhances the adherence and addition of serum to the growth medium inhibits the adherence[23,24].

Nature of Slime

It is composed of carbon fragment (polysaccharide) and amino acid. The amino acid could be glutamine, a rich component of casein[23].

BIOFILMS

Biofilms have been described in many systems since Anton van Leuwenhoek exclaimed the animalcules in the plaque on his own teeth in the 17th century.

Biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum / interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced and exhibit an altered phenotype with respect to growth rate & gene transcription[25].

Biofilm formation is a developmental process in which bacteria undergo a regulated lifestyle switch from a nomadic unicellular state to a sedentary multicellular state where subsequent growth results in structured communities and cellular differentiation[22].

Staphylococci are recognized as the most frequent causes of biofilm - associated infections. This exceptional status among biofilm - associated pathogens is due to the fact that staphyloccoci are frequent commensal bacteria on the human skin and mucous membranes. Thus they are the most likely germs to infect any medical device that penetrate these surfaces.

Biofilm formation has been described in the acquatic system, those on the spectrum of indwelling medical devices and of living tissues such as tooth enamel, heart valves, lung and middle ear[25].

Biofilm formation

It is a multifactorial process which considers the nature of the substratum and the characteristics of the cell surface. The characteristics of the substratum may have a significant effect on the rate and extent of attachment by microorganisms. In general,

the rougher and more hydrophobic materials (Teflon, various plastics, latex and silicon) will develop biofilms more rapidly than the hydrophilic substances like glass and various metals.

The characteristics of cell surface like glycocalyx and cell surface hydrophobicity in non motile cells like staphylococci contribute for microbial attachment[25].

Biofilm formation involves two steps[22]

1. Initial attachment of bacterial cells to the polymer surface
2. Maturation phase, which involves
    a. Intercellular aggregation
    b. Biofilm structuring

1. Attachment :

Attachment to human matrix proteins represent the first step in biofilm formation. This is mediated by MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), expressed on the CoNS, that have the capacity to bind to human matrix proteins such as fibrinogen, or fibronectin, and other matrix proteins.

Other proteins contributing for initial adhesion are the accumulation associated protein Aap and several non covalently bound surface proteins, such as autolysin Alt, which harbor binding sites for human matrix proteins.

Host matrix proteins cover the devices soon after insertion and therefore the specific interaction between these proteins and MSCRAMMs most likely is of much greater importance for colonization.

Maturation phase:

a. Intercellular aggregation

Polysaccharide intercellular adhesin (PIA) also called poly-*N*-acetylglucosamine (PNAG), is the main molecule responsible for intercellular adhesion in Staphylococci. It is a partially deacetylated polymer of beta-1-6-linked N-acetylglucosamine which together with other polymers such as teichoic acids and proteins forms slime or glycocalyx.

Mechanism of intercellular adhesion of PIA:[22]

The deacetylation of N-acetylglucosamine residues in PIA introduces a positively charged character in the otherwise neutral molecule by liberating free amino groups that become charged at neutral or acid pH, such as found in the natural habitat of staphylococci, the human skin. As the bacterial cell is negatively charged, PIA works like glue that sticks the cells together by electrostatic interaction.

Teichoic acids may represent the negatively charged molecules that interact with PIA on the cell surface.

PIA biosynthesis:[22]

PIA biosynthesis is accomplished by the products of the *ica* gene locus, which comprises an *N*-acetylglucosamine transferase (*icaA* and *icaD*), a PIA deacetylase (*icaB*), a putative PIA exporter (*icaC*), and a regulatory gene (*icaR*). Expression of ica gene is regulated by a variety of environmental and regulatory proteins.

PIA independent biofilm formation has been demonstrated, as PIA production does not seem to be of universal importance for biofilm production.

Cofactors like accumulation associated protein, *Aap*, is implicated in the biofilm formation. Formation of fibril like structures on the S.epidermidis surface is dependent on *Aap*. Another protein called Biofilm associated protein, *Bap* is involved in adherence. Teichoic acid also contributes to the adherence as it binds to fibronectin coated surfaces.

b. Biofilm structuring

A mature biofilm has a three dimensional structure, described to consist of towers or mushrooms. In between those towers, there are fluid filled channels that have a vital function in delivering nutrients to cells in deeper biofilm layers.
Differential expression of biofilm exopolysaccharide PIA to some degree contributes to biofilm structuring.

Staphylococci use quorum sensing controlled surfactant peptides to structure biofilms. eg. Phenol-soluble modulins (PSMs). The expression of PSM beta peptides leads to the detachment of cell clusters, leading to the formation of holes in the early biofilm and thereby to biofilm structuring.

Regulation of biofilm formation:[22]

- Environmental influences:

  Anaerobiosis, iron limitation and high osmolarity upregulates biofilm formation

- Regulation of attachment factors:

  Upregulation of adhesion factors such as MSCRAMMs when the cell density is low, and increasing activity of *agr* quorum sensing system which abolishes the expression of the no longer needed colonization factors.

- Regulation of Exopolysaccharide (PIA) expression:

Upregulators:

- Anaerobiosis
- Iron limitation
- High NaCl
- High temperature
- Repression of the TCA cycle especially by the latter stressors.
- TCA cycle inhibitor fluoroacetate
- Subinhibitory concentration of tetracycline, quinipristine-dalfopristine
- Glucose (causes repression of icaADBC, but enhances PIA production)
- SarA, a global regulatory DNA-binding protein, activates transcription of icaADBC.

Downregulators:

- icaR
- LuxS - dependent quorum sensing system agr quorum sensing system.

Human infections involving biofilms[25]

a. Native valve Endocarditis:
    - When endothelium is damaged, a thrombus formation takes place which is composed of platelets, fibrin and occasionally red blood cells
    - Fibronectin, secreted by endothelial cells, platelets and fibroblasts in response to vascular injury binds to fibrin, collagen, human cells and bacteria
    - Bacteria adhere to the edge of the thrombus and begin to multiply and form microcolonies in the platelet- fibrin matrix.
    - Bacterial colonies develop fibrin capsules and eventually form biofilms.
b. Otitis media
c. Chronic bacterial prostatitis

Biofilms on medical devices[25]

i. Prosthetic heart valves:

Surgical implantation of prosthetic valves results in tissue damage, leading to accumulation of the platelets and fibrin at the suture site. Microorganisms adhere to the fibrin matrix by expressing fibronectin receptors.

Colonization is more on the sewing cuff fabric of prosthetic valve and the valve annulus into which the prosthetic valve has been sewn, leading to separation of the valve and the tissue resulting in leakage.

ii. Central venous catheter:

CVCs pose a greater risk of device related infections than any other indwelling device. Organisms that colonize the CVC originate either from the skin insertion site, migrating along the external surface of the device, or from the hub, due to manipulation by health care workers, migrating along the inner lumen. Because the device is in direct contact with the bloodstream, the surface becomes coated with platelets, plasma, and tissue proteins such as albumin, fibrinogen, fibronectin and laminin.

iii. Urinary catheters:

The organisms that attach to the catheter and develop the biofilm originate from one of the several sources.

a. Organisms are introduced into the urethra or bladder as the catheter is inserted
b. Organisms gain entry through the sheath of exudates that surrounds the catheter
c. Organisms travel intraluminally from the inside of the tubing or collection bag.

Extracellular Slime like Substance (ESS) of *S. saprophyticus* may be a risk factor for the development of urinary stones, especially in urine in which the urea concentration is high. The urease of *S.saprophyticus* has been shown to be a major factor required for invasiveness in bladder tissue and is probably one of the reasons why this species is sometimes associated with urinary calculi[5].

RELATION SHIP BETWEEN BIOFILM FORMATION AND DISEASE:

Biofilms play a role in infectious diseases, cystic fibrosis, periodonititis and indwelling medical devices.

Suggested mechanisms[25].

1) **Detachment of cells or cell aggregate:** Cell may detach from biofilm, due to cell division or cell growth within the biofilm. Increase in shear stress occurring as a result of change in direction or rate of flow may result in blood stream or urinary tract infection.
2) **Resistance to the immune system:** Extracellular slime interferes with the macrophage phagocytic activity. Antibodies produced are ineffective in mediating phagocytosis and elimination of bacterial cells growing in biofilm microcolonies.
3) **Provision of niche**: Bacteria exchange plasmids by conjugation within biofilm and resistance factors may be carried on plasmids. Conjugation is favoured in biofilm because of close proximity of cells. This is relevant in case of indwelling medical devices.

Resistance to antimicrobial agents[25]

The nature of biofilm structure & physical attributes of organism confer an inherent resistance to antimicrobial agents.

Mechanisms for antimicrobial resistance:

i. Decreased penetration of antimicrobials
ii. Slow growth of biofilm microbes
iii. Genetic exchange through plasmids by conjugation
iv. Physical changes, toxic metabolites, nutrient limitations & low concentration of oxygen.

Intervention strategies[25]

Biofilm producing organisms have a inherent resistance to antimicrobial agents. The increasing number of antibiotic- resistant strains highlights the need for effective preventive strategies.

Interventional strategies currently used for biofilm control will either:

- Prevent initial device contamination
- Minimize initial microbial cell attachment to the device
- Increase penetration of biofilm matrix by the antibiotics and kill the biofilm associated cells
- Remove the device
- Use of antitiseptic impregnated devices. The various antiseptics used for impregnating central venous catheters are
  - Chlorhexidine combined with silver sulphadiazine
  - Minocycline with rifampin
  - Tridecylmethylammonium chloride plus cephalosporin
  - Silver ions coating
  - Heparin coating

- Control strategies used to inhibit biofilm formation on urinary catheters include:
  - Antimicrobial ointment and lubricants
  - Bladder instillation or irrigation
  - Antimicrobial agents in the collection bags
  - Impregnating the catheters with silver oxide and other antiseptics
- Systemic antibiotics for prophylaxis in catheterized patients.
- Enzymatic dissolution of matrix extracellular polymeric substance
- Disruption of the quorum sensing system.

DRUG RESISTANCE:

The continued emergence of antimicrobial drug resistance is a serious problem for the antibiotic treatment of patients with staphylococcal infections[26].

The national nosocomial survey found that from 1980 to 1989 that the proportion of nosocomial CoNS resistant to methicillin, oxacillin, or nafcillin increased from 20 to 60%. Most of these methicillin-resistant CoNS were also resistant to multiple additional antimicrobial agents[27].

Multiresistant CoNS also commonly colonize the skin of hospitalized patients and hospital personnel. Widespread skin colonization serves as a potential reservoir for multiresistant isolates that can cause infections, particularly infections of indwelling intravascular devices. In addition, these colonizing isolates serve as a reservoir for antibiotic resistance genes that can transfer among CoNS and be acquired by Staphylococcus aureus[27].

Infections caused by methicillin-resistant *Staphylococcus* strains are difficult to treat. In some cases, the isolates are only susceptible to glycopeptides and new drugs, such as linezolid, tigecycline, daptomycin and quinupristin/dalfopristin[26].

The resistance of *Staphylococcus* species to oxacillin is mediated by the production of a supplemental penicillin-binding protein (PBP 2' or PBP 2a) that has a low affinity for semi-synthetic penicillins. The protein is encoded by the *mecA* gene and this gene is identical in all *Staphylococcus* strains. Thus, *mecA* is a useful molecular marker of oxacillin resistance. The *mecA* gene is carried on a specific mobile genetic element, called the staphylococcal cassette chromosome *mec* (SCC*mec*)[26].

Methicillin was the predominant semisynthetic penicillin used in early therapy so the term "Methicillin resistance" become a generic term for resistance to beta-lactam antibiotics based on the inability of these agent to bind to the new penicillin- binding protein product of *mecA* PBP2a[11].

Methicillin resistance:

Methicillin resistant strains of CoNS were 1st reported in 1960. Resistant strains were recovered from nasal cultures obtained from newborns in a nursery in which methicillin was aerosolized in an effort to reduce S. aureus infections. Shortly, thereafter, methicillin-resistant strains of CoNS were recovered from children with ventriculoperitoneal shunt infections[4].

Beta lactamase production: It is due to "hyperproduction" of beta-lactamase enzymes, resulting in slow hydrolysis of the semisynthetic penicillins. 80- 90% of CoNS produce an inducible beta lactamase[27].

Penicillin binding protein 2a (PBP2a):

This is the most important mechanism for resistance to beta-lactams. Resistance is mediated by production of altered penicillin binding protein 2a which has low affinity for the beta lactamase antibiotics. The protein is encoded by mecA gene and is identical in all staphylococci. The mecA gene is carried on a specific mobile genetic element, called staphylococcal cassette chromosome mec (SCC mec)[27].

Resistance to methicillin is associated with in vitro cross resistance to other antimicrobials. CoNS with mecA is associated with resistance to erythromycin, clindamycin and co-trimoxazole.

Laboratory diagnosis:

1. **Specimens:**[7]

   CoNS can be isolated from pus, swabs from wounds and burns, urine, blood, CSF, tracheobronchial secretions, peritoneal fluid, pleural fluid and other sterile body fluids, catheter tips, ear swabs, cervical swabs.

2. **Collection and Transport of specimens:**[28]

   Specimens are to be transported to the laboratory without delay.

3. **Processing:**

   The specimen is processed as follows.

a. Gram's strain:[7]

Gram positive cocci measuring 0.5-1.5µm in diameter arranged in clusters, small chains, and singles.

b. Culture:[28]

Samples are seeded on the Nutrient agar, Blood agar and Mac Conkey's medium for samples other than urine. Urine samples are seeded on CLED agar and Mac Conkey's medium and incubated at 37^0C for 24-48 hours.

c. Colony morphology:[29]

CoNS appear as small to medium, low convex, smooth opaque, gray white colonies most of them non-haemolytic and some haemolytic on sheep blood agar.

d. Identification:[7,28]

CoNS are identified based on catalase, slide and tube coagulase, urease, phosphatase, voges-proskauer, nitrate reduction, ornithine decarboxylase, deoxyribonuclease (DNAse), thermonuclease, and carbohydrate utilization tests.

Methods to detect Slime:

1) Tube method.
2) Congo red method
3) Spectrophotometric method
    a. Adherence test.
    b. Micro assay.
4) Transmission electron microscopy.

Tube method[23,30,31]

It is a semi qualitative assessment of slime detection.

Here trypticase soy broth with 1% glucose in tubes are inoculated with a loopful of microorganism from overnight culture plates, and incubated for 24 hrs at 37^0 C. The tubes are decanted and washed with phosphate buffer saline and dried. The dried tubes are stained with crystal violet (0.1%), or safranin or tryphan blue. Slime

formation is considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the interface is not considered of the slime production.

Congo red agar method[10,30]

It is a screening method for slime formation.

Congo red agar is used which consists of BHI 37gm/l, sucrose 50gm/l, agar 10gm/l, and congo red stain 0.8gm/l. Congo red stain is prepared as a concentrated aqueous solution and autoclaved at 121^{o} C for 15 min separately and added to the growth medium cooled to 55^{o} C.

The test culture isolates are inoculated on to the CRA plates and incubated aerobically for 24- 48 hrs at 37^{0} C.

The test is read positive if there are black colonies with a dry crystalline consistency. Weak slime producers are indicated by pink colonies with occasional darkening at the centre of colonies. Non slime producing organisms produce pink colonies.

Spectrophotometric methods.

Adherence test:[32]

Adherence of each isolate to smooth (quartz) surface is determined quantitavely using spectrophotometer.

Overnight cultures of bacteria in trypticase soy broth are diluted in 1:100 fresh trypticase soy broth and 1 ml of each is taken in a separate quartz cuvetts. After overnight incubation at 37^{o}C, the cuvetts are washed 4 times with phosphate buffer solution at pH 7.2 & then fixed with Bouins fluid and stained with crystal violet. Excess stain with crystal violet is removed by decanting the cuvetts first & then

rinsing them gently with tap water. The optical density of the stained bacterial film is read with the help of a spectophotometer at 570nm. The cut off OD is calculated as 3X standard deviation above the mean OD of 10 blanks stained exactly by similar procedure.

Microtiter plate method[30]

It is performed in a sterile, polystyrene 96-well flat bottomed plates. Isolates from fresh agar plates are inoculated in TSB with 1% glucose and incubated for 18 hrs at 37^0C in stationary conditions and diluted 1 in 100 with fresh medium. Individual wells are filled with 0.2ml aliquots of the diluted cultures and only broth serves as control. The tissue culture are incubated for 18 and 24 hrs at 37^0C. After incubation, content of each well is gently removed by tapping the plates. The wells are washed four times with 0.2 ml of PBS to remove free floating planktonic bacteria. Slime formed with adherent sessile bacteria are fixed with sodium acetate 2% and stained with 0.1% of crystal violet. Excess stain is rinsed off by through washing with de ionized water and plates are dried. Adherent Staphylococcal cells and formed slime are uniformly stained with crystal violet. Optical density of stained adherent bacteria is determined with a microELISA autoreader at wavelength 570 nm. These OD values are considered as index of bacteria forming slime.

Transmission electron microscopy:[23]

Organisms are incubated in trypticase soy broth at 37^o C overnight. Then they are washed in PBS and fixed in 2.5% glutaraldehyde in 0.1ml PBS for 30min at 4^oC.The contents are centrifuged at 120g, pellets are collected and resuspended in PBS and centrifuged again. Then pellet is postfixed in 1% osmium tetroxide for 30min, dehydrated in ascending grades of acetone, filtrated & embedded in araldite

CY212. Thin sections of 60-70nm are cut, stained with aranyl acetate and alkaline lead citrate for 1 min and observed under transmission electron microscope.

Detection of *ica* ADBC genes[33]

Genomic DNA is extracted by boiling. Several colonies from an overnight grown culture on nutrient agar, are resuspended in 250µl double distilled water and placed in a boiling water bath for 20 mins before centrifugation at 12000 x g for 5 min. Detection of ica ADBC gene cluster is carried out by amplification of a DNA region partially spanning the icaA, icaD, and icaB genes to yield a 546 bp amplification product.

The primers used are: icaADB-F 1893-TTATCAATGCCGCAGTTGTC-1913 and icaADB-R 2388-GTTTAACGCGAGTGCGCTAT-2408. Reaction mixtures (25µl) contain 10µl genomic DNA, 40pM of each oligonucleotide primer, 0.2 mM dNTP mix, 1.25 u Taq polymerase, 10mM Tris – Hcl, and 1.5 M $Mgcl_2$.

Detection and measurement of biofilms[25]

1) **Measurement of biofilms by viable count**: Biofilm associated cells are removed by mechanical force, vortexing, sonication and then cells are plated on to solid medium & counted.

Methods:

- Roll plate technique
- Cather tip in PBS is votexed, plated
- Catheter tip in tripticase soy broth, sonicated, vortexed and cultured
- Alginate swab

- Endoluminal brush introduced into the implanted catheter, PBS, sonicated & plated.

2) **Microscopy :**

i. **Scanning electron microscope**:

This technique utilizes graded solutions (alcohol, acetone, and xylene) to gradually dehydrate the specimen prior to examination. The dehydration process results in significant sample distortion and artefacts; the extracellular polymeric substances, will appear as fibers than as a thick gelationous matrix surrounding the cells.

ii. **Transmission electron microscope**:

With specific polysaccharide stains like ruthenium red, allows both to identify the nature of extracellular fibers in biofilms and elucidate their association with cells.

iii. **Confocal laser scanning microscope**:

Examines biofilms in situ without the limitations of scanning electron microscope.

iv. **Epifluorescence microscope**:

Biofilms are stained with fluorescent nucleic acid stains such as acridine orange, Syto 9, 4,6-diamidino-2-phenylindone.

v. Other methods :

- Fluorescent antisera and fluorescent in situ hydridization probes
- Total protein absorbance at either 550 nm or 950 nm
- Tryptophan fluorescence

e. Antibiotic Susceptibility Testing:[34]

CoNS are tested for penicillin, ampicillin, cefotaxime, cephalexin, cefuroxime, piperacillin, cefoperazone, tetracycline, gentamycin, amikacin, ciprofloxacin, norfloxacin, ofloxacin, clindamycin, vancomycin, teicoplanin, erythromycin, azithromycin, linezolid, trimethoprim-sulphamethoxazole by Kirby Bauer disk diffusion method.

f. Detection of Methicillin resistance:[35,36]

The following methods are used for Methicillin detection:

1. Cefoxitin disc diffusion test:[35]

The isolates are subjected to cefoxitin disc diffusion test using a 30µg cefoxitin disc. A 0.5 Mac Farland standard suspension of the isolate is made and the lawn culture done on MHA plate. Plates are incubated at 37^0C for 18 hrs and zone diameters are measured. An inhibition zone diameter of ≤ 19mm are reported as oxacillin resistant and ≥ 20mm are reported as sensitive.

2. Oxacillin screen agar:[35]

MHA plates containing 4% NaCl and 6µg/ml of oxacillin are inoculated with 10µl of 0.5 Mc Farland of the isolate by streaking in one quadrant and incubated at 37^0 for 24 hrs. Plates are observed carefully in transmitted light for any growth. Any growth is considered as oxacillin resistant.

3. Broth Dilution method:[36]

The national committee for clinical laboratory standards recommended the use 2% NaCl, an inoculums of 5X10 CFU/ml and incubation for 24 hours at 35^0C for oxacillin, nafcillin or methicillin.

4. **Fluorescence Test:**[37]

It is a commercial qualitative screening test. A fluorescence indicator detects oxygen consumption by actively growing organisms in a microtiter panel. The first well, which is used as a growth control, does not contain any antibiotic, the second well contains oxacillin at a concentration of 4 mg/ml and the third well, as a negative control, contains vancomycin at a concentration of 16 mg/ml. After incubation the bacteria susceptible to oxacillin do not fluoresce, while resistant organisms, as a result of their metabolic activity, do fluoresce.

5. **Strip test:**[28]

Inoculated plates of isosensitest agar should be streaked with the organisms to be tested as well as controls. As many as five streaks per plate can be used. Methicillin (25 mg) strip is then put perpendicularly across the streaks, and incubated at 30^0 C for 18 hours.

6. **The E test:**[28]

The E test consists of strips impregnated with a continuous gradient of antibiotic which is laid on a plate inoculated with the test organism. An elliptical zone of growth inhibition is obtained after incubation and the MIC is read where the ellipse intersects the strip.

7. **Latex slide agglutination for PBP2a:**[36]

This is a rapid test performed according to the manufacturer's instruction. Bacterial cells (5ml) are obtained from a fresh sub culture and suspended in 4 drops of extraction reagent 1 and boiled for 3 min. The suspension is allowed to cool to room temperature, after which 1 drop of extraction reagent 2 is added and the mixture is vortexed thoroughly. The suspension is then centrifuged at 1500 X 9 for 5 min. A 50 µl aliquot of the supernatant is mixed with 1 drop of anti PBP2a

monoclonal antibody sensitized latex beads, placed on a shaker and gently mixed for upto 15 min. The resulting agglutination pattern is read at l0 min.

8. PCR amplification of the *mec A* gene:[35]

It is the gold standard for the detection of MR CoNS. DNA extraction is performed by QIAamp minikit (QIAGEN). The mec A gene is amplified using the primer 5'-GTAGAAATGACTGAACGTCCGATAA- 3'. A 50 µl PCR reaction mixture consists of 45µl of master mix containing PCR buffer (1X), dNTP mix (0.2 mM of each), primer 0.5 µM, Taq DNA polymerase (0.25 units), and $MgCl_2$ (1.5mM) with 5 µl of template DNA. Cycling conditions are - hot start at 94^0C for 4 minutes followed by 30 cycles of denaturation at 94^0C for 45 seconds, annealing at 50^0C for 45 seconds, and extension at 72^0C for 1 minute, and final extension step at 72^0C for 3 minutes. PCR products are visualized on 2% agarose gel with ethidium bromide dye under UV transilluminator. Amplicons of 310 base pairs are consistent with *mec A* gene amplifications.

TYPING METHODS

Several different epidemiological typing methods have been applied to study CoNS. Typing techniques are used in tracking sources, pathways of spreading infections and in determining genetic relatedness[14,38]. They have been used in the strain identification for establishing the clinical significance of CoNS[39].

The typing methods include:[14, 38,39,40,41]

1. Phenotypic methods:

a. Antibiogram typing: [14,38]

It involves the comparison of susceptibilities of isolates to a range of antibiotics. Isolates which differ in their susceptibilities are considered as different strains.

The identification of an unusual pattern of antibiotic resistance among isolates from multiple patients is considered as an indication of outbreak. The technique is easy to perform, inexpensive, gives rapid results and is easily available in routine microbiology laboratory. But has a poor discriminatory power and resistance pattern is influenced by a variety of factors.

b. Phage typing:[14,38]

Strains are classified according to their susceptibility to a set of phages selected. The technique requires maintainence of biologically active phages and is available only at reference centres. It has fair amount of reproducibility, discriminatory power, ease of interpretation and is cost effective. However it is time consuming and technically demanding. Valuable in identification of known epidemic strains among endemic strains.

c. Serotyping:[14,38]

It is based on the fact that the strains of the same species can differ in the antigenic determinants expressed on the cell surface. It is performed using several serological tests such as bacterial agglutination, co-agglutination and enzyme labeled assays. It is reproducible but has poor discriminatory power.

d. Biotyping:[7,14,38]

It makes the use of patterns of metabolic characteristics expressed by an isolate. It can be used alone or in combination with the antibiogram as a means of distinguishing strains of CoNS[7]. Several different biotyping systems were proposed for both taxonomic and epidemiological purposes, including those by Biard Parker, Holt and several others.

Of the various biotyping schemes, only the Biard-Parker scheme has received much attention. It deals primarily with strains from non clinical sources and in general places more emphasis on taxonomy[7].

e. Protein electrophoretic typing[14,38]

i. Whole cell protein typing:

In this method the proteins are extracted from the culture of a strain, separated by sodium dodecyl sulfate – polyacrlamide gel electrophoresis (SDS-PAGE) and stained to compare with those of other strains. A large number of bands are produced in whole cell protein electrophoresis but the differences, even between unrelated strains are small leading to poor discrimination.

ii. Immunoblotting:

In immunoblotting the electrophoresed products of SDS-PAGE are transferred to nitrocellulose membrane and then exposed to antisera raised against specific strains. The bound antibodies are then detected by enzyme-labelled anti-immunoglobulins. By this method though the number of bands produced are less as compared to whole cell protein electrophoresis, discriminatory power is still quite low.

iii. Multilocus enzyme electrophoresis (MLEE):

It involves extraction of enzymes from the strain, their separation by electrophoresis and examination by selective staining. This technique is labour extensive and the discriminatory power depends upon the number of enzymes extracted.

2. Genotypic methods:

They are more discriminative than phenotypic methods for the characterization of staphylococci[40].

a. Plasmid DNA analysis:[14,38]

In this technique the isolates are differentiated according to the number and sizes of plasmids they carry. The method is easy to perform and interpret, but poor in discrimination. It is also less reproducible due to existence of plasmids in different molecular forms such as supercoiled, nicked or linear, each of which migrates differently on electrophoresis. Related strains can exhibit different plasmid profiles since plasmids can be spontaneously lost or readily acquired. Some isolates may lack plasmids and will not be typeable by this method. The technique has not been found to be useful for the investigation of outbreak infections because of inherent mobility of extra chromosomal DNA and unstable plasmid profile of strains.

b. Southern blot analysis of RFLP:[38]

Here the restriction fragments which are generated by the digestion of DNA by endonucleases are separated by gel electrophoresis and transferred on to nitrocellulose membranes. The fragments containing specific sequences are then detected by labeled DNA probes. Variations in the number and sizes of the fragments detected are referred to as restriction fragment length polymorphism. Discrimination is better but the method is technically complex and the patterns generated are difficult to interpret.

c. Ribotyping: (rRNA restriction fragment length polymorphism)[38,40]

Is based on differences in the genes encoding the 16S, 5S, and 23S rRNA and flanking DNA. It is also based on southern hybridization, which involves the blotting of restriction enzyme digestion of ribosomal RNA and insertion sequences. Radiolabelled or biotinylated probes are used. Restriction enzyme

usually is Eco R1. The technique is reproducible but time consuming and technically demanding.

d. Pulsed field gel electrophoresis (PFGE):[38,41]

It is widely adopted method for typing. It is a variation of conventional agarose gel electrophoresis in which the orientation of the electric field across the gel is changed periodically. This modification enables large fragments to be separated accordingly to size, minimizing the overlapping of fragments.

Here, the Chromosomal DNA from the CoNS isolates is prepared and the DNA containing disks are restricted overnight with Sma I at 37^0C and loaded in a gel and run for 20 hours at 11.3^0C in a counterclamped homogenous electric field apparatus. Digestion patterns are visualized by staining with ethidium bromide.

All strains are typeable by this method. The technique is highly discriminatory and reproducible. The major difficulties associated with PFGE are the technical demands of the procedure, cost of the reagents and equipments and the time required to perform the test. Interpretation is little difficult.

MATERIALS AND METHODS

The present study was undertaken at the department of Microbiology, BLDEU's Shri B.M.Patil Medical College and Hospital, Bijapur during the period from 01-01-2012 to 30-06-2013. A total of 130 Coagulase negative Staphylococci isolated from various clinical samples were included in the study based on the inclusion and exclusion criteria mentioned below.

INCLUSION CRITERIA

Coagulase negative Staphylococci that will be isolated repeatedly from subsequent samples of the same patient will be included.

EXCLUSION CRITERIA

Coagulase negative Staphylococci which will not be re-isolated from repeat specimens from non-sterile sites, of the same patient will be excluded.

I. Specimens :

The specimens collected were pus, swabs from wounds and burns, blood, urine, ear swabs, endotracheal tubes, pleural fluid, CSF, cervical swabs and semen.

II. Transport :

Specimens were transported to the laboratory without delay.

III. Processing :

The specimens were processed as follows.

1. **Gram stain**:

The staining was done by using Modified Hucker's method[28].

Gram Positive Cocci arranged in clusters, short chains and single cells were seen.

Quality control:

Positive control: *Staphylococcus aureus* ATCC 25923

Negative control: *Escherichia coli* ATCC 25922

2. **Culture :**

The samples were seeded on Nutrient agar, Blood agar and Mac Conkey's medium for samples other than urine. Urine samples were seeded on CLED agar and Mac Conkey's medium. The media were incubated at 37^0C for 24-48 hours.

Colony morphology:

The colonies were identified based on size, shape, color (pigmentation), consistency, surface, and haemolysis.

IV. Identification:

The isolates were identified by a battery of tests given below.

1. **Catalase test:**[7]

Principle: The enzyme catalase mediates the break down of hydrogen peroxide (H_2O_2) into oxygen and water. Prompt effervescence indicates catalase production.

Procedure: A small amount of culture to be tested was picked from nutrient agar plate with a clean, sterile glass rod and inserted in hydrogen peroxide solution placed in a sterile tube.

Interpretation: Prompt effervescence indicated a positive reaction and negative reaction when there were no gas bubbles.

Quality control:

Positive control: *Staphylococcus aureus ATCC 25923*

Negative control: *Streptococcus pyogenes*

2. **Coagulase test:**

a. Slide Coagulase test[28]

Principle: This test detects bound coagulase (clumping factor) which is a surface component that causes the organism to clump when mixed with plasma. This factor reacts directly with fibrinogen in plasma causing rapid cell agglutination.

Procedure: Staphylococcal colony was emulsified in a drop of saline on a slide with minimum spreading. If the isolate did not form a smooth milky suspension, the test was not processed further. Similar suspensions of positive and negative controls were made. Flamed and cooled inoculating loop was dipped into the undiluted plasma and stirred in the staphylococcal suspension. Same procedure was repeated with the control suspension.

Interpretation:

Test was read as positive when a coarse clumping of cocci was visible to the naked eye within 10 seconds and considered as negative when there was absence of clumping within 10 seconds or a slow reaction seen after 10 seconds. Negative and slow reacting strains were re-examined by the tube test.

b. Tube Coagulase test:[28]

Principle: It detects free coagulase, a thrombin like substance, present in culture filtrate. Free coagulase reacts with serum substance (coagulase reacting factor) to form a complex that, in turn, reacts with fibrinogen to produce the fibrin clot.

Procedure: 1 in 6 dilution of plasma in saline (0.85% NaCl) was prepared and 1ml of this was placed in test tubes. Colony of the staphylococcus under test was emulsified in it. With each batch of tests, tubes with known coagulase positive and negative controls and a tube of unseeded diluted plasma to confirm that it did not coagulate spontaneously were included. Tubes were incubated at 37^0C, in a water bath, for up to 4 hrs. At 1, 2 and 4 hr intervals tubes were examined for clot formation by tilting the tube through 90^0. Negative tubes were left at room temperature overnight and re-examined.

Interpretation:

Positive: Any degree of visible clot formation.

Negative: When plasma remains wholly liquid or showed only a flocculent or ropy precipitate.

Quality Control:

Positive: *Staphylococcus aureus ATCC 25923*

Negative: Coagulase negative *Staphylococcus*

3. **Urease test:**[7]

Principle: This test determines the ability of microorganism to produce the enzyme urease. The occurrence of this enzyme can be tested by growing the organism in the presence of urea and testing for alkali (NH_3) production by using a suitable pH indicator.

Medium: Christensen's urease medium.

Method: A drop of 4-6 hours old broth culture was inoculated heavily over the entire slope surface. The medium was incubated at 37^0C and examined after 4 hrs and after overnight incubation.

Interpretation: The test was read as positive when the medium turned purple-pink and negative when there was no change in color.

4. **Phosphatase test:**[7]

Principle: The test is used to determine the ability of the organism to produce phosphatase enzyme. This enzyme acts on phenolphthalein diphosphate in the medium to liberate free phenolphthalein, which when exposed to ammonia vapours gives pink color.

Medium used: Phenolphthalein phosphate agar.

Procedure: Inoculum was spread over the plate to a width of 2-3 mm and incubated aerobically at 37^0C for 18 hrs. Few drops of ammonia solution were placed in the lid of petridish and covered with the plate.

Interpretation: If the colonies turned pink, when exposed to ammonia vapours within few minutes indicated production of phosphatase. No development of colour indicated negative reaction.

5. Voges – Proskauer test:[42]

Principle: The test is performed to determine the ability of the microorganism to ferment glucose with the production of acetyl methyl carbinol (acetion).

Medium: Glucose phosphate broth.

Method: Five ml of the medium was inoculated with the test isolate and incubated at 37^0C for 72 hrs. Subsequently 0.2ml of 40% potassium hydroxide

and 0.6ml of 5% α- naphthol solution were added and the contents were shaken vigorously for 30 seconds.

Interpretation: Reddening of the supernanant within 5-10 mins due to acetoin production was read as positive reaction. A negative reaction was indicated by the development of yellow color.

6. **Nitrate reduction test:**[7]

Principle: The test is used to determine the ability of an organism to reduce nitrate. The reduction of nitrate to nitrite is determined by adding sulfanilic acid and alpha-napthylamine. The sulfanilic acid and nitrite react to form a diazonium salt. The diazonium salt then couples with α-napthylamine to produce a red, water soluble azo dye.

Procedure:

One loopful of the test organism was inoculated in nitrate broth and incubated for 48 hours at 37^0C in ambient air. At the end of incubation 1ml of alpha-napthylamine & 1 ml of sulfanilic acid were added.

Interpretation:

Positive reaction was indicated by development of red colour within 30 secs. No colour indicated that nitrate was not reduced.

Quality control:

Positive control: *Escherichia coli ATCC 25922*

Negative control: *Acinetobacter baumannii ATCC 19606*

7. **Ornithine Deccarboxylase test (ODC):**[28]

Principle: This test is based on the ability of the organism to decarboxylate Ornithine.

Medium: Moeller's Ornithine Decarboxylase medium

Method: The test isolate was stab-inoculated into the medium, overlaid with a 5mm layer of sterile liquid paraffin, and incubated at 35^0C for 24 hours.

Interpretation: The production of violet color was read as positive and yellow color was read as a negative reaction.

8. **Carbohydrate utilization test:**[42]

Principle: The test is used to determine the ability of an organism to ferment a specific carbohydrate which is incorporated in a basal medium, and to produce acid or acid with visible gas. Sugars tested included Mannitol, Mannose, Maltose, Lactose, Sucrose, and Trehalose.

Medium: Sugar 1.0gm, Nutrient broth base 100ml, Phenol Red indicator 1.2 ml.

Method: The medium was inoculated with the isolate and incubated at 37^0C for 24 hrs.

Interpretation: A positive test was shown by yellow colouration of the medium; no color change was read as negative reaction.

9. **Polymixin – B sensitivity test:**[7]

It helps in identification of CoNS.

Medium: Mueller Hinton agar.

Procedure: A suspension of the organism equivalent to 0.5 McFarland turbidity standard was prepared in saline and swabbed onto the plate. A Polymixin B disk (300 units) was applied to the inoculum and the plate was incubated overnight.

Interpretation: zone of ≥10 mm was considered as susceptible whereas a zone of ≤10 mm as resistant.

10. Novobiocin sensitivity test:[7]

This test differentiates S. saprophyticus from other CoNS.

Medium: Mueller Hinton agar.

Method: A suspension of staphylococcal isolate, equivalent to 0.5 McFarland turbidity standard was prepared and spread over the plate by using a sterile swab. A 5µg Novobiocin disc was placed aseptically over the inoculum and the plate was incubated for 24 hrs at 35^0C.

Interpretation: The isolate was distinguished as S. saprophyticus if the inhibition zone was < 12 mm in diameter and other CoNS if the inhibition zone was > 12 mm in diameter.

V. Antibiotic sensitivity testing:[28]

All the isolates were tested for the following antibiotics according to CLSI 2007 criteria: Ampicillin 10mcg/disc, Cefuroxime 30mcg/disc, Cefotaxime 30mcg/disc, Azithromycin 15 mcg/disc, Gentamycin 10mcg/disc, Piperacillin-Tazobactum 100/10µg, Co-trimoxazole 25 mcg/disc, Tetracycline 30mcg/disc, Ciprofloxacin 5mcg/disc, Cefoperazone-sulbactum 75/10µg, Teicoplanin 30mcg/disc, Clindamycin 2mcg/disc, Amikacin 30mcg/disc, Vancomycin 30mcg/disc and Oxacillin 1mcg/disc, by Kirby- Bauer's disc diffusion method.

Procedure: Growth from an 18-24 hour culture was inoculated into peptone water using a sterile inoculating needle. The broth was incubated at 37^0C for

approximately four hours. The turbidity of the broth was matched with the turbidity of the 0.5 McFarland turbidity standards (i.e. 1.5 x 10^8 CFU/ml).

A swab was immersed into the broth, rolled and squeezed against the sides of the tube to remove excess broth. The swab was then used to inoculate the plate of Mueller-Hington agar, in 3 different directions to ensure an even and complete distribution of the inoculum over the entire plate. The plates were allowed to dry for 3-5 minutes. With the help of a sterile forceps, the antibiotic discs were applied within 15 minutes of inoculation of plate and the plate was incubated in inverted position overnight.

Commercially obtained Hi media discs were used. The strength of discs used and their interpretative zone size were read according to guidelines by NCCLS 2007[34].

VI. Detection of Methicillin resistant CoNS (MRCoNS):[34]

Procedure: Methicillin resistance was detected using Oxacillin by disc diffusion method. 1mcg Oxacillin disc was applied to the MHA plate swabbed with the test strain adjusted to 0.5 McFarland turbidity standard and incubated at 35^0C for 24 hours.

Interpretation:

A zone of ≥18mm was considered as sensitive, whereas a zone of ≤17mm was considered as resistant.

VII. Detection of slime production**:**

a. Christensen's Tube method[23,30,31]

It is a semi qualitative assessment of slime detection.

Procedure: Trypticase Soy Broth with 1% glucose were inoculated with a loopful of growth, in tubes and incubated at 37^0 C for 24 hrs. The tubes were decanted and washed with Phosphate buffer saline and dried. The dried tubes were stained with crystal violet (0.1%) for 5 minutes.

Interpretation:

Slime formation was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the interface was not considered of the slime production.

b. Congo red agar (CRA) method[10,30]

It is a screening method for slime formation.

Procedure: The isolates were inoculated on to the CRA plates and incubated aerobically at 37^0 C for 24- 48 hrs.

Interpretation: Positive result was indicated by black colonies with a dry crystalline consistency. Colonies of non slime producers usually remained pink.

Statistical analysis

The statistical analysis for comparison of different methods for slime production was done by Fischer's exact test using SPSS software.

Biochemical Characters of CONS

Species	*Coagulase*	Clumping Factor	V-P Test	Nitrate Red	Urease	*Phosphatase*	Novabiocin	Polymixin – B	ODC	Mannitol	*Maltose*	*Mannose*	*Lactose*	Sucrose	*Trehalose*
S.epidermidis	-	-	+	+	+	+	**S**	R	V	-	+	+	V	+	-
S.saprophyticus	-	-	+	-	+	+	**R**	**S**	-	V	+	-	V	+	+
S.hemolyticus	-	-	+	+	-	-	**S**	**S**	-	V	+	-	V	+	+
S.lugdunensis	-	+		+	V	-	**S**	**S/R**	+	-	+	+	+	+	+
S.schleferi	-	+	+	+	-	+	**S**	**S**	-	-	-	+	-	-	V
S.capitis	-	-	V	V	-	-	**S**	**S**	-	+	-	+	-	+	-
S.cohnii	-	-	V	-	-	-	**R**	**S**	-	V	V	V	-	-	+

PHOTO 1: SLIDE COAGULASE

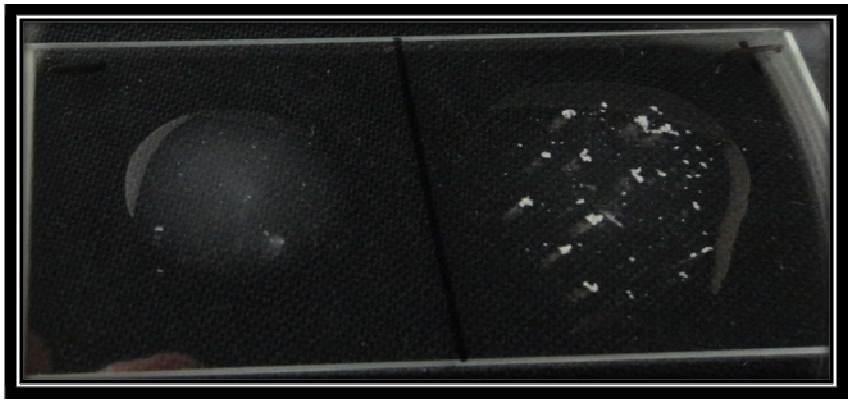


PHOTO 2: TUBE COAGULASE

PHOTO 3: MANNITOL SALT AGAR

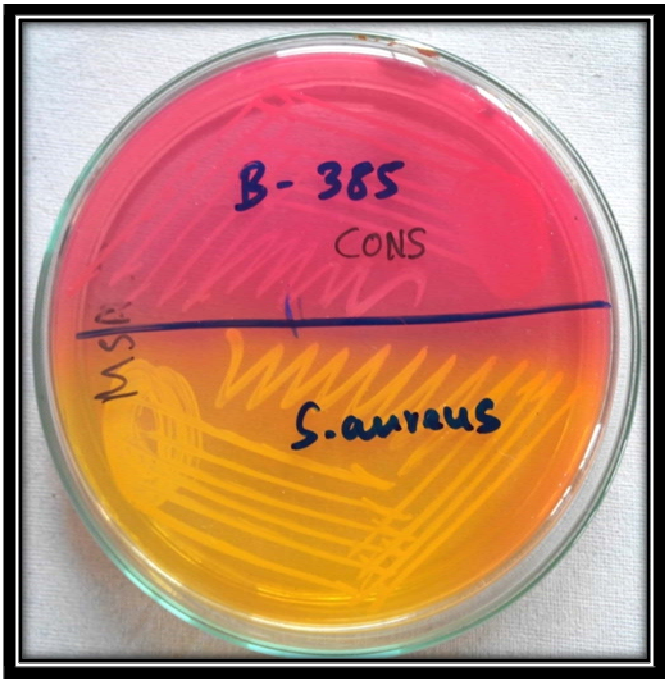


PHOTO 4: PHOSPHATASE AGAR

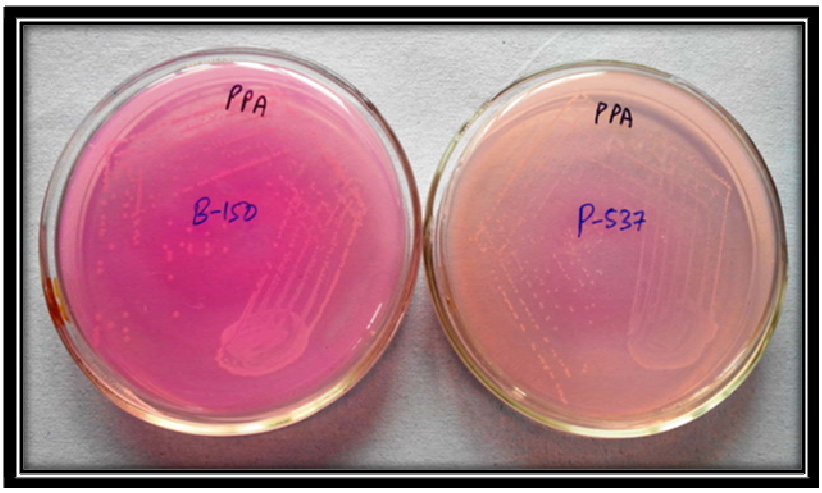


PHOTO 5: SUGAR UTILIZATION TEST

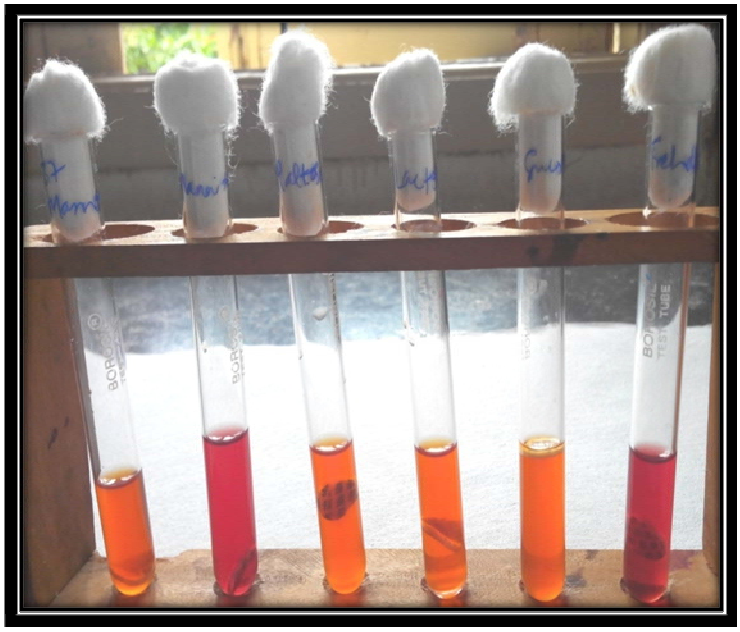


PHOTO 6: NITRATE REDUCTION TEST

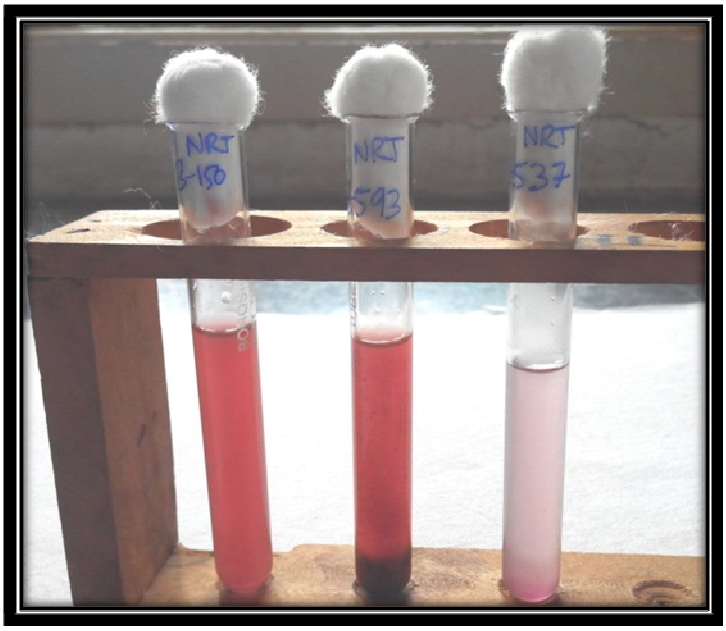


PHOTO 7 : UREASE TEST

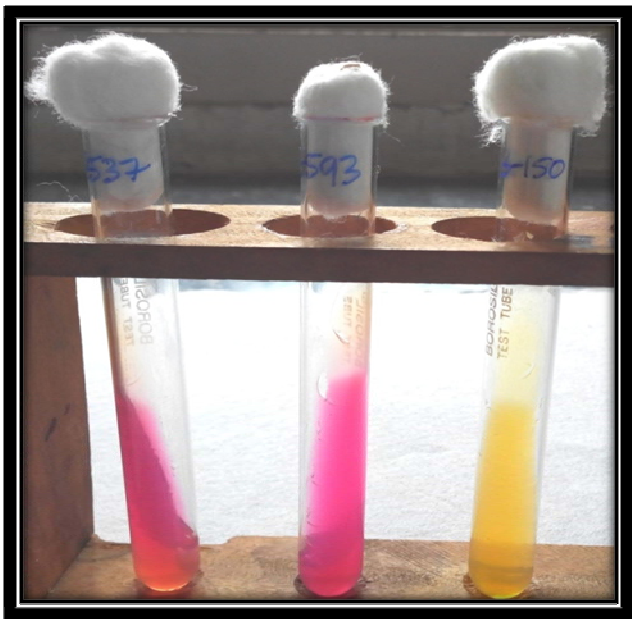


PHOTO 8 : ORNITHINE DECARBOXYLASE TEST

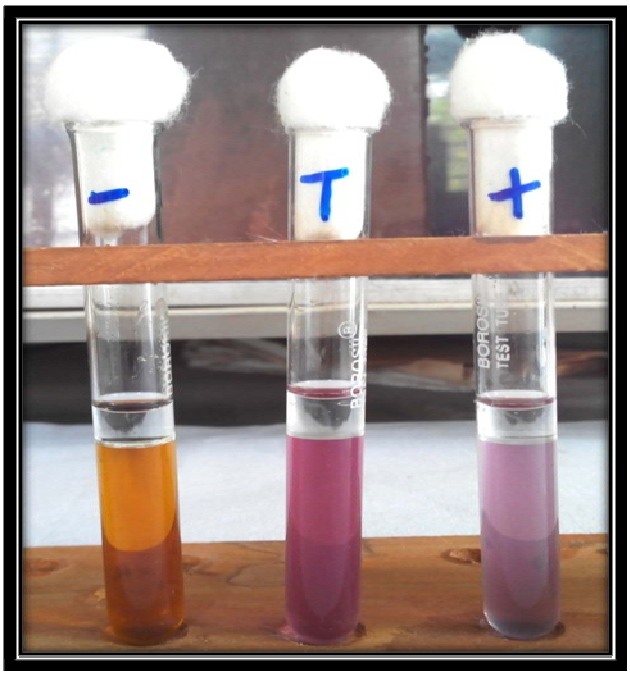


PHOTO 9 : CONGO RED AGAR

PHOTO 10 : CHRISTENSENS TUBE METHOD

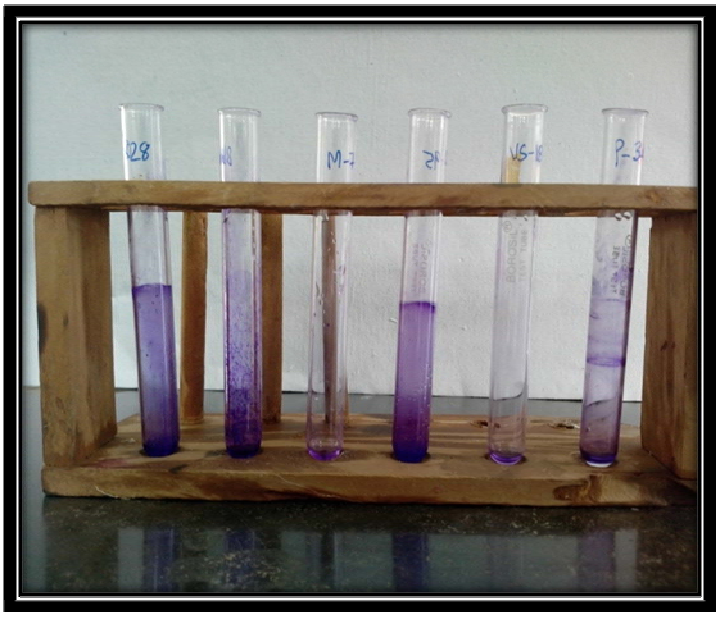


PHOTO 11: NOVOBIOCIN SENSITIVITY TEST

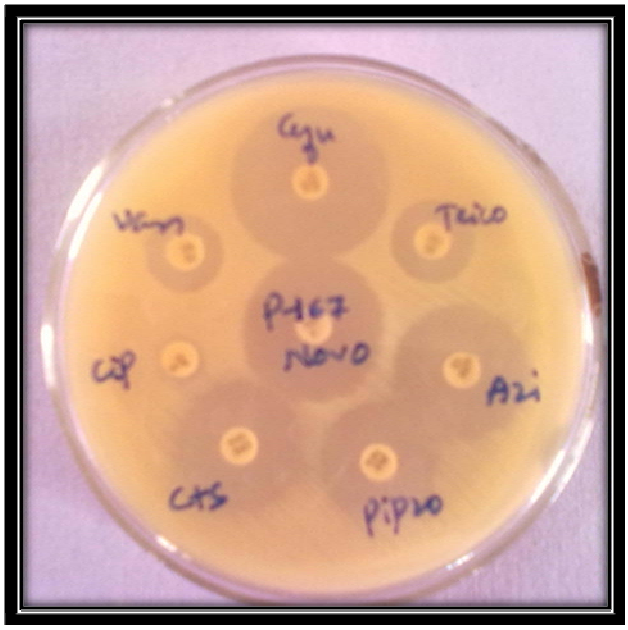
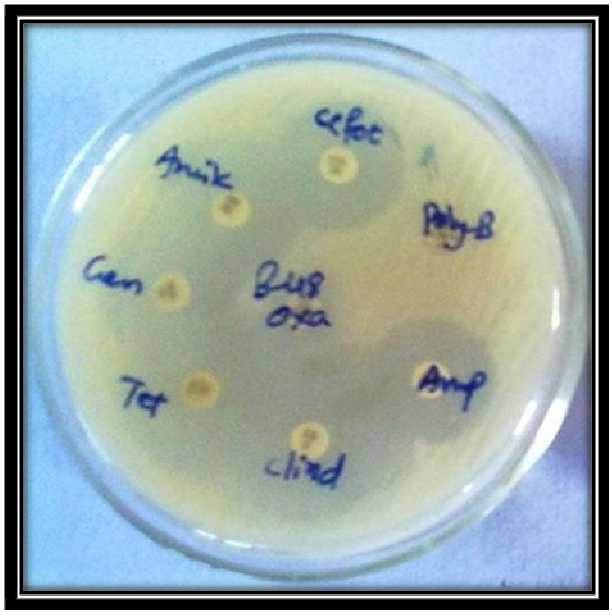


PHOTO 12: METHICILLIN RESISTANT CoNS



RESULTS

A total of 130 pathogenic CONS were isolated from various clinical samples. Most of the isolates were from pus samples 68 (52.3%) followed by urine 25 (19.2%), blood 24 (18.4%), cervical swab 06 (4.6%), pleural fluid 01(0.7%), semen 01(0.7%), and other samples 05 (3.8%) which included one sample each from throat swab, gastric aspirate, endotracheal tube, bronchial lavage and eye discharge.(Table -1).

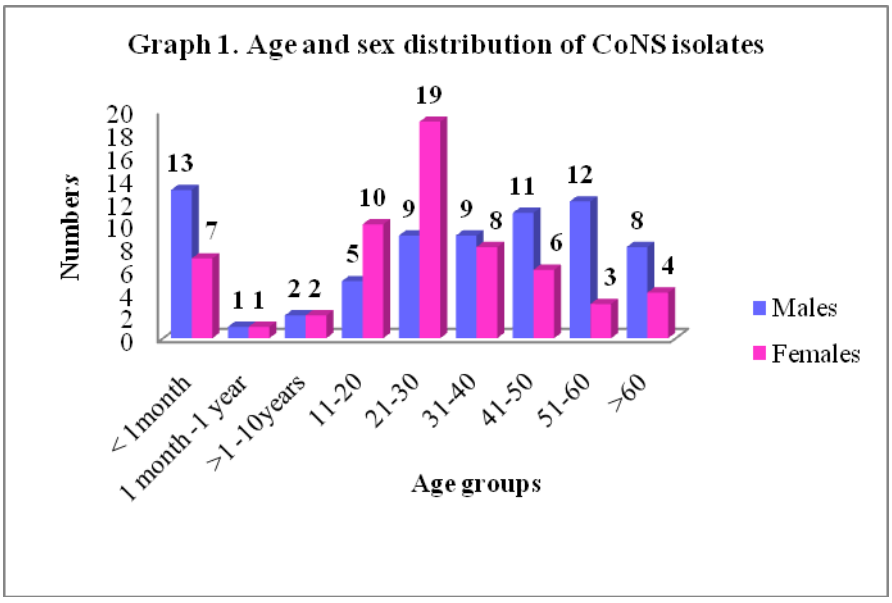
Table 1: Distribution of CONS Isolates from different Clinical Specimens

Specimen	No. of isolates (%)
Pus / wound discharge	68 (52.3%)
Blood	24 (18.4%)
Urine	25 (19.2%)
Cervical swab	06 (4.6%)
Pleural fluid	01 (0.7%)
Semen	01 (0.7%)
Others	05 (3.8%)
Total	**130 (100%)**

Distribution of CoNS in different age and sex groups:

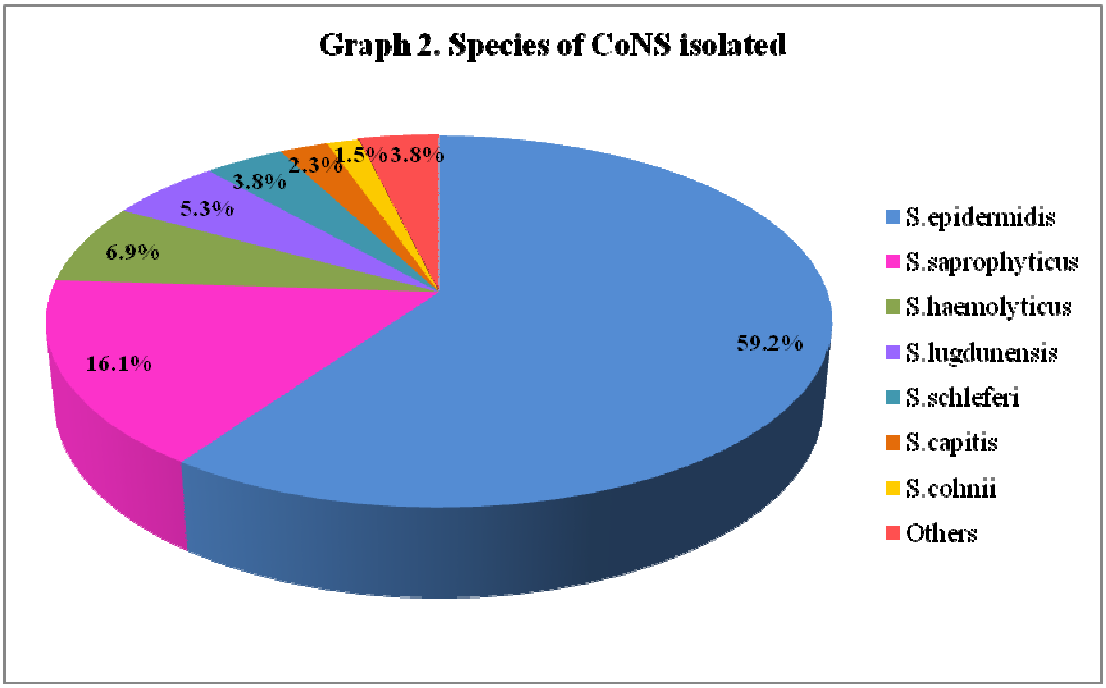
Of the 130 CoNS isolated, 70 (53.8%) were from males and 60 (46.1%) from females. In males 18.5% (13) of CoNS were isolated in neonates <1 month, and 17.1% (12) were from 51-60 age group (Graph 1).

Whereas, in females, 31.6% (19) of CoNS were isolated from 21-30 years followed by 16.6% (10) from 11-20 years age group.



Species of CoNS isolated

In the present study, of the 130 CoNS, *S.epidermidis* was the most common species isolated 77 (59.2%), followed by *S.saprophyticus* 21 (16.1%), *S.haemolyticus* 9 (6.9%), *S.lugdunensis* 7 (5.3%), *S. schleiferi* 5 (3.8%), *S.capitis* 3 (2.3%) and *S.cohnii* 2 (1.5%). Six (4.61%), of the isolates remained unidentified (Graph 2).



Distribution of CoNS species in clinical specimens:

Majority of the *S.epidermidis* isolates were from pus samples 42 (54.5%), followed by blood 18 (23.3%), cervical swab 6 (7.7%), urine 4 (5.1%), and one each (1.2%) from pleural fluid, semen, throat swab, gastric aspirate, endotracheal tube, bronchial lavage, and eye discharge.

Of the 21 *S.saprophyticus* isolates, 19 (90.4%) were from urine samples and 2 (9.5%) from pus sample.

Amongst the 9 *S. haemolyticus* isolates, 4 (44.4%) were from pus, 3 (33.3%) from blood and 2 (22.2%) from urine.

Five (71.4%) of the 7 *S.lugdunensis* isolates were from pus and 2 (28.5%) were from blood specimens.

We could isolate 5 (3.8%) *S.schleiferi*, 3 (2.3%) *S.capitis* and 2 (1.5%) *S.cohnii*, all from pus samples (Table 2).

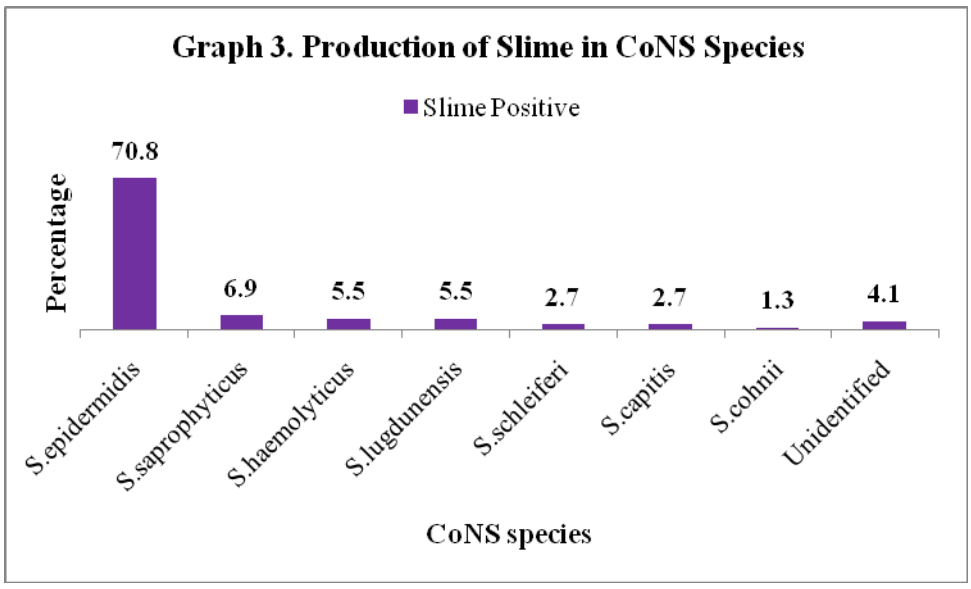
Six (4.6%), of the isolates remained unidentified, 5 from pus and 1 from blood sample.

Table 2: Distribution of CoNS species in Clinical Specimens

Species	*S.epidermidis*	*S.saprophyticus*	*S.haemolyticus*	*S.lugdunensis*	*S.schleiferi*	*S.capitis*	*S.cohnii*	*Others*	
Pus/Wound samples	**42 (54.5%)**	**02 (9.5%)**	**04 (44.4%)**	**05 (71.4%)**	**05 (100%)**	**03 (100%)**	**02 (100%)**	**05 (83.3%)**	**68 (52.3%)**
Blood	**18 (23.3%)**	**-**	**03 (33.3%)**	**02 (28.5%)**	**-**	**-**	**-**	**01 (16.6%)**	**24 (18.4%)**
Urine	**04 (5.1%)**	**19 (90.4%)**	**02 (22.2%)**	**-**	**-**	**-**	**-**		**25 (19.2%)**
Cervical swab	**06 (7.7%)**	**-**	**-**	**-**	**-**	**-**			**06 (4.6%)**
Pleural fluid	**01 (1.2%)**	**-**	**-**	**-**		**-**	**-**		**01 (0.7%)**
Semen	**01 (1.2%)**	**-**	**-**	**-**	**-**	**-**	**-**		**01 (0.7%)**
Throat swab	**01 (1.2%)**	**-**	**-**	**-**	**-**	**-**	**-**		**01 (0.7%)**
Gastric aspirate	**01 (1.2%)**	**-**	**-**	**-**	**-**	**-**	**-**		**01 (0.7%)**
Endotracheal tube	**01 (1.2%)**	**-**	**-**	**-**	**-**	**-**	**-**		**01 (0.7%)**
Bronchial lavage	**01 (1.2%)**	**-**	**-**	**-**	**-**	**-**	**-**		**01 (0.7%)**
Eye discharge	**01 (1.2%)**	**-**	**-**	**-**	**-**	**-**	**-**		**01 (0.7%)**
Total	**77 (59.2%)**	**21 (16.1%)**	**09 (6.9%)**	**07 (5.3%)**	**05 (3.8%)**	**03 (2.3%)**	**02 (1.5%)**	**06 (4.6%)**	**130**

Production of Slime in CoNS species

In the present study, out of 130 CoNS isolated, slime production was detected in 72 (55.3%). *S.epidermidis* (70.8%) was the commonest species in producing slime followed by *S. saprophyticus* (6.9%), *S. haemolyticus* (5.5%), *S.lugdunensis* (5.5%), *S.schleiferi* (2.7%), *S.capitis* (2.7%) and *S.cohnii* (1.3%). Three (4.1%) of the unidentified CoNS also produced slime (Graph 3).



Slime production was noted to be more in CoNS species isolated from pus/wound samples (56.9%), followed by the blood samples (20.8%) (Table 3).

Table 3: Distribution of slime producing CONS with regards to clinical specimens

Species (n)	Pus/ Wound	Blood	Urine	Cervical swab	Pleural fluid	Semen	Others	Total
S.epidermidis	28	12	03	03	01	01	03	51 (70.8%)
S.saprophyticus	-	-	05	-	-	-	-	05 (6.9%)
S.hemolyticus	03	01	-	-	-	-	-	04 (5.5%)
S.lugdunensis	02	02	-	-	-	-	-	04 (5.5%)
S.schleferi	2	-	-	-	-	-	-	02 (2.7%)
S.capitis	02	-	-	-	-	-	-	02 (2.7%)
S.cohnii	01	-	-	-	-	-	-	01 (1.3%)
Unidentified	03	-	-	-	-	-	-	03 (4.1%)
Total	41	15	08	03	01	01	03	72 (55.3%)

Comparison of Christensens tube method and Congo Red Agar method for detection of slime production:

Of the 130 CoNS isolates, 72 (55.3%) were detected by tube method, whereas Congo red agar method detected only 6 (4.6%) of the isolates to be positive.

The sensitivity and specificity for Christensen's tube method was 83% and 46% and for Congo red agar method it was 6.9% and 98%, respectively.

Antibiotic sensitivity and resistance pattern of CoNS

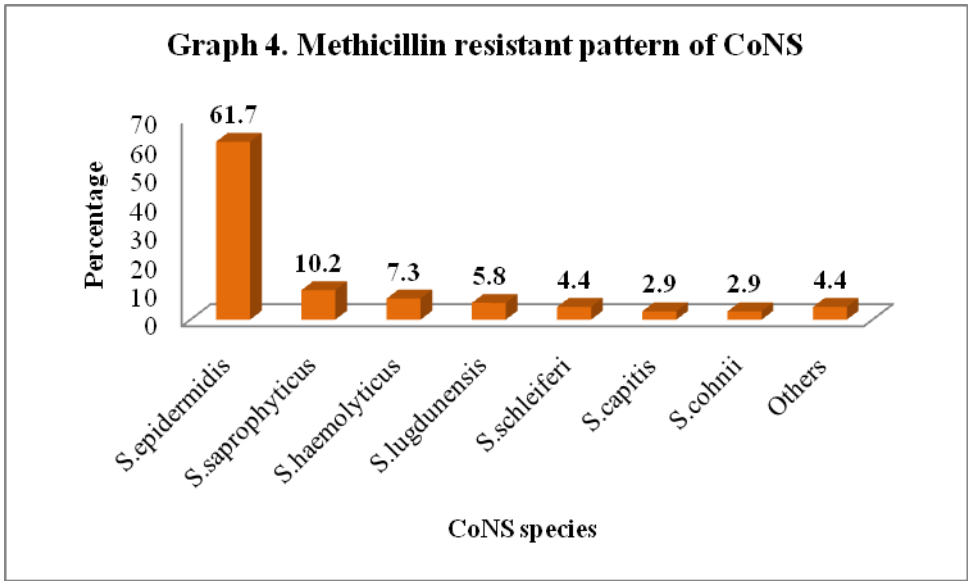
In the present study, of the 130 CoNS strains, 97 (74.6%) were resistant to ampicillin, 82 (63%) to erythromycin, 61 (46.9%) to azithromycin, 57 (43.8%) to gentamycin, 55 (42.3%) to ciprofloxacin, 43 (33%) to co-trimoxazole, 27 (20.7%) to tetracycline, 27 (20.7%) to cefoperazone +sulbactum, 30 (23%) to cefotaxime and cefuroxime, 21 (16.1%) to piperacillin+tazobactum, 25 (19.2%) to vancomycin and 17 (13%) to teicoplanin. Least resistance was seen to amikacin and teicoplanin -17 (13%) (Table 4).

Table 4: Antibiotic Sensitivity and Resistance Pattern of CONS Species

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	33 (25.3%)	97 (74.6%)
Clindamycin	105 (80.7%)	25 (19.2%)
Tetracycline	103 (79.2%)	27 (20.7%)
Gentamycin	73 (56.1%)	57 (43.8%)
Amikacin	113 (86.9%)	17 (13%)
Cefotaxime	100 (76.9%)	30 (23%)
Cefuroxime	100 (76.9%)	30 (23%)
Vancomycin	105 (80.7%)	25 (19.2%)
Teicoplanin	113 (86.9%)	17 (13%)
Piperacillin+ Tazobactum	109 (83.8%)	21 (16.1%)
Cefoperazone+Sulbactum	103 (79.2%)	27 (20.7%)
Azithromycin	69 (53%)	61 (46.9%)
Erythromycin	48 (36.9%)	82 (63%)
Ciprofloxacin	75 (57.6%)	55 (42.3%)
Cotrimoxazole	87 (66.9%)	43(33%)
Oxacillin	62 (47.69%)	68 (52.30%)

Methicillin resistance pattern of CoNS:

Out of 130 CoNS isolated in the present study, 68 strains (52.3%) were resistant to Oxacillin (Graph 4).



Susceptibility pattern of Methicillin Resistant CoNS (MRCoNS):

Of the 68 Methicillin resistant CoNS, 60 (88.2%) were resistant to ampicillin, 49 (72%) to erythromycin, 45 (66.1%) to gentamycin, 44 (64.7%) to ciprofloxacin, 38 (55.8%) to azithromycin, 23 (33.8%) to cefoperazone+sulbactum, 19 (27.9%) to vancomycin and cefuroxime, 18 (26.4%) to cefotaxime, 13 (19.1%) to amikacin and teicoplanin and 17 (25%) to piperacillin+tazobactum (Table 5).

Table 5: Susceptibility pattern of Methicillin Resistant CoNS (MRCoNS)

	MRCoNS		MSCoNS	
Antibiotic	Sensitive	Resistant	Sensitive	Resistant
Oxacillin	-	**68**	62	-
Ampicillin	8	**60**	25	**37**
Clindamycin	51	**17**	54	**8**
Tetracycline	49	**19**	54	**8**
Gentamycin	23	**45**	50	**12**
Amikacin	55	**13**	58	**4**
Cefotaxime	50	**18**	50	**12**
Cefuroxime	49	**19**	51	**11**
Vancomycin	49	**19**	56	**6**
Teicoplanin	55	**13**	58	**4**
Piperacillin+tazobactum	51	**17**	58	**4**
Cefoperazone+sulbactum	45	**23**	58	**4**
Azithromycin	30	**38**	39	**23**
Erythromycin	19	**49**	29	**33**
Co-trimoxazole	42	**26**	45	**17**
Ciprofloxacin	24	**44**	51	**11**

DISCUSSION

Coagulase negative Staphylococci are widely distributed over the surface of the human body, constituting the major commensal bacterial microflora. In the past, CoNS were considered non-pathogenic and their isolation in the laboratory was attributed to specimen contamination. Now they have emerged as important pathogens in hospital acquired infections due to widespread use of implanted medical devices like indwelling catheters, prosthetic joints, prosthetic valves, endotracheal tubes and other chronic implanted devices[9].

CoNS have historically been more resistant to antimicrobials, including beta lactam antibiotics. Some hospitals reveal rates of oxacillin resistance approaching 90% and decreased susceptibility to glycopeptides were reported from the developed countries in the late 1970's[11,43].

In this study, speciation of pathogenic CoNS isolated from various clinical samples and its antibiotic susceptibility pattern with special reference to methicillin resistance were studied.

Isolation of CoNS from clinical samples:

In this study, of the total 130 CoNS isolated, most of the isolates were from pus samples 68 (52.3%) followed by urine 25 (19.2%), blood 24 (18.4%), cervical swab 06 (4.6%), pleural fluid 01 (0.7%), semen 01 (0.7%), and other 05 (3.8%) samples which included one each from throat swab, gastric aspirate, endotracheal tube, bronchial lavage and eye discharge.

Our data correlates with the results of studies by Goyal et al. and Sewell CM et al.[44,45] (Table 6).

Table 6: Comparison of CONS species isolated from clinical samples

Samples	De Paulis et al.[46]	Goyal R et al.[44]	Sewell CM et al.[45]	Mohan U et al.[47]	Gill VG et al.[48]	Present Study
Pus/ wound swab	30 (15.0%)	39(38.2%)	84(43%)	34(17%)	118(17.4%)	**68 (52.3%)**
Blood	26 (13.9%)	15(14.7%)	52(26%)	9(4.5%)	336(49.5%)	**24 (18.4%)**
Urine	72 (36.0%)	29(28.4%)	24(12%)	93(46.5%)	46(6.7%)	**25 (19.2%)**
Endotracheal tube	22 (11%)	11(10.7%)	-	22(11%)	76(11.2%)	**1(0.7%)**
Cervical swab	-	-	-	-	-	**6 (4.6%)**
CSF	2 (1.0%)	4(3.9%)	16(8%)	-	-	-
Amniotic fluid	-	-	-	-	-	-
Pleural fluid	-	-	2(1%)	-	-	**1 (0.7%)**
Peritoneal fluid	19 (9.5%)	2(1.9%)	18(9%)	-		-
Sputum	-	-		-	-	-
Others	29 (14.5%)	2(1.9%)	2(1%)	42(21%)	102(15%)	**05 (3.8%)**
Total	200	102	198	200	678	**130**

However in results published by Gill VJ et al., blood was the most common source for CoNS, followed by wounds and abscesses and IV catheter tips[48]. Two other reports by De Paulis et al. and Mohan U et al. highlight the variability in prevalence of CoNS, wherein urine was the most common source, followed by pus, catheter tips/IV canulas, blood, aural swabs, peritoneal fluid and cerebrospinal fluid[46,47].

CONS SPECIATION:

Several methods exist for speciation of CoNS. Though the method of Kloos and Schleifer is a reliable technique for the identification and speciation of CoNS, it is relatively cumbersome, time consuming and requires the use of expensive reagents. Alternative approaches include use of several commercially available identification kits and automated instruments; however, several factors preclude their use. Among the different approaches, the five-test simple scheme by De Paulis et al.[46], along with the Simple Scheme, a two step procedure, used by Goyal R et al.[44] were employed in this study, which are convenient, reliable and inexpensive.

In the present study, *S.epidermidis* was the most common species isolated (59.2%). *S.epidermidis* was also the commonest species isolated in studies by Goyal R et al., De Paulis et al., Pal N and Ayyageri A, Kleeman KT et al. and Singh S et al.[44,46,49,50,51](Table 7).

Table 7: Distribution of CONS species

Species	Pal N and Ayyageri A et al.[49]	De Paulis et al.[46]	Goyal R et al.[44]	Kleeman KT et al.[50]	Singh S et al.[51]	Present Study
S.epidermidis	45 (60%)	101 (50.5%)	42 (41%)	322 (64.5%)	60 (40.0%)	**77 (59.23%)**
S.saprophyticus	3 (4.0%)	32 (16.0%)	17 (16.6%)	5 (1%)	21 (14.0%)	**21 (16.15%)**
S.hemolyticus	3 (4.0%)	37 (18.5%)	15 (14.7%)	67 (13.4%)	18 (12.0%)	**9 (6.92%)**
S.lugdunensis	--	12 (6.0%)	5 (4.9%)	14 (2.8%)	9 (6.0%)	**7 (5.38%)**
S.schleiferi	--	2 (1.0%)	2 (1.9%)	-	3 (2.0%)	**5 (3.84%)**
S.capitis	4 (5.3%)	4 (2.0%)	2 (1.9%)	18 (3.6%)	6 (4.0%)	**3 (2.3%)**
S.cohnii	10 (13.3%)	1 (6.5%)	-	3 (0.6%)	-	**2 (1.53%)**
S.simulans	1 (1.3%)	3 (1.5%)	-	12 (2.4%)	-	-
S.scuiri	--	2 (1%)	-	-	-	-
S.hominis	8 (10.7%)	1 (0.5%)	15 (14.7%)	37 (7.4%)	9 (6.0%)	-
S.xylosus	-	-	-	-	-	-
S.warneri	1 (1.3%)	5 (2.5%)	2 (1.9%)	20 (4%)	9 (6.0%)	-
Unidentified						**6 (4.61%)**

S.saprophyticus and *S.haemolyticus* formed the next common species in the present study as well as in the studies by Goyal R et al., De Paulis et al. and Singh S et al.[44,46,51]. However, *S.haemolyticus* and *S. hominis* were the next common species in studies by Sewell CM et al., Kleeman KT et al. and Eng RHK et al.[45,50,52].

SLIME PRODUCTION

Slime plays an important role in non specific mechanism of antibiotic resistance[22]. Many methods are available for detection of slime production.

Table 8: Comparison of slime production by CoNS

Studies	Kotilainen P[53]		Cunha MLR et al.[54]		Boynukara B et al.[55]		Present study	
Species	No. of Strains	Slime Positive	No. of Strains	Slime Positive	No. of Strains	Slime Positive	No. of Strains	Slime Positive
S.epidermidis	51	33 (65%)	24	4 (16.7%)	18	11 (61.1%)	**77**	**51 (70.8%)**
S.saprophyticus	-	-	-	-	2	2 (100%)	**21**	**5 (6.9%)**
S.haemolyticus	-	-	11	2 (18.2%)	4	3 (75%)	**9**	**4 (5.5%)**
S.lugdunensis	-	-	1	1 (100%)	-	-	**7**	**4 (5.5%)**
S.schleiferi	-	-	-	-	-	-	**5**	**2 (2.7%)**
S.capitis	1	0	-	-	2	0	**3**	**2 (2.7%)**
S.cohnii	-	-	2	0	-	-	**2**	**1 (1.3%)**
S.hominis	8	1 (12.5%)	5	0	20	11 (55%)	-	-
S.warneri	2	0	5	2 (40.0%)	6	3 (50%)	-	-
S.scuiri	-	-	-	-	5	3 (60%)	-	-
S.xylosus	-	-	2	0	6	4 (66.7%)	-	-
S.simulans	-	-	1	0	2	2 (100%)	-	-
Unidentified	2	0	-	-	-	-	**6**	**3 (4.1%)**
Total:	**64**	**34 (53%)**	**51**	**9 (17.6%)**	**65**	**39 (60.0%)**	**130**	**72 (55.3%)**

In the present study, out of 130 CONS isolated, slime production was detected in 72 (55.3%).

Comparable results have been published by Mathur T et al., Kotilainen P, and Boynukara et al.[30,53,55](Table 8).

In contrast, Cunha MLR et al. reported only 17.6% of CoNS to be slime producers[54]. On the other hand, Ishak MA et al. found that 92.8% of CoNS produced slime[56].

S.epidermidis 51 (70.8%) was the commonest species producing slime in our study. This was comparable to the studies of Kleeman et al. and Ishak et al., with 64.5% and 92% respectively[50,56].

However Cunha MLR et al. has reported 83.3% of *S. epidermidis* were non slime producers[54].

In the present study, majority of the slime positive *S.epidermidis* were isolated from pus (54.9%), followed by blood (23.5%). In contrast Boynukara et al. has reported that 54.6% of slime producing *S. epidermidis* were isolated from blood and 9.1% from pus[55].

Comparison of Christensen's tube method and Congo red agar method for detection of biofilm

The sensitivity and specificity for Christensen's tube method was 83% and 46%, whereas it was 6.9% and 98% for Congo red agar method respectively. Similar results have been published by Mathur et al[30].

Oliveria ADD et al. has reported 100% sensitivity and specificity by tube method and 89% sensitivity and 100% specificity by Congo red agar method[36].

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF CoNS

Antimicrobial susceptibility in CoNS became an issue in the 1970s when these species were isolated as pathogens in prosthetic valve endocarditis infections and cerebrospinal fluid shunt infections[11]. Methicillin resistance in Staphylococcus aureus first appeared in 1960s. Since then Methicillin resistant S.aureus and Methicillin resistant CoNS have spread worldwide[57]. During the 1970s methicillin resistance was more prevalent in CoNS rather than in S.aureus and multiresistance in CoNS was reported in 1980's[11].

In the present study, of the 130 CoNS, 74.6% were resistant to ampicillin, 63% to erythromycin, 46.9% to azithromycin, 43.8% to gentamycin, 42.3% to ciprofloxacin, 33% to co-trimoxazole, 20.7% to tetracycline, 23% to cefotaxime and cefuroxime, 19.2% to clindamycin, 13% to amikacin. Resistance to vancomycin and teicoplanin was seen in 19.2% and 13%.

Comparable results were published by Archer GL et al. which stated that majority of CoNS were resistant to erythromycin (75%), followed by gentamycin (61%), trimethoprim (50%), tetracycline (35%). However he observed clindamycin resistance in 60%[27].

Manijeh Mehdinejad et al. has reported maximum resistance to erythromycin (77.1%), followed by tetracycline (71.3%), clindamycin (61.1%), and vancomycin (21.6%)[58].

METHICILLIN RESISTANCE CoNS (MRCoNS)

In the present study 52.3% of the isolates were resistant to oxacillin, whereas Amita Jain et al. and Chaudhary et al. have reported oxacillin resistance of 66% and 68.4% and respectively[12,59].

The incidence of MRCoNS of various published reports has been compared in the Table. 13

High incidence of MRCoNS was reported by Archer GL et al. (80%), Hira V et al. (87%), and Singhal R et al. (72.3%)[27,60,61].

A 3 year study conducted by Deepa et al., revealed MRCoNS rates of: 41.5% (2008), 47% (2009) and 57.3% in (2010) [62] (Table 9).

Table 9: Comparison of Incidence of MRCoNS

Study	Incidence of MRCoNS
A Choudhary, AG Kumar	68.4%
Jain A, Agarwal Jand Bhansal S	66%
Deepa, Amruta, Venkatesh	48.72%
Hira V et al.	87%.
Singhal R et al.	72.3%
Present study 2013	**52.3%**

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF MRCONS

Of the 68 Methicillin resistant CoNS isolated, 60 (88.23%) were resistant to ampicillin, 49 (72%) to erythromycin, 45 (66.17%) to gentamycin, 44 (64.70%) to ciprofloxacin, 38 (55.88%) to azithromycin, 23 (33.82%) to cefoperazone+sulbactum, 19 (27.94%) to vancomycin and cefuroxime, 18 (26.47%) to cefotaxime. Least resistance was seen to amikacin and teicoplanin 13 (19.11%), and to piperacillin+tazobactum 17 (25%). Similar observations were made by Archer GL et al. who made the analysis of a study and stated that 61% of MRCoNS were resistant to gentamycin, 50% to trimethoprim, 75% to erythromycin, 35% to tetracycline[27]. Whereas Menijeh Mehdinejad et al. has reported that 61% of MRCoNS were resistant to clindamycin, 71.3% to tetracycline and 21.6% to vancomycin[58].

SUMMARY

The present study was conducted at the department of Microbiology, BLDEU's Shri B.M.Patil Medical college and Hospital, Bijapur, from 01-01-2012 to 30-06-2013.

Of the 130 CoNS isolated, 70 (53.8%) were from males and 60 (46.1%) from females. In males 18.5% (13) of CoNS were isolated in neonates <1 month,whereas in females, 31.6% (19) of CoNS were isolated from 21-30 years.

A total of 130 pathogenic CONS were isolated from various clinical samples. Most of the isolates were from pus samples 68 (52.3%) followed by urine 25 (19.2%), blood 24 (18.4%), cervical swab 06 (4.6%), pleural fluid 01(0.7%), semen 01(0.7%), and other samples 05 (3.8%) which included one sample each from throat swab, gastric aspirate, endotracheal tube, bronchial lavage, and eye discharge.

Of the 130 CoNS, S.epidermidis was the most common species isolated 77 (59.2%); followed by S.saprophyticus 21 (16.1%), S.haemolyticus 9 (6.9%), S.lugdunensis 7 (5.3%), S. schleiferi 5 (3.8%), S.capitis 3 (2.3%) and S.cohnii 2 (1.5%). Six (4.61%), of the isolates remained unidentified.

The majority of the isolates were from pus 68 (52.3%), followed by urine 25 (19.2%), blood 24 (18.4%), cervical swab 6 (4.6%), and one each (0.7%) from pleural fluid, semen, throat swab, gastric aspirate, endotracheal tube, bronchial lavage and eye discharge.

In the present study, out of 130 CONS isolated, slime production was detected in 72 (55.3%). S.epidermidis 51 (70.8%) was the commonest species in producing slime followed by S. saprophyticus 5 (6.9%), S. haemolyticus 4 (5.5%), S.lugdunensis 4 (5.5%), S.schleiferi 2 (2.7%), S.capitis 2 (2.7%) and S.cohnii 1 (1.3%).

Of the 130 CoNS isolates, 72 (55.3%) were detected positive for slime production by Christensen's tube method, whereas Congo red agar method detected only 6 (4.6%) of the isolates to be positive.

The sensitivity and specificity for Christensen's tube method was 83% and 46% and for Congo red agar method it was 6.9% and 98%, respectively. Of the 130 CoNS strains, 97 (74.6%) were resistant to ampicillin, 82 (63%) to erythromycin, 61 (46.9%) to azithromycin, 57 (43.8%) to gentamycin, 55 (42.3%) to ciprofloxacin, 43 (33%) to co-trimoxazole, 27 (20.7%) to tetracycline, 27 (20.7%) to cefoperazone +sulbactum, 30 (23%) to cefotaxime and cefuroxime, 21 (16.1%) to piperacillin+tazobactum, 25 (19.2%) to vancomycin and 17 (13%) to teicoplanin. Least resistance was seen to amikacin and teicoplanin -17 (13%)

Out of 130 CoNS isolated, methicillin resistance was seen in 68 (52.3%) strains.

CONCLUSION

1. In males 18.5% (13) of CoNS were isolated in neonates <1 month, whereas in females, 31.6% (19) of CoNS were isolated from 21-30 years
2. Majority of the isolates were from pus samples 68 (52.3%) followed by urine 25 (19.2%), blood 24 (18.4%).
3. *S.epidermidis* was the most common species isolated 77 (59.2%) and 42 (54.5%) were from pus samples.
4. Slime production was detected in 72 (55.3%).
5. S.epidermidis 51 (70.8%) was the commonest species in producing slime.
6. The sensitivity and specificity for Christensen's tube method was 83% and 46% and for Congo red agar method it was 6.9% and 98%, respectively for detection of biofilms.
7. Maximum number of isolates were resistant to ampicillin 97 (74.6%). Least resistance was seen to amikacin and teicoplanin 17 (13%).
8. Methicillin resistance was seen in 52.3% strains.

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ANNEXURES

PROFORMA

1. NAME :

2. AGE/SEX : IP NO:

SPECIMEN :

LAB NO : DATE:

MICROBIOLOGICAL STUDY

I. Microscopy- Gram stain:

II. Cultural study

BA :

NA :

MA :

CLED:

Colony Gram stain:

III. **Biochemical tests-**

1. Catalase test
2. Coagulase test Slide : Tube :
3. Ornithine decarboxylase

4. Urease
5. Nitrate reduction
6. Phosphatase
7. Sugar fermentation

   Mannose Mannitol Trehalose lactose
8. Acetoin production
9. Novobiocin(5µg) sensitivity test

IV. Final identification:

V. Antibiotic susceptibility testing

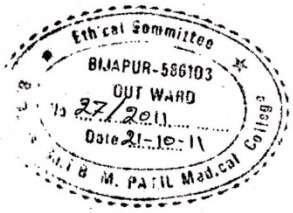
Ampicillin 10 µg	Clindamycin 2µg
Piperacillin-tazobactum 100/10µg	Cefoperazone-sulbactum 75/10µg
Cefotaxim 30µg	Cefuroxime 30µg
Gentamycin 10µg	Amikacin 30µg
Erythromycin 15µg	Azithromycin 15µg
Tetracycline 30µg	Ciprofloxacin 5µg
Trimethoprim-sulfamethoxazole 1.25/23.75µg	Norfloxacin 10 µg
Oxacillin 30µg	Vancomycin 30µg
Teicoplanin 30µg	

VI. Repeat specimen collection date:

VII. Species isolated:

VIII. Comments:

ETHICAL CLEARANCE CERTIFICATE



B.L.D.E. UNIVERSITY'S

SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103

INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 20-10-2011 *at* 10-30 am *to scrutinize the Synopsis/Research projects of postgraduate/undergraduate student/Faculty members of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis/Research project has been accorded Ethical Clearance.*

Title Speciation and antibiogram of coagulase nega-tive Staphylococci isolated from clinical speci-mens with special reference to biofilm production

Name of P.G./U.G. student/Faculty member Dr. Raj mohammed D. Malled
Dept of Microbiology

Name of Guide/Co-investigator Dr. A.G. Sajjan Assoc Prof. Microbiology

DR.M.S.BIRADAR,
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.
Chairman
Ethical Committee
BLDEA'S Shri. B.M. Patil
Medical College
Bijapur-586103

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

KEY TO MASTER CHART

P	: Positive
N	: Negative
S	: Sensitive
R	: Resistant
d	: Days
m	: months
E.disc	: Ear discharge
Ey.dis	: Eye discharge
G.asp	: Gastric aspirate
Br.lav	: Bronchial lavage
T.swab	: Throat swab
Pl.fld	: Pleural fluid
ET tube	: Endotracheal tube
S.sapro	: S. saprophyticus
TC	: Tube Coagulase
SC	: Slide Coagulase
MSA	: Mannitol salt agar
NRT	: Nitrate reduction test
PP4	: Phosphatase test
ODC	: Ornithine decarboxylase
VP	: Voges Proskauer test
Man	: Mannose
Mani	: Mannitol
Mal	: Maltose
Lac	: Lactose

Suc	:	Sucrose
Tre	:	Trehalose
Novo	:	Novobiocin
Pol-B	:	Polymixin B
Tube	:	Tube method
CRA	:	Congo red agar
Amp	:	Ampicillin
Tet	:	Tetracycline
Clind	:	Clindamycin
Gen	:	Gentamycin
Amik	:	Amikacin
Cefo	:	Cefotaxime
Cefu	:	Cefuroxime
Teico	:	Teicoplanin
Van	:	Vancomycin
P+T	:	Piperacillin + Tazobactum
C+S	:	Cefoperazone + Sulbactum
Cip	:	Ciprofloxacin
Azi	:	Azithromycin
Ery	:	Erythromycin
Cot	:	Co-trimoxazole.

MASTER CHART

S.No	Age/Sex	Specimen	Species	TC	SC	MSA	Urease	NRT	PP4	ODC	VP	Sugar Utilization test						Novo	Pol-B	Biofilm		Oxacillin	Amp	Tet	Clind	Gen	Amik	Cefo	Cefu	Teico	Van	P+T	C+S	Cip	Azi	Ery	Cot
												Man	Mani	Mal	Lac	Suc	Tre			Tube	CRA																
1	70/M	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	R	S	R	S	R	S	S	S	S	S	S	R	R
2	35/M	semen	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	14d/M	blood	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S
4	28/M	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	N	N	*R*	R	R	S	R	S	S	R	S	S	S	S	R	S	R	S
5	1d/M	G.asp	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S
6	23/F	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	S	S	R	S	R	R	R	R	S	S	R	R	R	R
7	21/F	E.disc	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	R	S	R	S	S	S	S	S	S	S	R	R	R	S
8	16/F	blood	S.epidermidis	N	N	N	V	P	P	N	N	P	N	P	N	P	N	S	R	N	N	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S
9	3d/F	blood	S.epidermidis	N	N	N	P	P	P	N	N	P	N	p	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
10	1m/F	blood	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
11	50/F	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
12	20/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	P	P	N	P	N	S	S	P	N	R	R	S	S	R	S	S	R	S	S	S	S	R	R	R	S
13	9/F	pus	S.epidermidis	N	N	N	V	P	P	N	N	P	N	P	N	P	N	S	R	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S
14	65/M	pus	?	N	N	N	N	P	P	N	N	P	P	P	P	P	N	S	R	N	N	R	S	R	S	R	S	S	R	S	S	S	S	R	R	R	R
15	12/F	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
16	25/F	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	N	N	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S
17	45/M	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

S.No	Age/Sex	Specimen	Species	TC	SC	MSA	Urease	NRT	PP4	ODC	VP	Sugar Utilization test						Novo	Pol-B	Biofilm		Oxacillin	Amp	Tet	Clind	Gen	Amik	Cefo	Cefu	Teico	Van	P+T	C+S	Cip	Azi	Ery	Cot
												Man	Mani	Mal	Lac	Suc	Tre			Tube	CRA																
18	28/M	blood	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	N	N	S	R	R	S	S	S	S	S	S	S	S	S	R	S	S	R
19	1d/M	blood	S.epidermidis	N	N	N	V	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	R
20	45/M	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
21	45/M	urine	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	R	S	S	R	S	S	R	S	S	S
22	52/M	pus	S.epidermidis	N	N	N	V	P	P	N	N	p	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	S	S	S	S	S	R	R	S	S	S
23	1d/M	blood	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	R	S	S	S	S	R	S	R	R	S
24	36/M	E.disc	S.epidermidis	N	N	N	N	P	P	N	P	P	N	P	N	P	N	S	R	P	P	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S
25	35/M	pus	S.epidermidis	N	N	N	N	P	P	N	P	P	N	P	N	P	N	S	R	N	N	R	R	S	S	S	S	S	R	S	S	S	R	S	S	S	S
26	20/F	urine	S.sapro	N	N	N	P	P	N	N	N	N	N	P	N	P	P	R	S	P	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S
27	24/F	pus	S.epidermidis	N	N	N	N	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	S	S	R	S	S	R	S	S	S	R	R	R	R	S
28	41/M	T.swab	S.epidermidis	N	N	N	V	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S
29	62/M	pus	S.capitis	N	N	P	N	N	N	N	N	P	P	N	N	P	N	S	S	N	N	R	R	S	S	S	S	S	S	S	S	S	R	R	S	S	S
30	1d/M	blood	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	N	s	S	N	N	R	R	S	R	R	S	R	R	S	S	S	R	R	R	R	R
31	55/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	R	S	R	R	S	R	R	R	S	S	R	R	R	S
32	3d/M	Ey.dis	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	R	S	R	S	S	R	S	S	R	R	S	R	R	R
33	1m/M	blood	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	S	S	S	S	S	R	S	S	R	S	S	S	S	S
34	26/M	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	S	R	R	S	R	S	R	R	S	R	S	S	S	R	R	R
35	1d/F	blood	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	R	R	R	R	S	R	R	R	R	R
36	13/M	Br.lav	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S

S.No	Age/Sex	Specimen	Species	TC	SC	MSA	Urease	NRT	PP4	ODC	VP	Sugar Utilization test						Novo	Pol-B	Biofilm		Oxacillin	Amp	Tet	Clind	Gen	Amik	Cefo	Cefu	Teico	Van	P+T	C+S	Cip	Azi	Ery	Cot
												Man	Mani	Mal	Lac	Suc	Tre			Tube	CRA																
37	75/M	pus	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	N	S	S	P	N	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
38	33/F	pus	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	N	R	S	S	R	S	S	S	S	S	S	S	S	S	S	R	S
39	69/M	pus	?	N	N	N	N	P	P	N	N	P	P	P	P	P	N	S	S	N	N	S	R	S	S	R	S	R	R	S	S	S	R	R	R	R	R
40	25/F	pus	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	N	N	R	R	R	S	R	R	S	R	R	R	S	S	S	S	S	R
41	20/F	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	P	R	R	S	S	S	S	S	S	S	S	S	R	S	R	R	S
42	8/M	E.disc	S.epidermidis	N	N	N	P	P	P	N	V	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
43	59/M	ET tub	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	R	S	R	R	R	R	R	R	R	R	R
44	2/F	pus	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
45	34/M	pus	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	N	N	S	R	S	S	S	S	R	S	S	S	S	S	S	S	R	S
46	28/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	P	N	R	S	R	S	R	S	S	R	S	S	R	R	R	R	R	R
47	2d/F	blood	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	N	N	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R	R
48	4d/F	blood	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	S	S	S	S	S	S	R	R	R	S
49	45/F	pus	?	N	N	N	P	N	N	N	N	N	N	P	P	P	P	S	S	N	N	S	R	R	S	R	S	R	R	S	S	S	S	S	R	R	R
50	42/F	urine	S.epidermidis		N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
51	55/M	pus	S.sapro	N	N	N	P	N	N	N	N	N	N	P	N	P	P	R	S	N	N	R	R	S	S	R	S	S	R	S	S	R	R	R	R	R	S
52	38/M	E.disc	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	R	R	S	R	S	S	S	S	S	S	R	R	S
53	23/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	N	S	S	S	S	S	S	S	S	S		S	S	S	S	S	S
54	50/F	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	S	R	S	R	S	S	S	S	S	S	R	R	R
55	55/M	pus	S.lugdunensis	N	P	N	N	P	N	P	N	P	N	P	P	P	P	S	R	P	N	R	R	R	R	R	S	R	S	S	S	S	S	R	R	R	R

S.No	Age/Sex	Specimen	Species	TC	SC	MSA	Urease	NRT	PP4	ODC	VP	Sugar Utilization test						Novo	Pol-B	Biofilm		Oxacillin	Amp	Tet	Clind	Gen	Amik	Cefo	Cefu	Teico	Van	P+T	C+S	Cip	Azi	Ery	Cot
												Man	Mani	Mal	Lac	Suc	Tre			Tube	CRA																
56	40/F	pus	?	N	N	N	N	P	P	N	N	P	P	P	P	P	P	S	S	P	N	R	R	R	R	R	S	R	S	S	S	S	R	R	R	R	R
57	40/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	P	S	R	S	S	R	S	S	S	S	S	S	R	S	R	R	R
58	12/F	blood	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	N	S	S	S	S	S	S	S	s	s	S	S	S	S	S	S	S
59	45/M	pus	S.schleiferi	N	P	N	N	P	P	N	N	P	N	N	N	P	N	S	S	P	N	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
60	55/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	S	R	S	S	R	R	R	R	R	S
61	18/F	urine	S.sapro	N	N	N	P	N	N	N	N	N	N	P	N	P	P	R	S	N	N	R	R	S	S	R	S	S	S	S	S	S	S	R	S	R	S
62	2d/F	E.disc	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	R	S	S	S	S	S	S	S	S	R	R	R	S
63	11/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S
64	26/F	cx.swb	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	R	R	S	S	S	S	S	S	R	R	R	R	S
65	31/F	urine	S.sapro	N	N	N	P	N	N	N	N	N	N	P	N	P	P	R	S	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
66	24/F	cx.swb	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
67	44/M	pus	S.cohnii	N	N	N	N	N	N	N	N	N	N	N	N	N	P	R	S	P	N	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
68	41/M	blood	S.lugdunensis	N	P	N	N	P	N	P	N	P	N	P	P	P	P	S	R	P	N	R	R	S	R	R	S	S	S	S	S	R	R	R	R	R	R
69	28/M	pus	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	P	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S
70	31/F	cx.swb	S.epidermidis	N	N	P	P	P	P	N	P	P	P	P	N	P	N	S	R	P	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S
71	29/M	pus	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	N	S	S	P	N	S	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
72	32/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
73	22/F	cx.swb	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
74	55/M	urine	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	N	S	S	N	N	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R

S.No	Age/Sex	Specimen	Species	TC	SC	MSA	Urease	NRT	PP4	ODC	VP	Sugar Utilization test						Novo	Pol-B	Biofilm		Oxacillin	Amp	Tet	Clind	Gen	Amik	Cefo	Cefu	Teico	Van	P+T	C+S	Cip	Azi	Ery	Cot
												Man	Mani	Mal	Lac	Suc	Tre			Tube	CRA																
75	4d/M	blood	S.epidermidis	N	N	N	P	P	P	N	P	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	S	S	S	S	S	S	R	S
76	4d/F	E.disc	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
77	43/F	pus	S.schleiferi	N	P	N	N	P	P	N	N	P	N	N	N	P	N	S	S	N	N	R	R	R	R	R	S	S	S	S	S	S	S	R	R	R	R
78	52/M	blood	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	N	S	S	N	N	R	S	R	R	S	S	S	S	S	S	S	S	R	R	R	S
79	29/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	R	S	R	R	R	R	R	R	S	S	R	R	R	R
80	50/M	pus	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	N	S	S	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
81	32/F	cx.swb	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	N	N	R	R	S	R	R	R	R	S	R	R	R	R	R	R	R	S
82	7/M	blood	?	N	N	N	P	N	N	N	N	N	N	P	P	P	P	S	S	P	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
83	60/M	pus	S.lugdunensis	N	P	N	N	P	N	P	N	P	N	P	P	P	P	S	R	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
84	20/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S
85	55/F	urine	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
86	15/M	pus	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
87	23/F	cx.swb	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	N	N	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	R
88	52/M	pl.fld	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
89	25/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	s	S	S	S	S	S	S	S	S
90	2d/M	blood	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	S	S	S	S	S	S	R	S	R	S
91	12/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
92	22/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	n	P	N	P	P	R	S	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
93	44/M	E.disc	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	N	S	S	R	S	R	R	R	R	S	R	S	R	R	R	R	R

S.No	Age/Sex	Specimen	Species	TC	SC	MSA	Urease	NRT	PP4	ODC	VP	Sugar Utilization test						Novo	Pol-B	Biofilm		Oxacillin	Amp	Tet	Clind	Gen	Amik	Cefo	Cefu	Teico	Van	P+T	C+S	Cip	Azi	Ery	Cot
												Man	Mani	Mal	Lac	Suc	Tre			Tube	CRA																
94	34/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	R	R	S	S	R	S	S	S	S	S	S	R	R	R	S
95	45/M	pus	S.capitis	N	N	P	N	N	N	N	N	P	P	N	N	P	N	S	S	P	N	R	R	R	R	R	S	R	S	R	R	R	R	R	R	R
96	21/F	pus	?	N	N	N	P	N	N	N	N	N	N	P	P	P	P	S	S	P	N	S	R	S	S	S	S	S	S	S	S	S	S	R	R	R
97	1d/M	blood	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	P	R	R	S	S	R	S	S	S	S	S	S	S	R	S	R	R
98	22/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	N	R	S	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S
99	53/F	pus	S.lugdunensis	N	P	N	N	P	N	P	N	P	N	P	P	P	P	S	R	N	N	R	S	S	S	R	S	R	S	S	S	S	S	R	S	R	S
100	14dF	blood	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	R	S	S	S	R	S	R	R	R	R
101	19/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	N	R	S	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
102	42/F	pus	S.epidermidis	N	N	N	P	P	P	N	N	N	N	P	N	P	N	S	R	P	N	R	R	S	S	R	R	S	S	R	R	R	R	R	R	R	S
103	43/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
104	51/M	pus	S.schleiferi	N	P	N	N	P	P	N	P	P	N	N	N	N	N	S	S	P	N	R	R	S	R	R	S	R	S	R	R	R	R	R	R	R	R
105	31/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	S	S	S	R	R	R	S	R	R	R	R	R	R	R	R	R
106	62/F	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
107	2d/M	blood	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	N	N	R	R	S	S	R	S	S	S	S	S	S	S	R	S	S	S
108	75/M	pus	S.lugdunensis	N	P	N	N	N	N	P	P	P	N	P	P	P	P	S	S	P	N	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
109	18/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	P	N	R	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S
110	65/M	E.disc	S.capitis	N	N	P	N	N	N	N	N	P	P	N	N	P	N	S	S	P	N	S	R	R	R	S	S	R	R	R	R	R	R	R	R	R	R
111	25/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	R	R	S	S	R	S	R	S	S	S	S	S	R	R	R	S
112	8d/M	E.disc	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	S	S	S	R	R	S	S	s	S	S	R	R	S

S.No	Age/Sex	Specimen	Species	TC	SC	MSA	Urease	NRT	PP4	ODC	VP	Sugar Utilization test						Novo	Pol-B	Biofilm		Oxacillin	Amp	Tet	Clind	Gen	Amik	Cefo	Cefu	Teico	Van	P+T	C+S	Cip	Azi	Ery	Cot
												Man	Mani	Mal	Lac	Suc	Tre			Tube	CRA																
113	57/F	pus	S.cohnii	N	N	N	N	N	N	N	N	P	N	N	N	N	P	R	S	N	N	R	S	S	R	S	S	S	S	S	S	s	S	S	S	S	S
114	66/F	blood	S.lugdunensis	N	P	N	N	P	N	P	P	P	N	P	P	P	P	S	S	P	N	S	R	S	R	R	S	R	R	S	S	s	S	S	R	R	S
115	60/M	urine	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	R	S	S	S	R	R	S	R	R	R	S
116	4d/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S
117	70/M	pus	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	P	S	S	P	P	S	R	R	R	R	R	S	R	S	S	R	S	R	S	R	R
118	27/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
119	23/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	P	N	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
120	13/F	pus	S.schleiferi	N	P	N	N	P	P	N	P	P	N	N	N	N	N	S	S	N	N	S	R	S	S	R	R	S	S	S	R	S	S	S	S	R	S
121	63/F	blood	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	P	S	S	P	N	R	R	R	S	R	R	R	S	S	R	R	S	R	R	R	R
122	27/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
123	38/M	pus	S.lugdunensis	N	P	N	N	P	N	P	P	P	N	P	P	P	P	S	S	N	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
124	1d/M	blood	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	S	S	R	S	S	S	S	S	S	S	S	S	R	S
125	65/F	urine	S.hemolyticus	N	N	N	N	P	N	N	P	N	N	P	N	P	P	s	S	N	N	R	R	S	R	R	R	S	S	S	R	S	S	R	R	R	R
126	29/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	R
127	40/M	pus	S.schleiferi	N	P	N	N	P	P	N	P	P	N	N	N	N	N	S	S	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
128	23/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S
129	38/M	pus	S.epidermidis	N	N	N	P	P	P	N	N	P	N	P	N	P	N	S	R	P	N	R	R	R	S	R	S	S	S	S	S	S	S	S	R	R	R
130	24/F	urine	S.sapro	N	N	N	P	N	N	N	P	N	N	P	N	P	P	R	S	N	N	S	R	S	S	S	S	S	S	S	S	S	S	S	R	R	S