"CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM PATIENTS ADMITTED IN DR.PRABHAKAR KORE HOSPITAL AND MRC WITH SPECIAL REFERENCE TO METHICILLIN RESISTANCE"

By

DR. RASHMI

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in

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Under the Guidance of

DR.MAHANTESH B. NAGMOTI MD, PhD

DEPARTMENT OF MICROBIOLOGY
J.N.MEDICAL COLLEGE, NEHRU NAGAR,
BELGAUM-590010.

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KLE UNIVERSITY, BELGAUM

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM PATIENTS ADMITTED IN DR.PRABHAKAR KORE HOSPITAL AND MRC WITH SPECIAL REFERENCE TO METHICILLIN RESISTANCE**" is a bonafide and genuine research work carried out by me, under the guidance of **Dr. MAHANTESH B. NAGMOTI** Professor, Department of Microbiology, J. N. Medical College, Belgaum.

Date:

Place: Belgaum. **Dr. RASHMI**

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This is to certify that the dissertation entitled **"CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM PATIENTS ADMITTED IN DR.PRABHAKAR KORE HOSPITAL AND MRC WITH SPECIAL REFERENCE TO METHICILLIN RESISTANCE"** is a bonafide research work done by **Dr. Rashmi** in partial fulfilment of the requirement for the degree of M.D Microbiology.

Date:

Place: Belgaum.

Dr. Mahantesh B. Nagmoti **MD, PhD**
Professor
Department of Microbiology,
J.N. Medical College,
Belgaum – 590010.

ENDORSEMENT BY THE HOD, PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM PATIENTS ADMITTED IN DR.PRABHAKAR KORE HOSPITAL AND MRC WITH SPECIAL REFERENCE TO METHICILLIN RESISTANCE"** is a bonafide research work done by **Dr. Rashmi** under the guidance of **Dr. Mahantesh B. Nagmoti,** Professor, Department of Microbiology, J. N. Medical College, Belgaum.

Dr. S.G.Kardesai MD, DCP
Professor & Head
Department of Microbiology,
J.N.Medical College,
Nehru Nagar,
Belgaum-590010

Date:
Place: Belgaum.

Dr. V. D. PATIL M.D, DCH
Principal
J.N.Medical College,
Nehru Nagar,
Belgaum-590010

Date:
Place: Belgaum.

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Place: Belgaum **Dr. Rashmi**

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Dr. Rashmi

LIST OF ABBREVIATIONS

agr	:	Accessory gene regulator
Bap	:	Biofilm associated protein
BC	:	Bacitracin
CFU	:	Colony forming unit
CONS	:	Coagulase-negative staphylococci
CSF	:	Cerebrospinal fluid
DltA	:	D alanine esterification of techoic acid
FX	:	Furazolidine
ica	:	Intercellular adhesion operon
MLEE	:	Multilocus enzyme electrophoresis
MR	:	Methicillin resistant
MR-CONS	:	Methicillin resistant Coagulase-negative staphylococci
MS-CONS	:	Methicillin sensitive Coagulase-negative staphylococci
NICU	:	Neonatal intensive care unit
Nv	:	Novobiocin
PBP	:	Penicillin binding protein
PBS	:	Phosphate buffer solution
PCR	:	Polymerase chain reaction
PIA	:	Polysaccharide intercellular adhesion
PMS	:	Pyrolysis mass spectrometry
SBA	:	Sheep Blood Agar
ST	:	Sulfamethoxazole
TMP	:	Trimethoprim
UTI	:	Urinary tract infection

ABSTRACT:

INTRODUCTION:

CONS inhabitants of skin and mucous membranes are known to be opportunistic pathogens causing infections in immuno compromised hosts or in patients with medical devices such as intravascular and peritoneal dialysis, catheters, prosthetic heart valves and orthopaedic prosthesis. Today it is well established beyond doubt as the causative agents of various diseases. They cause infections like septicemia, endocarditis, osteomyelitis, urinary tract infection and peritonitis. This study has been undertaken with a view to a speciate the CONS, and also to detect the production of slime and beta-lactamase. As slime is one of the virulence marker, slime is believed to make the micro organism insusceptible to antibiotics.

We have taken the CONS isolated from various clinical specimens like pus, urine, blood, endotracheal tip, cervical swab, pleural fluid, amniotic fluid and CSF during one year period. Antibiotic sensitivity pattern studied with special reference to methicilline.

MATERIAL AND METHODS

The present study was undertaken at the Department of Microbiology, J N Medical College, Belgaum during the period from 01-01-09 to 01-01-10. A total of 120 CONS were isolated from various clinical samples were included in the study. CONS were isolated based on, which showed gram positive cocci in cluster with pus cells on gram stain, pure growth on culture media. Slide coagulase and tube coagulase negative were taken.

All these were speciated according to the Kloos and Schleifer flow chart. These isolates were also tested for slime production by tube method and beta-lactamase production by chromogenic cephalosporin method using nitrocefin disk.

Antibiomicrobial sensitivity testing was performed by Kirby Bauer disc diffusion method on Muller-Hilton agar plate.

RESULTS

Out of 120 isolates, 78 (65.5%) were *S.epidermidis*, 18 (15%) *S.saprophyticus*, 12 (10.83%) *S.haemolyticus*, 04 (3.33%) *S.sciuri*, 02(1.67%) each of *S.hominis*, *S.xylosus* and *S.cohnii* and 01 (0.83%) *S.simulans*.

The majority of the isolates were from pus/wound discharge 58(48.33%) followed by blood 27(22.50%) and urine 23(19.17%). Out of 120, 67(55.83%) were slime producing organisms. *S.epidermidis* showed maximum slime production. 45(37.5%) isolates showed beta-lactamase production. 48(40%) species showed resistance to methicillin. Most of the species showed resistance to penicillin 90(75.17%) followed by ampicillin 87(72.5%) and gentamycin 68(56.66%).

CONCLUSION:

This study concludes that CONS isolated from clinical specimen showed more resistant to penicillin, ampicillin, gentamycin even to methicillin. This has become a serious clinical and epidemiological problem, as resistance to methicillin implies resistance to all β-lactum drugs.

TABLE OF CONTENTS

Sl.No.	Particulars	Page
1.	INTRODUCTION	1-3
2.	AIMS AND OBJECTIVES	4
3.	REVIEW OF LITERATURE	5-50
4.	MATERIAL AND METHODS	51-73
5.	RESULTS	74-82
6.	DISCUSSION	83-92
7.	CONCLUSION	93
8.	SUMMARY	94-95
9.	BIBLIOGRAPHY	96-103
10.	PROFORMA	104-105
11.	CONSENT FORM	106-107
12.	MASTER CHART	

LIST OF TABLES

		Caption	Page
Table 1	:	Distribution of CONS Isolates from different Clinical Specimens	74
Table 2	:	Distribution of CONS species in different age & sex groups	75
Table 3	:	Distribution of Species of CONS in Clinical Specimens	76
Table 4	:	Predisposing factors of CONS species	77
Table 5	:	Production of slime with regards to CONS species & Clinical specimens	77
Table 6	:	Production of slime with regards to CONS species	78
Table 7	:	Production of β-Lactamase with regards to CONS species	79
Table 8	:	Biochemical Characters of CONS	80
Table 9	:	Antibiotic Sensitivity and Resistance Pattern of CONS Species	81
Table 10	:	Methicillin resistant pattern of CONS species	82
Table 11	:	Susceptibility pattern of Methicillin Resistance CONS	82
Table 12	:	Comparison of CONS species isolated among clinical samples	84
Table 13	:	Comparisons of distribution of CONS species	86
Table 14	:	Comparison of slime production by CONS	87
Table 15	:	Comparison of Incidences of MRCONS	91

LIST OF GRAPHS

		Caption	Page
Graph 1	:	Distribution of CONS from different Clinical Specimens	74
Graph 2	:	Distribution of CONS species in different age & sex groups	75
Graph 3	:	Distribution of Species of CONS	76
Graph 4	:	Slime production by CONS species	78
Graph 5	:	β-Lactamase production by CONS species	79
Graph 6	:	Antibiotic Sensitivity and Resistance Pattern of CONS Species	81

LIST OF PHOTOGRAPHS

		Caption	Page
Photo 1	:	Gram stain showing gram-positive cocci in clusters	66
Photo 2	:	CONS colonies on nutrient agar	66
Photo 3	:	S.hemolyticus colonies on blood agar showing beta hemolysis	67
Photo 4	:	Slide coagulase test	67
Photo 5	:	Tube coagulase test	68
Photo 6	:	Furazolidone disc test showing sensitive	68
Photo 7	:	Novobiocin disc test showing resistance	69
Photo 8	:	Bacitracin disc test showing resistance	69
Photo 9	:	S.saprophyticus on SBA showing resistance to novobiocin and bacitracin, sensitive to furazolidone.	70
Photo 10	:	S.epidermidis on SBA showing resistance to bacitracin, sensitive to novobiocin and furazolidone	70
Photo 11	:	Test showing O/F fermentative, nitrate reduction and sugars fermentations	71
Photo 12	:	Phosphatase test	71
Photo 13	:	Beta Lactamase test	72
Photo 14	:	Slime test	72
Photo 15	:	Antibiogram of CONS	73

INTRODUCTION

Coagulase negative Staphylococci (CONS) form the major part of the skin flora of man and other mammals and were considered to be non-pathogenic in past. However infections with these organisms have been reported since 1950 with increasing frequency[1]. They have been implicated as the causative agents of infections in immuno-compromised hosts particularly patients with cancer, burns, end stage renal disease and renal transplantation and of infections related to indwelling catheters, shunts and prosthetic devices[2].

In recent years CONS have emerged as major opportunistic pathogens. They cause infections like septicaemia, endocarditis, osteomyelitis, urinary tract infection and peritonitis[3].

CONS includes *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*[4].

The most frequently encountered CONS species associated with human infections is *S.epidermidis*, in particular in association with intravascular catheter. It is the predominant agent of nosocomial bacteremia, endocarditis, urinary tract infection, surgical wound infection, ophthalmic infections, intravenous catheter infection and infection of various prosthetic devices. The second most frequently encountered CONS species is *S.haemolyticus*. It has been implicated in native-valve endocarditis, septicemia, peritonitis & wounds, bone & joint infections. Similarly *S.saprophyticus* causes urinary tract infections in young healthy sexually active women. Infection with *S.lugdunensis* is seen in endocarditis with massive valve destruction[4].

In *S.epidermidis*, production of slime, a complex glycoprotein, is important in the ability of these strains to colonize on foreign bodies such as vascular catheters or indwelling prosthesis. Slime formation involves both host and bacterial factors. It is an important factor for the establishment of *S.epidermidis* as a pathogen, as it is considered as the one of virulence marker. Slime producing colonies become encased in an extracellular glycocalyx rendering them insusceptible to antibiotics[5].

In recent years, an increase in the number of methicillin resistant coagulase negative Staphylococci (MRCONS) strains has become a serious clinical and epidemiological problem as resistance to this antibiotic implies resistance to all beta – lactum antibiotics. Accuracy and promptness in the detection of methicillin resistance are the key importance in ensuring correct antibiotic treatment in infected patients and control of MRCONS in hospital environments[6].

There are several phenotypic methods, such as MIC determination by agar dilution, broth dilution and E test, the oxacillin screen agar method and disc diffusion test for the detection of MRCONS .The *mecA* gene detection by PCR is useful marker of methicillin/oxacillin resistance. It is the gold standard test for detection of MRCONS isolates. Methicillin resistance is the important factor in the establishment of CONS as a nosocomial pathogen. CONS strains produce an inducible beta-lactamase in 80 - 90% and some of them are methicillin resistant[6].

Because of the increasing clinical significance of CONS, accurate species identification is highly desirable to permit a more precise determination of host-pathogen relation of CONS. Early detection of methicillin resistance strains will help in prevention and control of complications resulting from such infection. Detection of slime producing & beta-lactamase producing strains help in establishment of CONS as a pathogen.

Hence the present study has been undertaken to identify, speciate and to know the antibiotic sensitivity pattern of CONS with special reference to methicillin resistant from patients of KLE'S Dr. Prabhakar Kore Hospital and MRC, as they are considered to be one of the most important pathogens causing severe debilitating infections.

This helps in determination of etiological role of various CONS in the causation of diseases, by characterization of CONS and antimicrobial sensitivity done to prevent MRCONS in our hospital. In addition to this isolates were tested for slime production and beta-lactamase production.

AIMS AND OBJECTIVES

1. Speciation of CONS obtained from various clinical samples like blood, sputum, pus, urine and others.

2. Antibiotics susceptibility pattern of CONS with special reference to methicillin resistance.

3. To test for beta-latamase production

4. To test for Slime production

REVIEW OF LITERATURE

Staphylococci were first observed in human pyogenic lesions by Von Recklinghausen in 1871. Sir Alexander Ogston, a Scottish surgeon described Gram-positive cocci associated with wound infections in 1880. Rosenbach named this organism as *Staphylococcus* and divided into two species, *aureus* and *albus*, based on the colour of the colonies[7].

Staphylococcus aureus was subsequently found in many wound and skin infections. *S.albus*, later called *S.epidermidis*, was considered a non pathogenic commensal organism. Subsequently, the coagulase test was used to separate the two species. Passet described *Staphylococcus citreus*. Alexander Ogston also gave the name Staphylococcus (Staphyle, in Greek, meaning 'bunch of grapes'; kokkos, meaning a berry). Loeb described coagulase test and found that Staphylococcus aureus were capable of producing coagulase and the one which did not produce coagulase are called as coagulase negative staphylococci[7].

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[4].

According to the new edition of Bergery's manual of systemic bacteriology, the staphylococci are in the phylum Firmicutes and comprise of genus I in family V (Staphylococcacea) order I (Bacillalus) in class III Bacilli. Planococci species are

marine cocci that are able to grow in media containing 15% NaCl. Micrococci are psychrophilic Gram-positive cocci that are slightly larger than Staphylococcus cells. Micrococci are 1 to 1.8 μm in diameter. The cells are arranged mostly as pairs, tetrads and irregular clusters. Stomatococci are encapsulated Gram-positive coccus that are a part of the normal human respiratory tract flora[4].

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 investigations 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[4].

In 1963, Baird Parker 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 character like coagulase reaction, mannitol fermentation with acid production both aerobically and anerobically and phosphatase activity[7].

The currently accepted taxonomy of the Staphylococci was proposed by Kloos and Scheifer in the mid 1970s, using biotyping to subdivide the coagulase negative Staphylococci into nine different species. Further evaluations have added six additional species to the genus for a total of 15 DNA homology studies confirm the genetic differences among these biotypes[7].

Several commercially available kits can be used to allow the microbiologist to identify the species of coagulase negative Staphylococci according to the Kloos and Scheifer classification. The Automicrobic system Gram-positive identification card (AMS GPI card, Vitek systems) uses 27 biochemical tests and is automatically interpreted by a reader -incubator module after24 hours. The API Staph Ident system consists of a plastic strip with ten microcouples prefilled with dehydrated reagents. A concentrated suspension of organism is added and manually interpreted from six hours later. The API strip gives consistent results, especially for most common isolates, without overnight incubation. These systems correctly identify 70% - 80% of clinical coagulase negative Staphylococcal isolates[7].

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 hold the most promise 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[7].

Coagulase-negative staphylococci usually behave as saprophytes of low virulence, without causing disease. In the right setting, however, they can indeed be pathogenic. They are emerging as significant pathogens, especially in nosocomial infections CONS infections occur in patients with impaired host defenses, particularly granulocytopenic patients, neonates, patients with implanted foreign bodies.

Antibiotic resistance is common among the coagulase negative staphylococci isolates from such patients[7].

By 1985 there were 19 recognized species of CONS, 8 of which exhibited a possible association with human infections[7]. By 2002 there were 32 CONS species with half of these isolated from human tissue and blood samples[3]. The genus *Staphylococcus* is currently 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*[8].

Staphylococci are non motile, non spore forming, catalase-positive, Gram-positive cocci. These organisms are arranged as single cells, pairs, tetrads and short chains but appear predominantly in grape like clusters, facultative anaerobes, except S.saccharolyticus[4].

Species of CONS (Found in humans)[4]

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 found in animal[4]

S.intermedius

S.hyicus

S.chromogenes

S.gallinarum

S.lentus

S.equorum

S.delphine

S.felis

S.lutrae

S.vitulinus

S.muscae

CONS found in environment[4]

S.carnosus

S.kloosii

S.arlettae

S.piscifermentans

S.condimenti

S.saccinis

S.fleurettii

Evidence emerged suggesting that particular species of CONS may be associated with specific disease, addressing the question of a need for speciation.

Species of CONS and their usual environmental sources[7]

ORGANISM	**ENVIRONMENTAL SOURCE**
S.epidermidis	Human skin
S.haemolyticus	Monkey and human skin
S.saprophyticus	Human skin & urine
S.hyicus	Swine with epidermatitis, cows with mastitis, healthy pigs.
S.simulans	Human skin
S.xylosus	Human skin
S.cohnii	Human skin
S.Warneri	Human skin
S.hominis	Human skin
S.capitis	Human skin, above waist
S.auricularis	Human external auditory canal
S.carnosus	Dry sausage
S.caseolyticus	Cows milk
S.scuiri	Milk, chickens, squirrels, racoons, dog, goat and Human skin.
S.intermedius	Noses of pigeons, mink, horses and dogs
S.lugdunensis	Human skin
S.pasteuri	Food
S.cohnii	Human skin
S.saccharolyticus	Human mucous membrane
S.gallinarum	Poultry
S.lentus	Skin flora of sheep and goat.

STAPHYLOCOCCUS EPIDERMIDIS[4]:

Gram stain: Gram-positive cocci measuring 0.5 - 1.5 µm in diameter in clusters & singles.

Culture characters on Blood agar:

Small to medium, low convex, smooth opaque, gray white colonies, most colonies are non-haemolytic[9].

Biochemical characters:

These are catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, urea hydrolysed, acetoin produced, alkaline phosphatase produced, nitrates are reduced to nitrites, resistant to polymyxin B, sensitive to novobiocin, ferments glucose, maltose, sucrose, fructose, lactose and mannose.

Most frequently recovered organism accounting for 50 - 80% of CONS isolates. It causes prosthetic valve endocarditis, urinary tract infection, surgical wound infection, infection of various prosthetic devices, CSF shunts, ophthalmic infections, peritoneal dialysis related infections. These have a capacity to produce biofilm.

Heilmann and co-workers identified an autolysin adhesion coded for by a gene called *atlE* that works in concert with polysaccharide adhesion in initial adherence of *S.epidermidis* to biomaterials.

Rupp and co-workers showed that a wild type of *S.epidermidis* strain was significantly more likely to cause abscess formation, less likely to be eradicated by host defense and more adherent to implanted plastic than an isogenic PIA negative mutant strain using a mouse foreign body model.

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. 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.

Pathogenesis:

These bacteria produce cell surface and extracellular macromolecules that initiate and subsequently enhance bacterial adhesin to the plastic surface of foreign bodies to form a biofilm. Specific adherence of most of *S.epidermidis* strains to plastic surfaces mediated by capsular polysaccharide adhesin called PS/A protect the organism from complement mediated phagocytic killing. Following initial adhesion to biomaterial there occurs adhesion between cells mediated by a polysaccharide called PIA (Polysaccharide intracellular adhesion).This is how they are associated with polymer Staphylococcal infection[4].

Some strains of *S.epidermidis* produce a fatty acid–modifying enzyme 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[4].

Over the past several years resistance of the coagulase negative Staphylococci to many classes of antimicrobial agents like oxacillin, methicillin has emerged. Use of vancomycin has led to the emergence of CONS with decreased susceptibility to vancomycin[4].

Vancomycin resistant S.epidermidis strains appeared in mid to late 1980s. In 1991, vancomycin resistant *S.epidermidis* strains was isolated from a patient with

peritonitis in England. In 1999 the first case of bacteremia due to *S.epidermidis* with increased susceptibility to vancomycin was reported in US[4].

STAPHYLOCOCCUS SAPROPHYTICUS:

Gram Stain: : Gram-positive cocci measuring 0.5 - 1.5 µm in diameter in clusters and singles.

Culture characters on blood agar: Large low convex with entire edge, very glossy smooth opaque butyrous convex and non haemolytic colonies.

Biochemical reactions:

Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, does not reduce nitrate to nitrites, alkaline phosphatase produced, novobiocin resistant, acetoin produced, urea hydrolysed, ferments glucose, maltose, lactose, mannitol, sucrose, trehalose and fructose.

This is a well documented pathogen causing UTI in young healthy sexually active women. Among young women of college and child bearing age. *S.saprophyticus* is the second most common cause of uncomplicated cystitis after *Escherichia coli*. It accounts for 41-86% of upper urinary tract infection. In urine specimens from these patient, the organism is frequently present in quantities ≤1000,000 CFU/ml, but will be detected in sequential specimens from infected patients.

It is known to cause UTI and urethritis in men and women, catheter associated urinary tract infections, prostatis in elderly men and rarely bacteremia, sepsis and endocarditis[4]. In 1999, Hell and colleagues reported the isolation of *S.saprophyticus* as a cause of nosocomically acquired pneumonia. Rupp and colleagues studied the prevalence of urogenital colonization in 276 women, rectum was the most frequent

site of colonization by *S.saprophyticus* (40%), urethra (30%), urine (20%) and cervix (10%). Hedman and colleagues found *S.saprophyticus* in 16.4% of 11331 food specimens with a prevalence of 34% in raw beef and pork[4].

Pathogenesis:

S.saprophyticus has special affinity to uroepithelium. So it adheres to uroepithelium, urethral and periurethral cells in greater number than other Staphylococci and does not adhere to other cell types. A surface exposed 160 kd protein with hemagglutinin or adhesion properties explain the tropism for kidney colonization. This binds to the fibronectin on uroepithelium cells. Colonization occurs and micro-colonies are formed. Then it elaborate urease, which causes tissue damage and invasion. Another 95 kd surface associated fibrillar protein, present in >98% plays a role as an intercellular adhesion favoring formation of multilayered colonies[3].

Slime production has not been a consistent attribute of *S.saprophyticus* strains. Hjelm and Lundell Etherden found that only 9 of 30 *S.saprophyticus* strains produced slime in trypticase soy broth, all 30 produced slime in urine. These workers proposed that both the preserve of urine and urease production were essential for slime production by this organism. Almost all of these are sensitive to B-lactam drugs and others except nalidixic acid[4].

STAPHYLOCOCCUS HAEMOLYTICUS[4]:

Gram Stain : Gram-positive cocci measuring 0.5 - 1.5 µm in diameter in clusters and singles.

Culture characters on Blood agar: Medium smooth butyrous, white opaque, beta haemolytic colonies[9].

Biochemical reactions:

Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, urea not hydrolysed, acetoin produced, alkaline phosphatase not produced, polymyxin sensitive, novobiocin sensitive, ferments glucose, maltose, sacrose, trehalose, fructose, and mannitol[4].

S.haemolyticus is part of the human skin flora and is also found in non-human primates. It is implicated to cause primary and nosocomial bacteremia, wound and soft tissue infections, urinary tract infections, nosocomial paediatric and neonatal infections. *S.haemolyticus* produces slime and an extracellular lipase which helps in the pathogenesis of disease[4].

Methicillin and quinolone resistant isolates of have been reported. 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. Glycopeptide resistance in *S.haemolyticus* is usually expressed heterogeneously with both susceptible and resistant sub-populations existing within a single culture[4].

The emergence of antibiotic resistant has underscored the importance of correct identification and susceptibility testing of these isolates and monitoring of their spread within the hospital environment[4].

STAPHYLOCOCCUS WARNERI

Gram stain: Gram-positive cocci measuring 0.5 - 1.5 µm in diameter arranged in cluster, pairs and singles.

Culture characters on blood agar: Medium to large smooth, glossy with entire edge slightly domed center, non-haemolytic colonies[9].

Biochemical reaction:

Catalase positive, modified oxidase negative, slide and tube coagulase negative, urea hydrolysed, acetoin produced, alkaline phosphatase not produced, sensitive to polymyxin B & novobiocin, does not reduce nitrate to nitrites ferments glucose, fructose, maltose, sucrose, trechalose, mannitol (variable)[4].

This species represents about 1% of the Staphylococci, normally found on human skin. It is now a well recognized cause of catheter-related bacteremia, native-valve endocarditis, haematogenous vertebral osteomyelitis, ventriculo-peritoneal shunt associated meningitis. These strains produce an extracellular enzyme that has both lipase and phospholipase activity[4].

STAPHYLOCOCCUS HOMINIS

Gram Stain : Gram- positive cocci measuring 0.5 - 1.5 µm arranged in cluster, pairs and singles.

Culture Characters on Blood agar: medium to large, smooth, butyrous opaque non-haemolytic colonies[9].

Biochemical character:

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 polymyxin, ferments glucose, maltose, sucrose, fructose, trehalose and lactose(variable)[4].

Found on the skin of humans. It has reported to cause endocarditis, catheter related sepsis in immune compromised hosts. In 1998 the species was divided into two subspecies, *S.hominis* subsp. *hominis*, *S..hominis*. subsp. *novobiosepticus*.

Subspecies *novobiosepticus* are more resistant to antimicrobial agents than subspecies *hominis*[4].

STAPHYLOCOCCUS SIMULANS[4]

Gram stain – Gram- positive cocci measuring 0.5 - 1.5 µm arranged in cluster, pairs and singles

Culture characters on Blood agar: Large raised circular, non pigmented with entire edge, smooth slightly glistening, non-haemolytic or haemolytic colonies.

Biochemical characters:

Catalase-positive, modified oxidase-negative, nitrates are reduced to nitrites, urea hydrolysed, alkaline phosphatase variable, slide and tube coagulase-negative, sensitive to novobiocin and polymyxin B, ferments fructose, mannose, maltose(variable) lactose, sucrose, trehalose and mannitol.

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. Methicillin resistant strains of *S.simulans* have been reported and they posses an additional penicillin binding protein that exhibits a low affinity for methicillin, cephalothin and cefamandole and has amino acid sequences analogues to *PBP2a* found in methicillin resistant S.aureus isolates.

STAPHYLOCOCCUS LUGDUNENSIS

Gram stain: Gram-positive cocci measuring 0.5 - 1.5 µm arranged in cluster and pairs.

Culture characters on blood agar: medium to large, smooth, glossy entire edge with slightly dome centre, non haemolytic colonies[9].

Biochemical characters:

Catalase-positive, modified oxidase-negative, slide coagulase-negative, Tube coagulase positive, ornithine decarboxylase positive, pyrolidonyl arylamidose activity present, urea hydrolysis variable, acetoin produced, alkaline phosphatase not produced, sensitive to polymyxin and Novobiocin. Ferments glucose maltose, sucrose mannose and trehalose[4].

S.lugdunensis species was first described in 1988. It forms part of the normal skin flora and has considered potential opportunistic pathogen in human. It causes skin and soft tissue infection. In recent years this pathogen has been reported to cause a wide variety of infections like brain abscess, meningitis, sepsis, chronic osteomyelitis, spondyloisis, endocarditis. It is an aggressive coagulase negative Staphylococcus. *S.lugdunensis* produces variety of potential virulence factors, like haemolysis, lipases to esterases[4].

S.lugdunensis isolates are usually susceptible to beta-lactam antibiotics. beta-lactamase production occurs in 24-29% of S.lugdunensis. Oxacillin-resistant strains of *S.lugdunensis* carrying the *mecA* gene have also been reported and characterized[4].

STAPHYLOCOCCUS SCHLEIFERI

Gram stain: Gram- positive cocci measuring 0.5 - 1.5 µm arranged in cluster, pairs

Culture Characters on Blood agar: medium to large smooth, glossy slightly convex with entire edge, unpigmented and non-haemolytic colonies[9].

Biochemical characters:

Catalase-positive, modified oxidase-negative, slide coagulase-negative, tube-coagulase positive, acetoin produced, nitrates are reducted to nitrites, Pyrrolidonyl arylamidase activity present, urea not hydrolysed, alkaline phosphatase produced, sensitive to polymyxin and novbliocin, ferments glucose and mannose[4].

S.schleiferi was first described in 1988. It causes human infections like brain emphysema, wound infections, bacteremia, indwelling catheter infection, nosocomial urinary tract infection, prosthetic valve endocarditis, and osteomyelitis. In 1990, the species was divided into two subspecies *S.schleiferi* subsp. *schleiferi* and *S.scheiferi* subsp. *coagulans*. Virulence factors like glycocalyx, lipases, esterases, haemolysins are produced by this species which contribute to pathogenicity[4].

STAPHYLOCOCCUES CAPITIS:

Gram stain: Gram- positive cocci measuring 0.5 - 1.5 µm arranged in cluster and pairs

Culture characters on blood agar: Small to medium smooth, slightly convex opaque glistening with entire edge and non haemolytic colonies[9].

Biochemical characters:

Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrates are reduced to nitrites, alkaline phosphatase produced, acetoin

production variable, sensitive to polmyxin and Novobiocin, ferments fructose, mannose, sucrose and mannitol[4].

S.capitis species is part of the normal human flora and found surrounding the sebaceous gland on the scalp and forehead. In 1991, this species divided into two subspecies, *S.capitis* subsp. *capitis* and *s.capitis* subsp. *urealyticus*. In 1992 this species has been reported as a cause of native and prosthetic valve endocarditis[4].

S.capitis caused sepsis in critically ill premature infants in 17 cases. In these cases, all of the isolates were resistant to oxacillin, erythromycin and clindamyan. These strains also showed hetero-resistance to vancomycin[4].

STAPHYLOCOCCUS COHNII:

Gram stain: Gram -positive cocci measuring 0.5 - 1.5 µm arranged in cluster and pairs

Culture characters on blood agar: Small to large convex with entire edge, circular smooth glistening opaque pigmented or unpigmented, some show haemolysis and some do not[9].

Biochemical characters:

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* hydrolyse urea. sensitive to polymyxin, resistant to Novobiocin, acetoin not produced, ferments fructose, mannose, maltose, trehalose and mannitol[4].

S.cohnii was first described in 1975. In 1991 it was divided into two subspecies *S.cohnii* subsp. *cohnii* and *S.cohnii* subsp. *urealyticum*. These are 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 due to *S.cohnii* have also been reported[4].

STAPHYLOCOCCUS XYLOSUS:

Gram stain: Gram- positive cocci measuring 0.5 - 1.5 µm arranged in cluster and pair

Culture characters on blood agar: Large raised to slightly convex circular smooth to rough, opaque, dull to glistening. Some colonies show yellow pigmentation, non haemolytic colonies[9].

Biochemical reactions:

Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrates are reduced to nitrites, akalaine phosphatase produced, acetoin produced, urea hydrolysed, sensitive to polymyxin, resistant to novobiocin, ferments fructose, mannose, xylose, arabinose, maltose, sucrose, trehalose and mannitol[4].

S.xylosus has been reported as a cause of upper and lower urinary tract infections ,endocarditis with intravenous drug use[4].

STAPHYLOCOCCUS SCIURI:

Gram stain: Gram- positive cocci measuring 0.5 - 1.5 µm arranged in clusters and pairs.

Culture characters on blood agar :medium to large, raised, smooth glistening circular opaque haemolytic or non-haemolytic colonies[9].

Biochemical reactions:

Catalase-positive, modified oxidase-negative, slide and tube coagulase-negative, nitrates are reduced to nitrites, urea hydrolysed, resistant to novobiocin, sensitive to polymyxin, acetoin not produced, ferments glucose, sucrose, mannitol and trehalose[4].

S.sciuri was first described by Kloos and colleagues in 1976 and is considered one of the most ancestral and dispersed *Staphylococcal* species with a wide range of habitats that includes the skin of several animals as well as environment reservoir like sand water. It is a commensal species in rodents, marsupials, cetaceans[4].

It is known to cause wound skin soft tissue infections, endocarditis, UTI, septic shock, endophthalmitis, peritonitis. In 1998, a case of S.sciuri endocarditis, was reported. It has also been isolated as a cause of peritonitis in a patient on dialysis. S.sciuri produces a variety of putative virulence factors like biofilm, exotoxins, DNAase and proteolytic activity[4].

The rate of isolations of these bacteria from human ranges from 0.042% from urinary tract to 0.087% from female genital tract. They constitute about 0.79 - 4.3% of the total number of CONS isolated from clinical sample. 21.4% of the *S.scuiri* group isolated were found to be clinically significant[4].

Oxacillin-resistant *S.scuiri* are emerging and these are frequently resistant to other antibiotics. *S.scuiri* have been found to harbor a homolog of the *S.aureus* methicillin resistance gene *mecA*[4].

Vijayalaxmi N et al studied biological characters and antimicrobial sensitivity of *S.epidermidis* isolated from human source at AIIMS New Delhi. The following biochemical and physiological tests were performed: production of acid from glucose mannitol, sucrose, maltose, lactose, xylose, xylitol, fructose, arabinose, trehalose, and

ribose, arginine, acetoin and reduction of nitrate. The isolates were classified based on Baird and Parker classification. All the 75 isolates were identified and classified as a S.epidermidis and were further subtyped[1].

In a study conducted by Pal & Ayyageri, 75 strains of CONS from different clinical specimens were classified using the Baird Parker system. *S.epidermidis* was the commonest isolate (60%). Twenty seven (36%) untypable strains typed using Kloos and Schleiferi classification. These strains were identified as *S.chonii*(10), *S.hominis*(8), *S.capitis*(4) *S.haemolyticus*(3), *S.simulans*(1) and *S.Warneri*(1)[13].

Christensen GD et al did the characterization of clinically significant strains of CONS. An outbreak of catheter associated sepsis with CONS was studied and it reported the repeated isolation of CONS in 2 or more culture of the same strain. Characterization was done using three different methods : Phage typing, Baird Parker biotyping and API STAPH IDENT system[14].

Vandessch et al reported 5 cases of human infection with *S.caprae* 2 from UTI ,1 from mitral valve endocarditis and 2 from bacteremia associated with vascular catheters.[15]

Gill V J et al isolated 678 CONS strains from different sources like blood, intravenous catheter tips, 75% of isolates were *S.epidermidis* with *S. haemolyticus* and *S hominis* being second and third respectively[16].

Joseph et al applied a new scheme for identification of CONS to 138 consecutive urinary isolates of CONS. Isolates were classified by the method of Kloos and Schleifer most common species were *S.epidermidis*(53%), *S.hominis*(12%), *S.haemolyticus*(10%) and *S.saprophyticus*(5%)[17].

Wilkinson B J et al did the classification and characterization of coagulase-negative methicillin resistant Staphylococci. Out of 65 clinical isolates of CONS;

S.epidermidis accounted about 63.0%, phosphatase negative *S.epidermidis*(12.3%), *S.haemolyticus*(6.2%), *S.hominis*(3.1%), *S.warneri*(1.5%). Five of the organisms(7.7%) could not beclassified[18].

In a study by Goel MM et al, 96 CONS were speciated by simplified Kloos and Scheifer scheme. These were obtained from urine(41), CSF(8), peritoneal fluid(1), ocular swabs(1), semen(1), urethral swab(1) and catheter tip(1)[19].

In another study by Wesely EK et al, characterization of CONS was done using key characters like coagulase, haemolysis, nitrate reduction and acid production from fructose, xylose, arabinose ribose, maltose lactose sucrose, trehalose mannitol and xylitol aerobically. Novobiocin susceptibility, lysostaphin susceptibility, phosphatase activity anaerobic growth pattern in thioglycollate broth and colony morphology were included to improve the accuracy in identification[20].

Seetha KS et al isolated 180 strains of CONS from blood and CSF. Out of 180, 124 (68.88%) were *S.epidermidis* and 56(31.11%) were *S.saprophyticus*[2].

Aggarwal P et al did the characterization and antibiotic sensitivity testing of CONS from various clinical samples. Out of 192 strains of CONS, *S.epidermidis* 158(82.29%) was the commonest followed by *S.saprophyticus* 30(15.62%)[21].

In a study done by Adriana N, 200 coagulase negative staphylococci were identified using five simple tests: urease test, pyrrolidonyl arylamidase, ornithine decarboxylase and arobic acid from mannose. This method was validated with reference method of Kloos and Schleifer. Of the 200 strains, the most frequent clinical CONS species were *S.epidermidis*(50.5%), *S.haemolyticus*(18.5%), *S.saprophyticus*(16%), *S.lugdunensis*(6.0%) and *S.warneri*(2.5%). This simple method has demonstrated an excellent correlation in the identification of the three most frequent species isolated[22].

In another study by Goyal R et al, Speciation of 102 clinically significant isolates of CONS was done by a scheme utilizing slide and tube coagulase test, urease test, ornithine deccorboxylase, novobiocin susceptibility and acid from mannose. 98(97%) of CONS isolates were identified by the above scheme. 4 species are not identified. *S.epidermidis* (41%) was the commonest, followed by *S.saprophyticus* (16.6%), *S.haemolyticus* (14.7%), *S hominis* (14.7%), *S.lugdunensis* (4.9%), *S.schleiferi* (1.9%) and *S.cohnii* (1.9%)[23].

Peggy et al isolated 68 strains of CONS from urine sample with patients suffering from UTI, cystitis, pyelonephritis in pregnant women, below 20 years of age Out of 68 strains, *S.saprophyticus* was most commonly isolated that is 64 isolates and all these showed resistance to novobiocin[24]

Chiquet C et al reported a case of acute post oprerative endopthalmitis caused by Staphylococcus lugdunensis[25].

Kaabia N et al did molecular identifications of Staphylococcus lugdenensis in a patient with meningitis[26].

Philippe et al developed a new oligonucleotide array to identify *Staphylococcal* strains to species level. It provides an accurate and rapid method for the identification of *staphylococcal* strains, isolated from clinical environmental or food samples at species level[27].

The study conducted by Khatib R et al determined the frequency of strain relatedness among randomly selected patients with coagulase negative staphylococcal infections in multiple blood culture by plasmid typing, determination of species and antibiotyping strain relatedness was demonstrated in 21 of 47 episodes of bacteremia (44.7%) among 34 patients, with a similar percentage among patient with two one

positive blood culture in 24 hours same strain bacteremia was more frequent in cases of infection among patients with a corresponding fever[28].

Heikens EL et al compared the genotypic and phenotypic methods for species level identification of clinical isolates of CONS. 47 clinical isolates of CONS, 10 CONS ATCC strains, and a *S. aureus* clinical isolate were identified using the APID staph ID test, BD phoenix Automated microbiology system and 16S rRNA gene sequencing. It demonstrated that *tuf* gene sequencing is the best method for the identification of CONS, but the API staph ID test is a reasonably reliable phenotypic method[8].

In a study done by Carretto E et al the performance of automated ribotyping was evaluated for identification of coagulase negative staphylococci other than *S.epidermidis*. In total of 177 isolates tested, 166 were identified by automated ribotyping. The identification results were compared with API 20 staph system, standard phenotypic and molecular methods as reference[29].

Singh S et al speciated 150 CONS isolated from NICU by using conventional microbiological methods. 90% isolates were conventionally identified. *S.epidermidis* (40%), *S.saprophyticus* (14%), *S.haemolyticus* (1.6%), *S.schleiferi* (2%), *S.capitis* (4%), *S.wanneri* (6), *S.lugdunensis* (6%) and *S.hominis* (6%). 15 isolated were not identified to the species level[31].

Singhal R et al did speciation and antimicrobial susceptibility of CONS in a tertiary care hospital by conventional tests like slide coagulase, Phosphatase, mannitol and trehalose fermentation, urease production, Voges-Proskauer test and resistance to novobiocin. A total of 83 CONS isolated from clinical samples, *S.epidermidis* (33.7%) was the commonest followed by *S.haemolyticus* (13.3%) and *S.lugdunesis* (13.3%), *S.schleiferi* (12.1%), *S.capitis* (8.96%), *S.xylosus* (5.6%), *S.hominis* (2.4%),

S.warneri (1.29%). Seven isolates remained unidentified. The most common source for all the species was blood[31].

SLIME

Slime is an extracellular polysaccharide and is important in the ability of these strains to colonize on foreign bodies such as vascular catheters, indwelling prostheses. This polysaccharide is a polymeric homoglycan consisting of repeating units of β-1,6 N-acetylglucosamine, termed polysaccharide intercellular adhesins (PIA). Synthesis of PIA is linked to the biosynthetic enzyme – coding genes located in the intercellular adhesuss operan (icc ADBC)[32].

Slime producing colonies become encased in a extracellular glycocalyx making them insusceptible to antibiotic. It as regarded as one of the virulence marker[5].

Methods to detect Slime:

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

1. Tube method:

A loopful of organisms from a blood agar plate was inoculated into 5ml of trypticase soy broth & incubated at 35^0 Celsius for 48 hours then the contents of the tubes stained with saffranin for 30 minutes. If visible saffranin stained film seen on

the wall of the tube is taken as positive for slime production. If a visible film seen at air-liquid was not considered as slime producing strain[33].

2. Congo Red method:

The test is done on Congo red media. It consists of brain heart infusion both 37g/L, sucrose 50g/L, agar 10g/L, & Congo red 0.8g/L. Congo red stain was 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.

One colony of a studied grown on 18-2 hour trypticase soy agar was spread on Congo red- color plate & incubated aerobically for 24 hours at 37^{o} C. A positive result was indicated by black colonies. Non slime producing organism remains pink color[34].

3. Spectrophotometric methods.

3a) Adherence test:

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

Overnight cultures of bacteria in trypticase soy both were diluted in 1:100 fresh trypticase soy both and 1 ml of each was taken in a separate quartz cuvetts. After overnight incubation at 37^{o}C, the cuvetts were 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 removed by decanting the cuvetts first & then rinsing them gently with tap water. The optical density of the stained bacterial film was read with the help of a spectophometer at 570nm. The cut off OD was calculated as 3X standard deviation above the mean OD of 10 blanks stained exactly by similar procedure[35].

3b) Microassay:

It is performed in sterile, polystyrene 96-well flat bottomed plates The basis of the assay is that slime positive cells adhere to polystyrene and cohered to each other, forming a biofilm whose density was measured spectoscopically at a wavelength of 492nm with a micro ELISA autoreader after staining[35].

4. Transmission electron microscopy:

Organism incubated in trypticase soy both at 37^{o} C overnight. Then washed in PBS and fixed in 2.5% glutaraldehyde in 0.1ml PBS for 30min at 4^{o}C. Centrifuge at 120g, pellets were 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 This section of 60-70nm were cut, stained with aranyl acetate and alkaline lead citrate for 1 min and observed under transmission electron microscope[33].

In a study conducted by Christensen GD et al, Slime production by CONS on intravascular catheter showed 63% strain positive for slime when cultured in trypticase soy broth & 37% randomly from blood culture. Transmission and scanning electron micrographs showed slime producer to be encased in an adhesive layer on the catheter surface while non-slime producer were not encased[33].

In one study the usefulness of a test for slime production as a maker for clinically significant infection with CONS and its implications for therapy were examined. 81% of 59 infections episodes were due to slime positive CONS. In contrast, 22 non-infectious episodes were due to slime positive and negative isolates. 32% patients with slime positive organisms improved by treatment with antibodies

alone. But 93% patients with slime positive organisms improved significantly on prosthetic device removal in addition to antibiotic treatment[36].

In another study slime production was observed in all CONS except *S.capitis*. Slime production strains were mostly from blood (38.5%), tracheal aspirations (20.5%) and reported 60% of CONS to be slime producer[37].

In the similar study, the prevalence rate of slime productions among CONS species was 40%. Among the CONS isolated, *S.epidermidis* species showed 62% to be slime production followed by *S.saprophyticus* (38.6%) and *S.hominis* (36%)[38].

In another study, slime production was detected by a new Congo red method and compared it with routinely used Christensen method. Both gave the same results in 124 strains of CONS except one strain which was negative by Christensen method, but positive by Congo red method[39].

Kotilainen P et al reported 65% slime production by pathogenic strains & 40% in non-pathogenic strains[40]. In the study conducted by Mohan U et al, slime production in 48.7% of S. epidermidis & 26.6% in *S.saprophyticus* strains[21].

Koksal F et al showed resistance to methicillin in 81% of slime positive and 57% of slime negative strains[41]. Boynukara B et al reported 60% of CONS to be slime producer[37].

BIOFILM:

Bioflims have been described in many system since van Leuwenhoek, exclaimed the animaleules in the plaque on his own teeth in the 17th centaury.

Marshall in 1976 noted the involvement of "very fine extracellular polymer fitrills that anchored bacteria to surfaces. Consterton et al in 1978 observed that communities of attached bacteria were found to be enclosed in a glycocayx matrix which is polysaccharide in nature. Costerton et al in 1987 stated that biofilm consists of single cells & micro colonies all embedded in a highly hydrated predominantly anionic exopolymer matrix. Characklis and Marshall in 1990 described the biofilm in term of spatial and tensorial herterogenicity and involvement of inorganic or abiotic substances held together is the biofilm matrix. Costerton et al in 1995, emphasized that biofilm could adhere to surface and interfaces to each other at the same time. Consterton & Lappin-Scott stated that adhesion triggered expression of genes controlling production of bacterial components necessary for adhesion and biofilm formation[42].

Biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substation / interface or to each other, are embedded in a matrix, a extracellular polymeric substances, that they have produced & exhibit an altered phenotype with respect to growth rate & gene transcription[42].

Biofilm Formation:

Biofilm formation is multifunctional & the ability to form a biofilm makes the strains much better able to survival in the normally hostile environment of tissue and blood. Biofilm formation appears to be a bacterial survival strategy that is turned on

when, for example oxygen & Fe+ ions become limited, sublethal concentration of certain antibiotics are present or other stress situation emerge[43].

Involves 2 Steps

1) The attachment of the bacterial cells to the polymer surface, which may occur very rapidly.
2) The growth dependent accumulation to form multilayered cell clusters.

PIA production plays a major role in generating a biofilm synthesis of PIA is linked to the biosynthetic enzyme-coding genes located in the intercellular adhesion operon (ica ADBC) and biofilm production involving PIA is known to be ica-dependent and is considered the primany mechanism. PIA leads to intercellular adhesion[32]. Other ica- independent mechanisms of biofilm development have been identified such as major cell wall autolysin (AtlE) which promote the initial adhesion of *S.epidermidis* to surfaces.

More recently it has found that atlE is influenced by the accessory gene regulator (agr) two component system. Cofactors such as the accumulation associated protein (AAP) and the biofilm-associated proteins (Bap) or the D-alanine esterification of techoic acid (DltA) helps in biofilm formation. Once a biofilm is formed the cells are nearly invulnerable, they are then shielded from the immune system & resist antibiotic treatment. Therefore it is very important to understand the various aspects of biofilm formation.

Three basic ingredients of biofilm are:

- Microbes
- Glycocalyx
- Surface

Distribution of Biofilm:

1) Natural aquatic environment
2) Industrial aquatic system
3) Medical biomaterials like central venous catheters, pacemaker, mechanical heart valves, Foleys catheter, Intravenous devices, endotracheal tubes etc[42].

RELATION SHIP BETWEEN BIOFLIM FORMATION AND DISEASE:

Bioflim have role in infectious disease, cystic fibrosis, periodonititis, indwellimg medical devices.

Suggested mechanism:

1) **Detachment of cells or cell aggregate:** Cell may detach from bioflim, due to cell division or cell growth within the bioflim. Increase in shear stress occurring as a result of change in direction or rate of flow. This may result in blood stream or urinary tract infection.
2) **Resistance to the immune system:** Extracellular slime produced 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 bioflim and resistance factors may be carried on plasmids. Conjugation is favoured in bioflim because of close proximity of cells. This is relevant in case of indwelling medical devices[42].

Native valve endocarditis:

Results from the interaction between vascular endothelium of valves of heart & bacteria.

Mechanism[42]:

Endothelial injury

↓

Thrombus formation

↓

Fibronectins
(platelets, endothelial, cells & fibroblasts)

↓

Bind to collagen, human cells & bacteria through fibronectin receptor.

↓

Bacteria start multiplying & form microcolonies on the platelet fibrin matrix.

↓

Bioflim on the valves

<u>Bioflim on medical devices:</u>

Urinary catheters:

Organism introduced into urethra or bladder as the catheter is inserted. Sheath of exudates forms around the catheter and travel intraluminally from inside part of

tubing or collecting bag. Initially colonization occur & urease produced by organisms increases the local pH & leads to precipitation of minerals[42].

Central venous catheters:

Organism gain access through insertion site of external surface of device, from hub due to manipulation and migration along the inner lumen. The device is in direct contact with the blood stream surface. Surface gets coated with platelet, fibrinogen and fibronectin. Organism adheres to the fibronectin. Organism also produces adhesin & forms aggregation of microbes resulting in biofilm[42].

Diagnosis of biofilm:

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, sonicate vortexed ,cultured.
   - Sonicate, vortex, homogenized cultured
   - Alginate swat
   - Endoluminal brush introduced into the implanted catheter, PBS, sonicate & plated.

2) **Visualization of biofilm:**

   A) Scanning electron microscope: Utilizes graded solvents (alcohol, acetone) to dehydrate the specimens. Sample disorted, polymeric matrix appears as fibril, used for examination and characterization of biofilm on medical device.

   B) Confocal laser scanning microscope: Biofilm can be examined insitu without dehydration.

   C) Epifluorescent microscope: Biofilm is stained with fluorescent stain[42].

Resistance to antimicrobial agents:

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

1) Decreased penetration of antimicrobials
2) Slow growth of biofilm microbes
3) Bacteria exchange plasmids by conjugation
4) Physical changes, toxic metabolites, nutrient limitations & low concentration of oxygen[42].

Intervention strategies:

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

Prophylactic antibiotic therapy to cover the surgical insertion of most biomaterials and temporary intravascular device insertion was the commonest practice. But Sieradzki and colleagues reported on the emergence of an *S.epidermidis*

strain with elevated vancomycin tolerance following vancomycin prophylaxis in dialysis patients.

Alternative strategies to inhibit bacterial attachment or colonization or both, of implanted biomaterials are now the focus of many investigations

- Prevent initial device contamination
- Remove the device
- Increase penetration of biofilm matrix by the antibiotics
- Minimize initial microbial cell attachment to the device
- Combining antibiotic therapy with the passage of a low voltage electric current through the implanted biomaterial to prevent bacterial attachment or colonization or both.
- Generation of electrolytes such as protons,hydroxyl ions,reactive oxygen & hydrogen appears to enhance antibiotic killing bacterial antibiotic killing of device surface & is termed the bioelectric effect.
- Use of antiseptic impregnated catheters
- Prophylactic antibiotic therapy to cover surgical insertion of biomaterials[43].

Strategies to develop biomaterials with resistance to bacterial colonization focus on achieving an optimum concentrating of the antimicrobial agent in the biomaterial, in order to deliver either a short term high concentration or a long term constant concentration of the particular agent[43].

DRUG RESISTANCE

Empirical treatment of CONS infections has become more difficult because of increasing resistance to multiple antimicrobial agents. In early reports, almost all coagulase negative staphylococci were very susceptible to most commonly useful antimicrobials. These included penicillin, cephalosporins, tetracyclines, macrolides and aminoglycosides. Unfortunately more recent reports have shown increasing resistant to multiple antimicrobials. Transfer of plasmid carrying genes for antibiotic resistance between staphylococci has been demonstrated. This occurs between staphylococci of both the same and different species. A gentamycin resistant plasmid in *S.epidermidis* strain has been isolated from various nosocomial sources. The mechanism for the transfer of such plasmid appears to be by bacterial conjugation[44].

In 1970's antimicrobial susceptibility in CONS had become an issue, when these species were isolated as pathogens in prosthetic valve endocarditis. There were earlier harbingers that new resistance genes were emerging, specifically those whose products would inhibit the binding of semisynthetic penicillin 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[45]. Also in 1970's methicillin resistance was more prevalent in CONS than methicillin resistant S aureus, an observation that continuous to be true todays antibiotics. Methicillin resistant CONS are typically resistant to a variety of antimicrobials, including quinolones, fobromycin, trimethoprim-sulfamethoxozole, tetracycline and erythromycin. Therefore before any drug other than vancomycin is used, susceptibility of the isolate must be confirmed[45].

Hussain et al in 2002 found that CONS with the *mecA* gene were resistant to erythromycin, clindamycin and co-triamoxazole, but the tetracycline was equally active against *mecA* positive and *mecA* negative isolates. During the early 1980's the problem of multi resistance in CONS was reported primarily in cases of device related infections, particularly prosthetic valve endocarditis. Archer in 1978 reported 56% of methicillin resistance in S-epidermidis isolates associated with device infection. Archer and Climo in 1994 reported increase in resistance for almost all antimicrobial classes excluding glycopeptides[45].

Vancomycin is the antibiotic most likely to be active against S.epidermidis including MRCONS strians. *S.saprophytius* has an unusual antimicrobial susceptibility pattern. It is almost always susceptible to most antibiotics except nalidixic acid[45].

CONS are ubiquitous in nature residing on skin and mucous membrane when exposed to medical devices, the CONS anchor themselves to a polymer surface via van-der waal's forces, hydrophobic interactions and polarity ultimately forming a thick biofilm. Production of biofilm reduces the organism susceptibility to specific antimicrobials. The problem of antibiotic biofilm interaction has recently become a stimulus to develop antibiotics that can inhibit microbial adhesion to polymer surfaces[45].

In recent years an increase in the number of methicillin- resistant coagulase negative staphylococci strains (MRCONS) has become a serious clinical and epidemiological problem, as resistance to this antibiotic implies resistance to all beta-lactam antibiotics.

Accuracy and promptness in the detection of methicillin resistance are of key importance in ensuring correct antibiotic treatment in infected patients and control of MR-CONS in the hospital environments.

METHICILLIN RESISTANCE

1. Hyperproduction of beta-lactamase:

It is due to acquisition of plasmid borne gene element causing resistance. It is due to "hyperproduction" of beta-lactamase enzymes, resulting in slow hydrolysis of the semisynthetic penicillins and borderline methicillin/oxacillin resistance. This is termed as borderline methicillin resistance[46].

2. Role of Penicillin-binding protein 2a (PBP2a):

Bacteria harbor surface-localized penicillin binding proteins with binding affinity for beta-lactam antibiotics. PBPs of Penicillin susceptible Staphylococci include five proteins designated PBP1, PBP2, PBP3, & PBP4 with decreasing molecular from 87KD4 to 41KD4

Resistance is associate with production of altered penicillin binding protein, PBP2a with low binding affinity for beta-lactamase antibiotics and cephalosporin PBP2a results from acquisition of a chromosomal gene called *MKA*. Once the normally present PBPS have been inactiviated by a beta-lactam agent, PBP2a continues to function and allows the synthesis of a stable peptidoglycan structure, thereby allowing the organism to grow and divide. The *mecA* gene may be in a given population, so resistance mediated by altered PBP is termed as hetero-resistance.

Strains that are resistant to methicillin/oxacillin by altered penicillin binding protein usually have higher levels of oxacillin resistance and resistance to multiple antibiotics like clindamycin, erythromycin and cephalosporins[46].

3. Genes involved in methicillin resistance:

Mec A gene, PBP2a is encoded by the chromosome linked *mecA* gene (2130 bp) with putative gene product of 76.462 KDa. The gene is nearly always harboured by isolates of methicillin resistant strains but not by methicillin sensitive strains. This has enabled gene based identification of methicillin resistance by *mecA* detection using gene-specific DNA probes or PCR based on *mecA* specific sets. Tests for mecA or for the protein encoded by mecA PBP2a are the most accurate methods for prediction of resistance to oxacillin.

TRIMETHOPRIM RESISTANCE:

The incidence of sulfamethoxazole(ST) and trimethoprim(TMP) resistance is very high and also ST resistance in CONS is closely related to trimethoprim (TMP) resistance. This prompted to investigate the TMP resistance genes in CONS isolates[47].

Archer and colleagues have found homologus *Tpr* genes on conjugative plasmids in geographically diverse *S.aureus* and *S.epidermidis* isolates. They have also described that 17% of CONS have shown resistance to trimethoprim as compared to *S.aureus* in which they found 10% resistance[48].

AMINOGLYCOSIDE RESISTANCE:

It is mediated by enzyme modification The bifunctional 61acetylating and 21 phosphorylating enzyme is most common. Resistance to amikacin and tobramycin is

mediated by the domain having 61 acetylating activity, where as resistance to gentamicin results from the 21 phosphrylating activity towards carboxy terminal of protein. Genes are encoded by both plasmids and transposable DNA elements[49].

Major Resistance mechanisms[49]:

Aminoglycosides	Enzyme modification
Choramphenical	Enzyme modification
Clindamycin	Altered target
Erthromycin	Altered target
Penicillins	Enzyme modification
Methicillin	Altered penicillin-binding proteins.
Tetracycline	Altered target, active import
Trimethoprim	Insensitive target
Quinolone	Insensitive target, reduced uptake
Rifampin	Altered target

DETERMINATION OF METHICILLIN REISTANCE

Rapid accurate, detection of methicillin resistance is important therapeutically and epidemiologically for health care institutions.

Following are the various methods.

1. Disc Diffusion method[5]:

The disc diffusion method by Kirby Baeur method is reliable for detection of methicillin resistance if the proper antibiotic and temperature are

used. Resistance is readily detected for oxacillin (1mg/disc) at 30^{o} c and 35^{o} c but may be missed at 37 c.

Sensitivity - 99.4%

Specificity - 91.8%

Media used:

Isosensitest agar incubation at 30^{o} c for 24 hours.

Muller-Hinton, Nutrient or Columbia agar, incubated at 30^{o} c for 4 hours.

Muller -Hinton or Columbia agar supplemented with an additional 5% sodium chloride.

2. Broth Dilution method[50]:

Before the development of a standardized method, upto 50% of resistant strains tested were susceptible my microdilution MIC. However if appropriate conditions are used 95% of resistant strains can be detected by this method. 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 c for oxacillin naficillin or methicillin.

3. Agar screen method[51]:

In the agar screen test; an inoculum of 10^{4} CFO is spotted onto Muller-Hinton agar supplemented with 4% NaCl containg 6 mg of oxacillin/ml. After 48 hour of incubation at 35^{o} c , the agar is inspected for growth of colonies, grow of even an single colony is indicative of resistance.

Sensitivity - 98%

Specifically - 100%

4. Fluorescence Test[52]:

A commercially available fluorescence test for the rapid detection of methicillin resistance has been used. Here the fluorescence indicator detects oxygen consumption by actively growing organisms in microtitre panel. The susceptible organism do not fluorensce, where resistant organism, as a result of their metabolic activity, do fluorensce.

5. Strip test[5]:

Inoculated plates of isosensitest agar should be streaked with the organisms to be tested to 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 C for 18 hours.

6. The E test[5]:

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

Sensitivity - 100%

Specificity - 85%

5. Latex slide agglutination for PBP2a[50]:

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

50 µl aliquot of the supernatant was mixed with 1 drop of anti PBP2a monoclonal antibody sensitized latex beads. A negative control test was performed by using 50µl of supernant mixture 1 drop of negative control latex. The isolates then placed on a shaker and gently mixed for upto 15 min. The resulting agglutination pattern was read at l0 min.

Sensitivity - 100%

Specificity - 98.5%

6**. Automated systems[50]:**

The automated systems are appearing because results can be obtained in a few hours. Unfortunately for any of these systems the sensitivity for detection of methicillin resistance has been low, especially for heteroresistance. The specificity of these systems are high.

Commercially available automated (GPS – SA cards) Vitek system are used.

8. **Polymerase chain reaction for mecA gene detection[50]:**

The *mecA* gene detection by PCR is a useful marker of methicillin /oxacillin resistance. It is the gold standard for the detection of MR CONS isolates.

Hackbarth CJ et al studies showed 59.6% CONS were resistance to penicillin & *S.epidermidis* was resistant than *S.saprophyticus* in their study[51].

Seetha KS et al studied 180 strains of CONS isolated from blood & CSF during the period of 2 years and observed resistant in penicillin (70%), ampicillin (80.64%) in *S.epidermidis* species & resistant to penicillin (82.1%), ampicillin (87.5%) oxcillin (87.5%) in S.saprophyticus species[2].

Blood culture isolates of CONS in NICU were studied for methicillin resistance in KGMU Lucknow using Kirby Bauer disc diffusion technique and by

PCR. Among the blood culture isolates 68% were MRCONS & 32% MSCONS by Kirby Bauer disk diffusion technique. Detection of *mecA* gene was carried out in 43 randomly selected blood isolates. The mecA gene was present in CONS isolates & its presence correlated with the phenotypic expression of methicillin resistance[53]. Phatak J et al reported a maximum resistance of CONS against penicillin (77.3%) & minimum to gentamycin (21.6%)[54].

Hussain Z et al detected methicillin resistance CONS in primary blood culture isolates by PCR, slide agglutination, disk diffusion method. Disk diffusion method showed 99.4% sensitivity & 91.8% specificity[50]. Eric S et al evaluated three methods for detection of MR using NCCLS new interpretive criteria PCR was taken as gold standard to evaluate disk diffusion with Muller-Hinton agar, mannitol salt agar & Scepter system for the detection of *mecA* genes. Sensitivity on Muller-Hinton agar (90%), mannitol salt agar (82.6%), scepter system (72%). Specificity on Muller-Hinton agar (79.2), mannitol salt agar (95.8%), scepter system (92.2%). Muller-Hinton agar of MR approaches the accuracy of PCR[55].

Amstrong DG et al studied methicillin resistant coagulase negative staphylococcal in osteomyelitis & its relationship to broad spectrum oral antibiotic in cases of diabetic population. Prevalence of CONS was 40% of which 63% methicillin resistant. Patients with oral antibiotic were treated with ciprofloxacin for more than 2 weeks and found 54% methicillin resistance and 15% showed methecllio sensitive[56].

Koksal F et al studied antibiotic resistance pattern in CONS causing nosocomial bacteremia. 200 isolates of CONS strains from blood samples were studied. Resistance to methicillin was detected in 67.5% of CONS isolates. MRCONS strains were determined to be more resistant to other antibiotics than MSCONS strain[56].

Vijaylaxmi et al studied 75 strains of CONS isolated in pure culture. All the strains were sensitive to oxacillin, novobiocin, nalidixic acid. 86 - 9.77% were sensitive to methicillin cephalexin, gentamycin, cephalosporins. Only one strain was sensitive to penicillin[1].

TYPING SYSTEMS

Hospital practices are facing a new menace in the form of CONS infection which are increasing day by day. Typing methods can be used as epidemiological marker to identify the potential sources of the varied strains to evaluate the role of human carriers, and to differentiate the epidemic and endemic strains.

1. Antibiogram typing

Antibiogram typing was performed by qualitative method i.e. resistance, profiles and quantitative method i.e. similarity analysis of inhibition zone diameters. This is done by the conventional Kirby Bauer disc diffusion test. Antibiograms of CONS have been found to vary with geographical location.

2. Biotyping

Based on numerous biochemical or physiological tests, various schemes of biotyping for CONS have been developed. However, there have been no attempts made to standardize the tests or methods used to compare the different schemes used.

Bioptyping can be done by using commercial identification systems such as API Staph IDENT, ATB 32 Staph system[57,58].

3. Reverse Phage Typing

Extraction of free phage from the strains is done with mitomycin C. After incubation and centrifugation, the supernatant was spotted on propagating for the CONS phages and released phages were detected. Combined with the antibiogram and biotyping, phage typing credibility can be increased and it has been suggested that phage typing should be reserved for epidemic rather than endemic situations[59].

4. Ribotyping

A selection of isolates for which clarification of relatedness was sought were further characterized by ribotyping. Whole cell DNA was digested with a restriction enzyme. DNA fragments were separated by horizontal agarse gel electrophoresis and transferred to nylon membrane. Hybridization was performed with plasmid containing an RNA operon *Escherichia coli*. The DNA probe was labeled with biotin. Bands of hybridization were detected with Blue Gene Kit[60].

5. Serotyping

The serotyping of *S.epidermidis* as an epidemiological tool has not developed compared to phage typing. This is due to the difficulties in preparing specific antisera, although serotyping in combination with biotyping has been used successfully in identifying strains of S.epidermidis possessing a particular agglutinogen or polysaccharide B or thermostable and thermolabile antigens[61].

6. Molecular Analysis of Plasmids

Agarose gel electrophoresis has been used to detect plasmids and estimate their molecular weights when combined with restriction enzyme digestion of

plasmid DNA, it is possible to "type" plasmids. More definitive evidence of the extent of homology between two plasmid molecules is obtained by DNA[61].

7. DNA hybridization.

Restriction enzyme analysis and nucleic acid hybridization provides additional information on the nucleotide sequences within the plasmid and makes it possible to "type" plasmids to determine the identity between them[61].

8. Whole – cell protein typing:

Sodium dodecyl sulphate – polyacrylamide gel electrophoresis was performed by a modification of the method of Clink and Pennington. Samples were prepared by broth culture, centrifugation and sonication. Electrophoresis was performed on 10% polyacrylamide gel in a discontinuous buffer system and gels were stained. Then the gels were scanned by a densitometer. The output voltage from the densitometer was converted into a series of integer values and this information was transmitted to a micro computer. The strains were regarded as indistinguishable if the percentage relatedness was equal to or greater than that for the control strains of *S.aureus*[59].

9. Pyrolysis Mass Spectrometry:

Pyrolysis mass spectrometry (PMS) has been reported to be a rapid, sensitive and reproducible method for inter strain comparison of microorganisms. Another potential use of PMS is in comparing several isolates from an individual patient[60].

10. Multilocus enzyme electrophoresis (MLEE):

MLEE detects changes in the electrophoretic mobilities of the individual soluble metabolic enzymes. Such variations, referred to as electromorphs, identify allelic alterations in the chromosomal genes encoding the enzymes, distinctive

combinations of electromorphs are designated as electrophoretic types (ETs). The evaluation of multiple constitutive metabolic enzymes ensures that all isolates are typable[60].

MATERIAL AND METHODS

The present study was undertaken at the department of Microbiology, J.N Medical college, Belgaum during the period from 01/01/2009 to 01/01/2010 and a total of 120 CONS isolated from clinical samples such as pus, urine, blood, endotracheal tube tip, cervical swab, pleural fluid, amniotic fluid and CSF were included in the study based on the inclusion and exclusion criteria mentioned below:

Inclusion Criteria:

1. When Gram positive cocci in clusters seen in a Gram stained smear along with pus cells.
2. When CONS strain repeatedly isolated from the same lesion.
3. When these CONS found relevant by critical appraisal of clinical picture.

Exclusion Criteria:

1. CONS isolated along with other bacteria (mixed culture)
2. CONS isolates with no clinical correlation of symptoms.

A detailed clinical history was obtained from each patient

The isolates were characterized by a battery of tests as follows:

1. Gram stain for morphology
2. Study of cultural characters

    On Blood agar at 37c

    On MacConkey agar at 37c

3. Catalase test
4. Oxidase test
5. Slide coagulase test
6. Tube Coagulase test

7. Urease test

8. Phosphatase test

9. Oxidative and fermentative test

10. Carbohydrate utilization test

11. Antibiotic sensitivity testing by Kirby-Bauer's disk diffusion method.

12. Furazolidine Susceptibility test

13. Bacitracin Susceptibility test

14. Novobiocin Susceptibility test

1 Gram's strain[5]:

Procedure: Isolated colony was selected for staining.

A drop of saline taken on the slide and the isolated colony form the solid media was picked up and emulsified in the saline using a sterile loop. The smear was allowed to air dry and then heat fixed by passing it through the flame 3 times.

The staining was done using Hucker's modification as follows

The slide was flooded with crystal violet and kept for 1 minute

The slide washed in running water.

The mordant Gram's Iodine was then poured into the slide and left for one minute.

The slide was then washed under running water and decolourised using spirit. One or two drops of spirit were put onto the smear and washed within 1-2 seconds to avoid over decolourisation. This was then immediately washed in running water and the counterstain 1% safranine was added and left for 30 seconds. After washing under running water, it was allowed to air dry.

The slide was then observed under oil immersion objective of the microscope for the morphology of the organisms.

Interpretation:

CONS were identified as –Gram positive cocci in cluster, pairs and singles.

Quality control:

Positive control: *Staphylococcus aureus* ATCC 25923

Negative control: *Escherichia coli* ATCC 25922

2. Colony Morphology following culture:

Procedure: Samples were cultured on Nutrient agar plate and Blood agar plate.

After 24 hrs of incubation at 37^0c, colony morphology were noted as below

Interpretation:

Nutrient Agar plate – CONS colonies were 2-4mm in diameter, circular, smooth whitish, and opaque.

Blood agar plate: CONS colonies were 2-4mm in diameter, circular, smooth, and opaque few showed beta-haemolysis, many showed no haemolysis.

3. Catalase test[5]:

Principle: The enzyme catalase mediates the break down of hydrogen peroxide (H_2O_2) into oxygen and water. The presence of the enzyme in a bacterial isolates is evident when a small inoculum is introduced into H_2O_2, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production

Procedure: A small amount of culture to be tested was picked from a nutrient agar plate with a clean sterile platinum loop or, a clean, thin glass rod and was inserted in hydrogen peroxide solution (10 volume) placed in a clean, small tube.

Interpretation: Positive reaction was noted when gas bubbles from the surface of the solid culture material and negative reaction when there were no gas bubbles.

Quality control:

Positive control: *Staphylococcus aureus*

Negative control: *Streptococcus pyogenes*

This test was used to differentiate Staphylococci from Streptococci

4. Modified Oxidase test[4]:

Principle: To determine the presence of an enzyme oxidase, which catalyse the transport of electrons between electron donors in the bacilli and redox dye. The dye is oxidized to indophenol blue producing deep purple colour.

Procedure: The reagent used was Kovac's reagent 1% tertramethyl-p-phenylenediamine dihydrochloride with dimethyl sulfoxide (DMSO). A filter paper was moistened with the Kovac's reagent and speck of the colony was rubbed on it with a sterile glass rod.

Interpretation: A deep blue to purple colour appearing within 10 seconds was considered a positive result. A colour appearing between 10-60 seconds was considered a delayed positive result. Any colour appearing after 60 seconds was considered negative. *Staphylococcus* were oxidase negative and *Micrococcus* were oxidase positive.

Quality Control:

- Positive control: *Pseudomonas aeruginosa* ATCC 27853
- Negative control: *Escherichia coli* ATCC 25922

5. Furazolidone disk test[4]:

Principle: Susceptibility to the compound furazolidone (FX) helps in the identification of staphylococci from micrococci

Reagents: Furazolidone disk (FX) 100µg. Sheep blood agar

Procedure: Suspension of the organism to be tested was prepared in sterile distill water or broth. The broth was incubated at 37^0c for approximately one hour. After this period, the turbidity of the broth was matched with the turbidity of the 0.5 McFarland turbidity standard (i.e 1.5 x 10^8 CFU/ml). A swab was immersed in to the matched broth and then rolled and squeezed against the slides of the tube to remove excess broth. The swab was then used to inoculate onto one-half of a blood agar plate. Aseptically FX disk was placed in the center of the inoculated area, and gently disk was tamped so it adhered to the agar surface. Plate incubated at 35^0c in an ambient-air incubator for 18 to 24 hours.

Interpretation:

Micrococcus species were FX resistant (6mm/no zone to 9mm)

Staphylococcus species were FX susceptible (≥15mm)

6. Novobiocin disk test[4]:

Principle: Coagulase-negative Staphylococci can be divided into novobiocin –susceptible & novobiocin –resistance species, Among the novobiocin-resistance S.saprophyticus is the one commonly recovered from human as a cause of urinary tract infections. Therefore, screening coagulase –negative staphylococci isolated from quantitative urine culture for susceptibility to novobiocin provides a reliable presumptive identification of this species.

Reagents: Novobiocin disk 5µg, Sheep blood agar

Procedure: Suspension of the organism to be tested was prepared in sterile distilled water or broth. The broth was incubated at 37^0c for approximately one hour. After this period, the turbidity of the broth was matched with the turbidity of the 0.5 McFarland turbidity standard (i.e 1.5 x 10^8 CFU/ml). A swab was immersed in to the matched broth and then rolled and squeezed against the slides of the tube to remove excess broth. The swab was then used to inoculate onto one-half of a blood agar plate. Aseptically Novobiocin disk was placed in the center of the inoculated area, and gently disk was tamped so it adhered to the agar surface. Plate incubated at 35^0c in an ambient-air incubator for 18 to 24 hours

Interpretation:

S.saprophyticus were novobiocin resistance (6mm/no zone to 12mm)

Other CONS & *S.aureus* were novobiocin suspectible ((≥16mm)

7. Bacitracin sensitivity[4]:

Principle: Susceptibility to low concentration of the polypeptide antibiotic bacitracin provides as method for presumptive identification of staphylococcus species.

Reagents- Taxo Bacitracin disc 0.04 units, Sheep blood agar

Procedure: Suspension of the organism to be tested was prepared in sterile distilled water or broth. The broth was incubated at 37^0c for approximately one hour. After this period, the turbidity of the broth was matched with the turbidity of the 0.5 McFarland turbidity standard (i.e 1.5 x 10^8 CFU/ml). A swab was immersed in to the matched broth and then rolled and squeezed against the slides of the tube to remove excess broth. The swab was then used to inoculate onto blood agar plate. Aseptically bacitracin disk containing 0.04 units was placed in the center of the inoculated area,

and gently disk was tamped so it adhered to the agar surface. Plate incubated at 35^0c in an ambient-air incubator for 18 to 24 hours

Interpretation:

Staphylococcus species were Bacitracin resistance (<10mm/no zone)

Micrococcus species were Bacitracin susceptible (10mm- 20mm)

8. Coagulase test[5]:

a. Slide Coagulase test

Principle: Staphylococcal coagulase is a protein that has prothrombin like activity which can convert fibrinogen to fibrin. A visible clot will result. Slide coagulase test detects bond coagulase which is attached to the bacterial cell wall and is not present in culture filtrate. Fibrin strands are formed between the bacterial cells when suspended in plasma (fibrinogen), causing them to clump into visible aggregates.

Procedure: Staphylococcal colony was emulsified in a drop of water on a microscope slide with a minimum spreading. If the isolate did not form a smooth milky suspension, the test was not processed further. Similar suspensions of control positive and negative strains were made to confirm the proper relativity of the plasma. Flamed and cooled straight inoculating wire was dipped into the undiluted plasma at room temperature, withdraw, and stirred the adhered traces of plasma (not a loopful) onto staphylococcal suspension on the slide. Same procedure was repeated with the control suspension.

Interpretation:

Read as positive when a course clumping of cocci was visible to the naked eye within

10 seconds. Read as negative when there was absence of clumping of any reaction within 10 seconds or slow reaction was seen after 10 seconds. Negative and slow reacting stains were re-examined by the tube test.

b. Tube Coagulase test[5]:

Principle: 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 placed 1ml volumes of the diluted plasma in small tubes. Colony of the staphylococcus under test was emulsified in a tube of diluted plasma. With each batch of tests, tubes with known coagulase negative culture as controls were included and a tube of unseeded diluted plasma to confirm that it did not coagulated spontaneously. 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*

Negative: Coagulase negative *Staphylococcus*

9. Urease test[5]:

Principle: Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by means of enzyme urease.

$NH_2CO\ NH_2 + H_2O \rightarrow 2NH_3 + CO_2$

The occurrence of this enzyme can be tested for by growing the organism in the presence of urea and testing for alkali (NH_3) production by means of a suitable pH indicator.

Procedure: Christensen's urease medium was used and was inoculated heavily over the entire slope surface and incubated at 37^0c. This was examined after 4 hrs and after overnight incubation.

Interpretation:

Positive: when the indicator turned to purple–pink.

Negative: no change in colour.

Quality control:

Positive control: *Proteus* species

Negative control: *Escherichia coli*

10. Phosphatase test[5]:

Principle: The test is used to determine the ability of the organism to produce free phosphates performed on Phenolphthalein phosphate agar. Free Phosphates is produced by some CONS with addition of Ammonia vapour and indicated by appearance of pink colour on the colony.

Procedure:

Inoculum loop was spread over the plate to a width of 2-3 mm and incubated aerobically at 370c for 18 hrs. 0.11 ml of ammonia solution was placed in the lid of petridish and covered with the plate.

Interpretation:

Pink colour colony within a few minutes when observed indicated production of phosphate. No development of colour indicated no phosphate production.

11. Voges - Proskauer Test[5]:

Principle: This test is used to determine the ability of an organism to produce neutral end products like acetyl methyl carbinol or it reduction product 2,3 butylene glycol from glucose fermentation.

Medium: Glucose phosphate peptone water

Procedure: Media was inoculated and incubated at 370C for 48 hrs. 1ml of 40% potassium hydroxide and 3ml of 5% solution of α-naphthol in absolute alcohol were added.

Interpretation: Positive reaction was indicated by the development of pink colour in 2-5 minutes, and later turned to crimson red in 30 minutes

Quality control:

Positive control: *Enterobacter aerogenes*.

Negative control: *Escherichia coli*.

12. Chromogenic cephalosporin test using Nitrocefin disk for β-Lactamase production[4]:

Principle: Commercially available filter paper disk impregnated with nitrocefin were used.

Procedure: A loopful of a colony was smeared on the disk wetted with normal saline & was placed in a closed Petri dish to prevent rapid desiccation.

Interpretation: Organism that contains β-lactamase changed the colour of the disk from yellow to red. The reaction usually showed within 30 seconds but tests were read after 15 min.

13. Nitrate Reduction Test[9]:

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 nitrate react to form a diazonium salt. The diazonium salt then couples with α-napthylamine to produce a red, water soluble azo dye

Procedure:

Nitrate broth containing Durham tube inoculated with 1-2 drops of young broth culture. Tube was incubated for 48 hours at 370c in ambient air. At the end of incubation 1ml of alpha-napthylamine & sulfanilic acid added in that order.

Interpretation:

Positive reaction was indicated by development of red colour within 30 sec & represents for nitrate reduction.

No colour indicated that nitrate have not been reduced or reduced to product other than nitrites, such as ammonia, molecular nitrogen, nitric oxide or nitrous oxide &

hydroxylamine. Addition of zinc dust to all negative tests was necessary. Zinc ions reduced nitrates to nitrites & development of red colour after addition of zinc dust indicated the presence of residual nitrates.

Quality control:

Positive control: *Escherichia coli*

Negative control: *Acinetobacter baumannii*.

13. Oxidation/ Fermentation test[5] (Modified Hugh & Leifson)

Principle: This test determines whether an organism uses carbohydrate substrate to produce acid byproducts either oxidative or fermentative process.

Procedure: Test culture was inoculated into two tubes of the medium by stabbing their whole length with a long wire loop. One of pair of stab cultures was covered with a layer of sterile liquid paraffin at least 2.5 cm, both tubes were incubated at 370c for 5 days

Interpretation: Yellow colour developed indicates acid production from glucose.

Positive: Indicated by yellow colouration in both tubes through out the length

Negative: No colouration in both tubes or yellow colouration only in the upper part of unsealed tube

14. Slime test[33]:

Procedure: A loopful of organisms from a blood agar plate inoculated in to 5ml of trypticase soya broth & incubated at 35c for 48 hours .After contents of tubes were aspirated & the tubes stained with saffranin for 30 min .

Interpretation:

Slime positive- visible saffranin stained film along the walls of the tube

Slime negative- visible saffranin stained film at the liquid-air interference.

14. Carbohydrate utilization test[5]:

Principle: These media are used to determine the ability of an organism to ferment a specific carbohydrate that is incorporated in a\basal medium, thereby producing acid with or without visible gas.

Procedure: The test was performed on conventional culture media with test sugar. Sugars tested were maltose, Sucrose, Mannitol, Trehalose, Xylose, Arabinose and Lactose, all with the concentration of 1%. Each tube with culture media inoculated with 1 drop of an 18-24 hour broth culture. Incubated at 37^0c for 1-2 days & then examined for acid production as indicated by colour change.

Interpretation:

Pink colour indicates acid production

15. Antibiotic sensitivity testing[5]:

Antibiotic sensitivity was tested 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 one hour. After this period, 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 in to the matched broth and then rolled and squeezed against the slides 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 antibiotic discs were applied within 15 minutes of inoculation of plate and the plate inverted for incubation.

Commercial obtained Himedia discs were used. The strength of discs used and their zone size interpretative standards were according to guidelines by NCCLS guidelines standards.

Below table shows Antibiotics used with strength per disc

1. Amplicllin	10mcg/disc
3. Cephalexin	30mcg/disc
4. Erythromycin	15 mcg/disc
5. Gentamycin	10mcg/disc
6. Penicillin G	10units/disc
7. Co-trimoxazole	25 mcg/disc
8. Tetracycline	30mcg/disc
9. Ciprofloxacin	5mcg/disc
10. Novobiocin	10mg/disc
11. Bactiracin	0.04units/disc
12. Oxacillin	1 mcg/disc

The plates were incubated at 37^0c overnight. The next day, the zone of inhibition for each disc was measured by a scale against a dark background in reflected light. The measurement was made of the diameter of the circle of inhibition, through the centre of the disc, including the diameter of the disc.

In place of Methicillin, Oxacillin 1µgm discs were used.

Interpretation:The interpretation of sensitivity was done as follows:

Antibiotic	**Resistant(mm/less)**	**Intermediate**	**Sensitive(mm/more)**
Penicillin	28	-	29
Ampicillin	20	21-28	29
Erthromycin	13	14-22	23
Gentamycin	12	13-17	18
Tetracyclin	14	15-18	19
Co-triamazole	11	11-14	15
Cephalexin	12	13-17	17
Methicillin	09	10-13	14

PHOTOGRAPHS

Photo 1: Gram stain showing gram-positive cocci in clusters

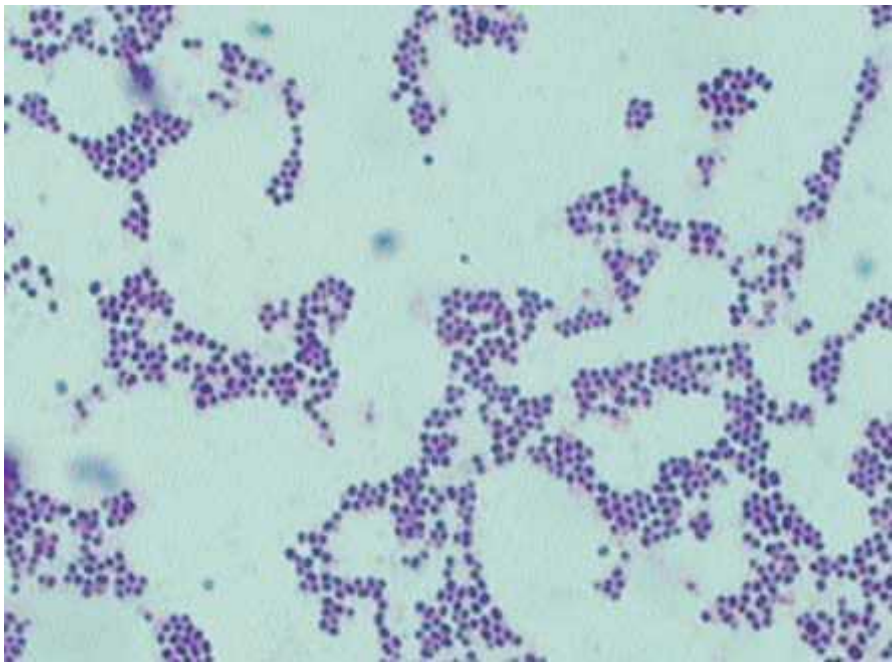


Photo 2: CONS colonies on nutrient agar

Photo 3: S.hemolyticus colonies on blood agar showing beta hemolysis

Photo 4: Slide coagulase test

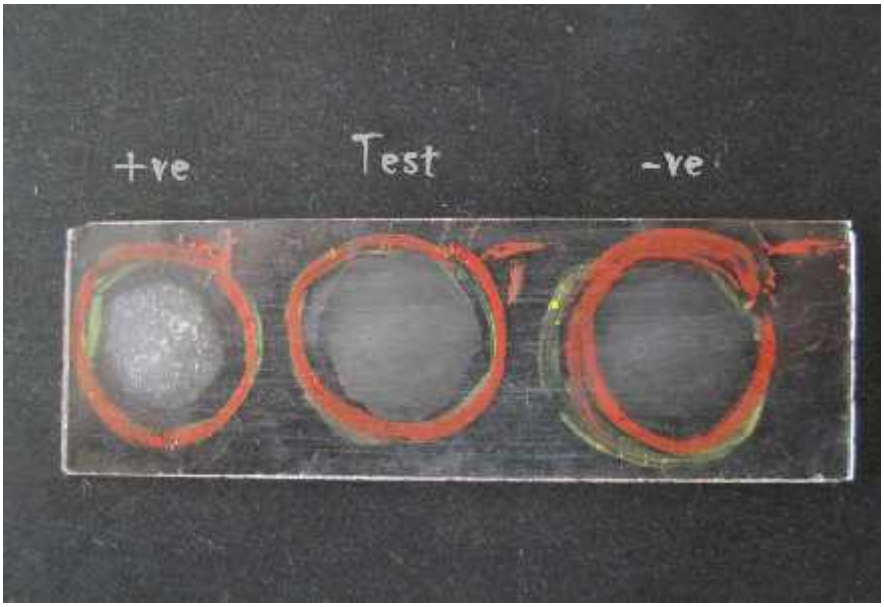


Photo 5: Tube coagulase test

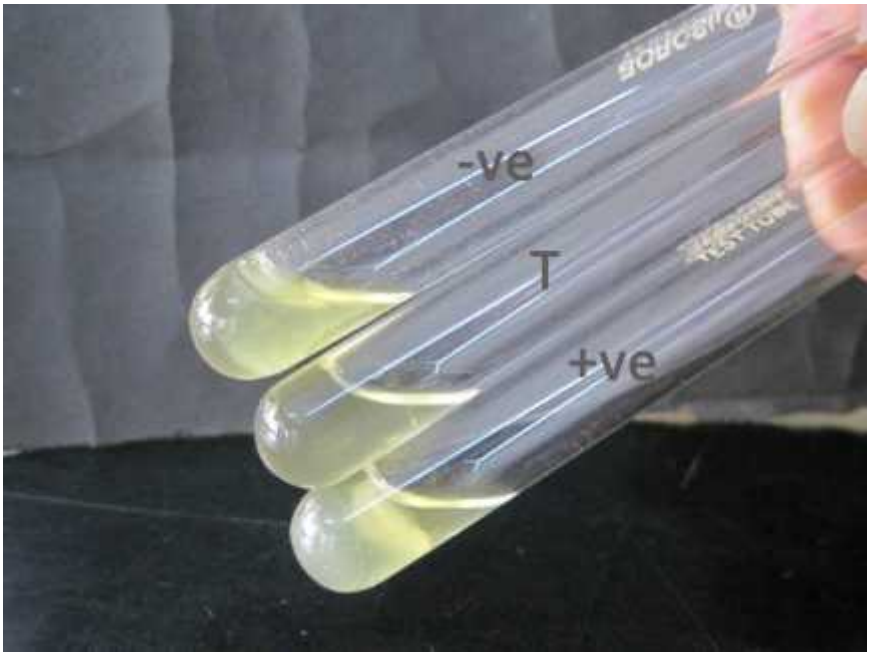


Photo 6: Furazolidone disc test showing sensitive

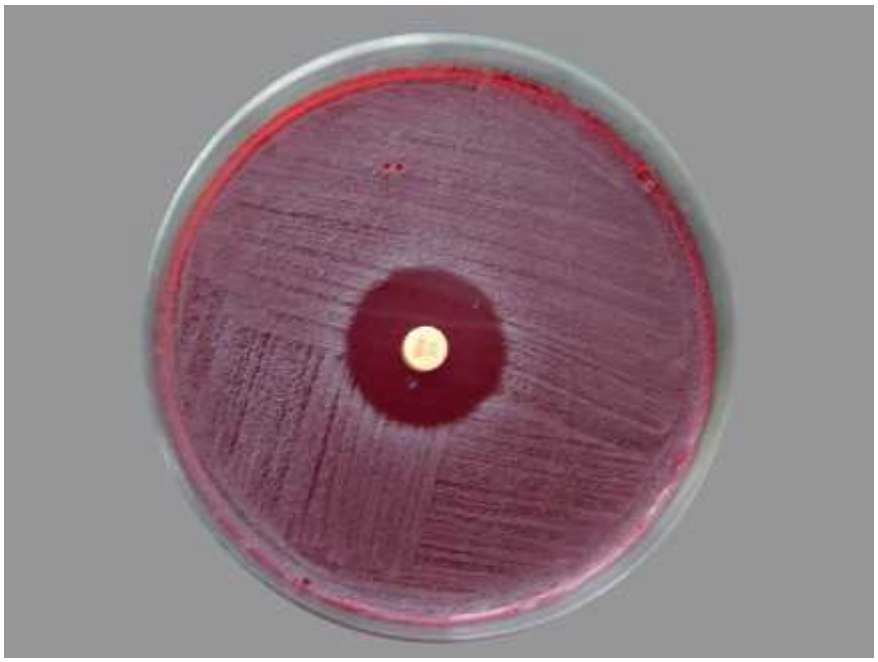


Photo 7: Novobiocin disc test showing resistance

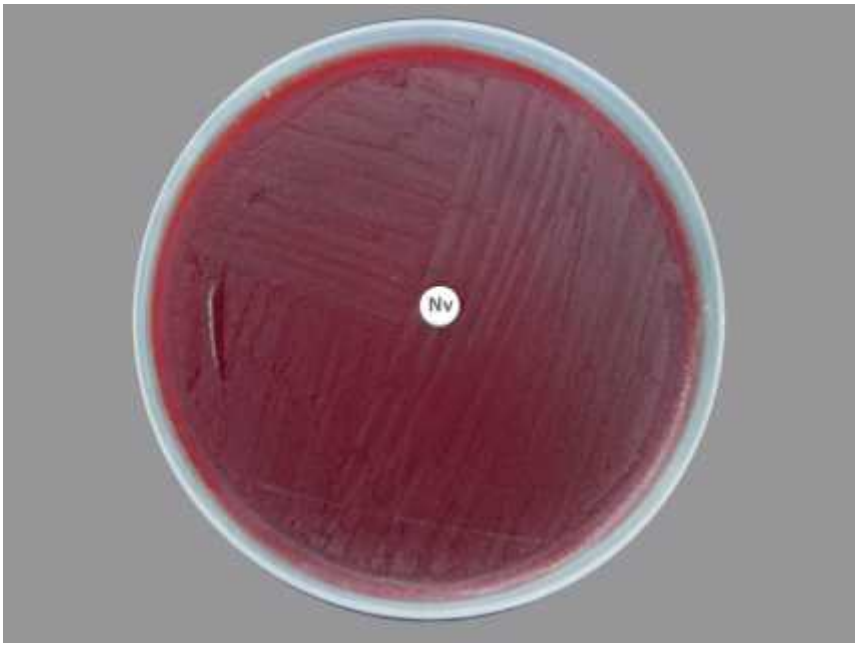


Photo 8: Bacitracin disc test showing resistance

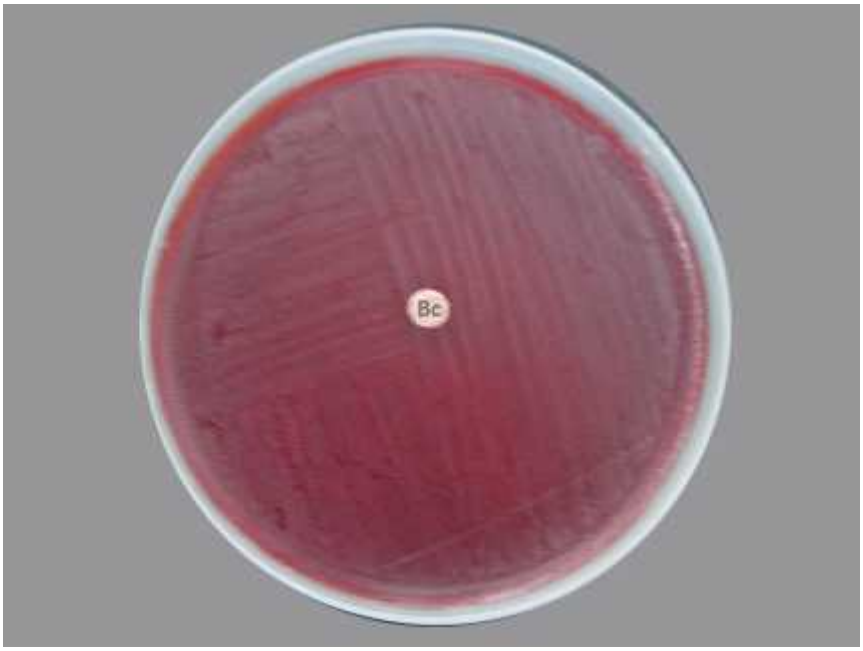


Photo 9: S.saprophyticus on SBA showing resistance to novobiocin and bacitracin, sensitive to furazolidone.

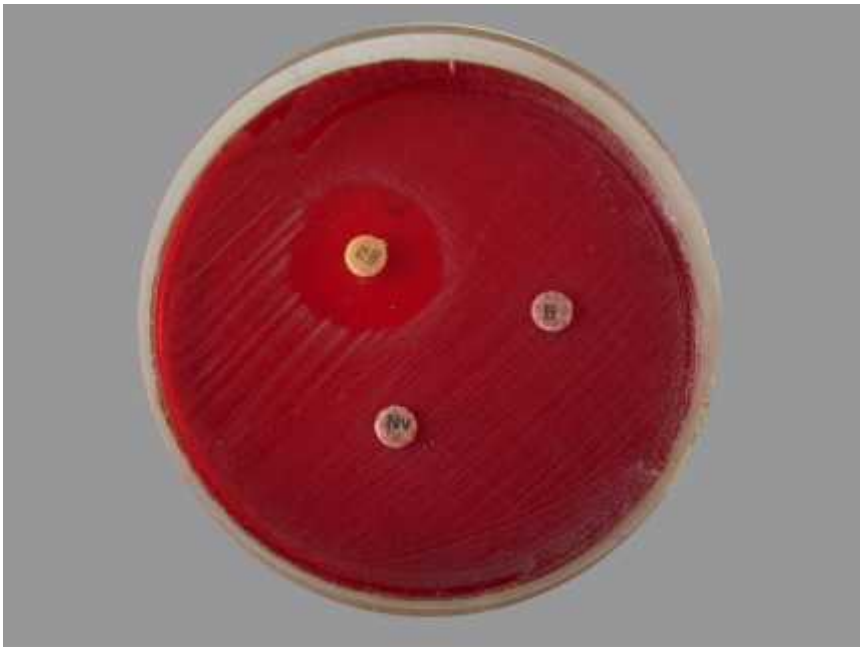


Photo 10: S.epidermidis on SBA showing resistance to bacitracin, sensitive to novobiocin and furazolidone.

Photo 11: Test showing O/F fermentative, nitrate reduction and sugars fermentations

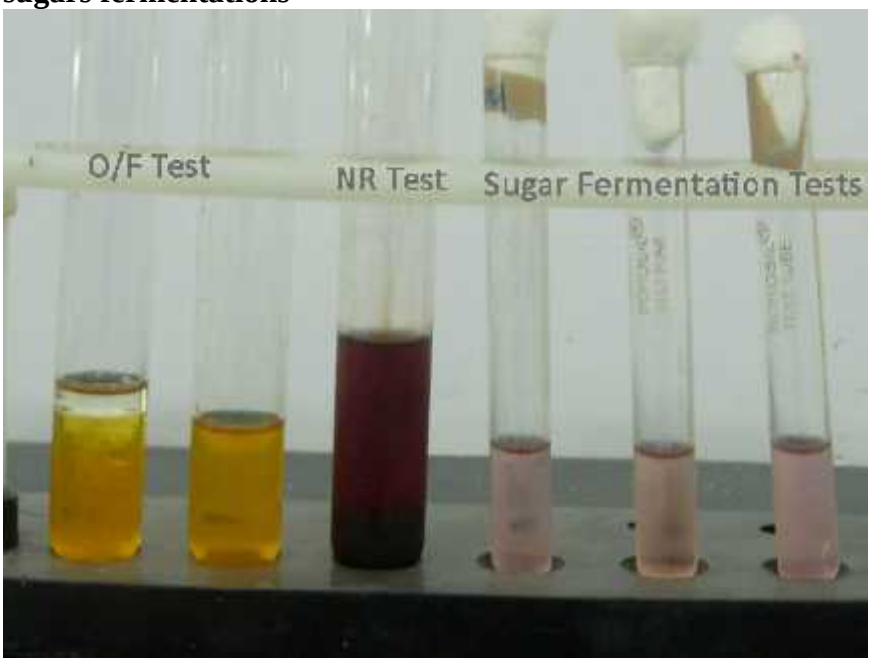


Photo 12: Phosphatase test

Photo 13: Beta Lactamase test

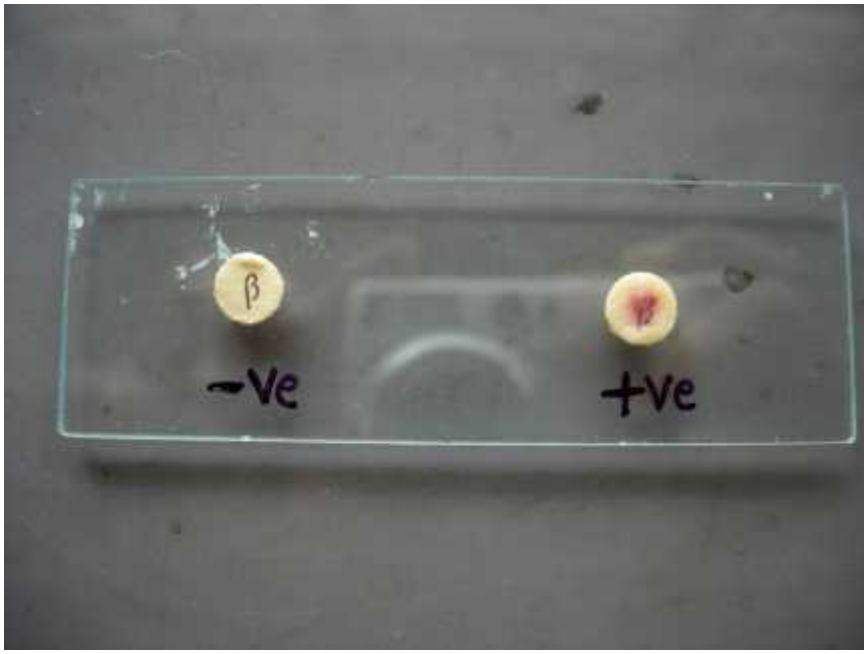
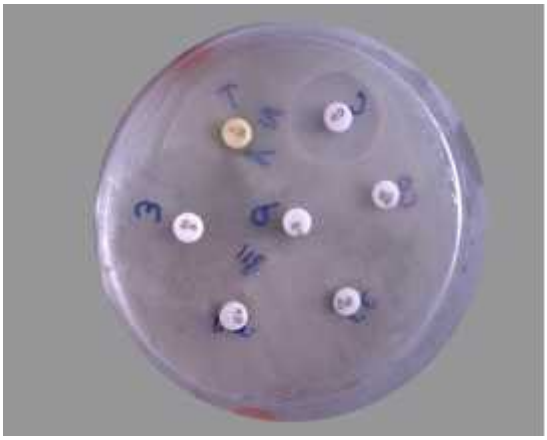


Photo 14: Slime test

Photo 15: Antibiogram of CONS



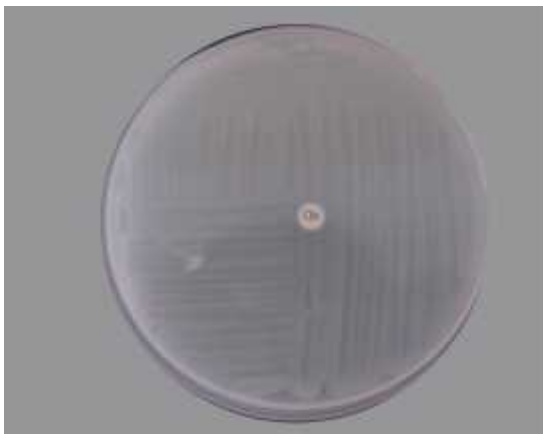


Table 1: Distribution of CONS Isolates from different Clinical Specimens

Specimen	No	%
Pus / wound discharge	58	48.33
Blood	27	22.50
Urine	23	19.17
ET tube	05	4.17
Cervical swab	04	3.34
Pleural fluid	01	0.83
Amniotic fluid	01	0.83
CSF	01	0.83
Total	**120**	**100%**

Graph 1: Distribution of CONS from different Clinical Specimens

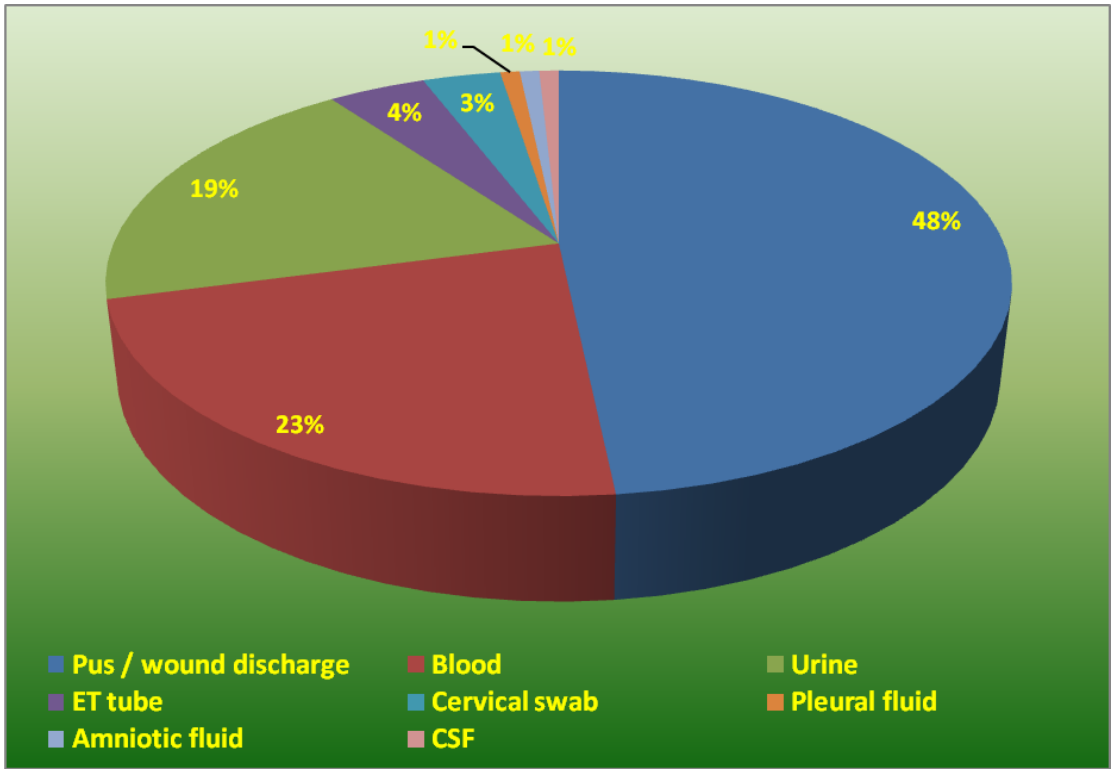


Table 2: Distribution of CONS species in different age & sex groups

Years Sex	0-10	11-20	21-30	31-40	41-50	51-60	>60	Total
Male	16	06	07	19	05	04	02	**59 (49%)**
Female	07	11	25	11	04	02	01	**61 (51%)**
Total %	**23 (19.17)**	**17 (14.17)**	**32 (26.66)**	**30 (25.0)**	**09 (7.20)**	**06 (5.0)**	**03 (2.5)**	**120**

Graph 2: Distribution of CONS species in different age & sex groups

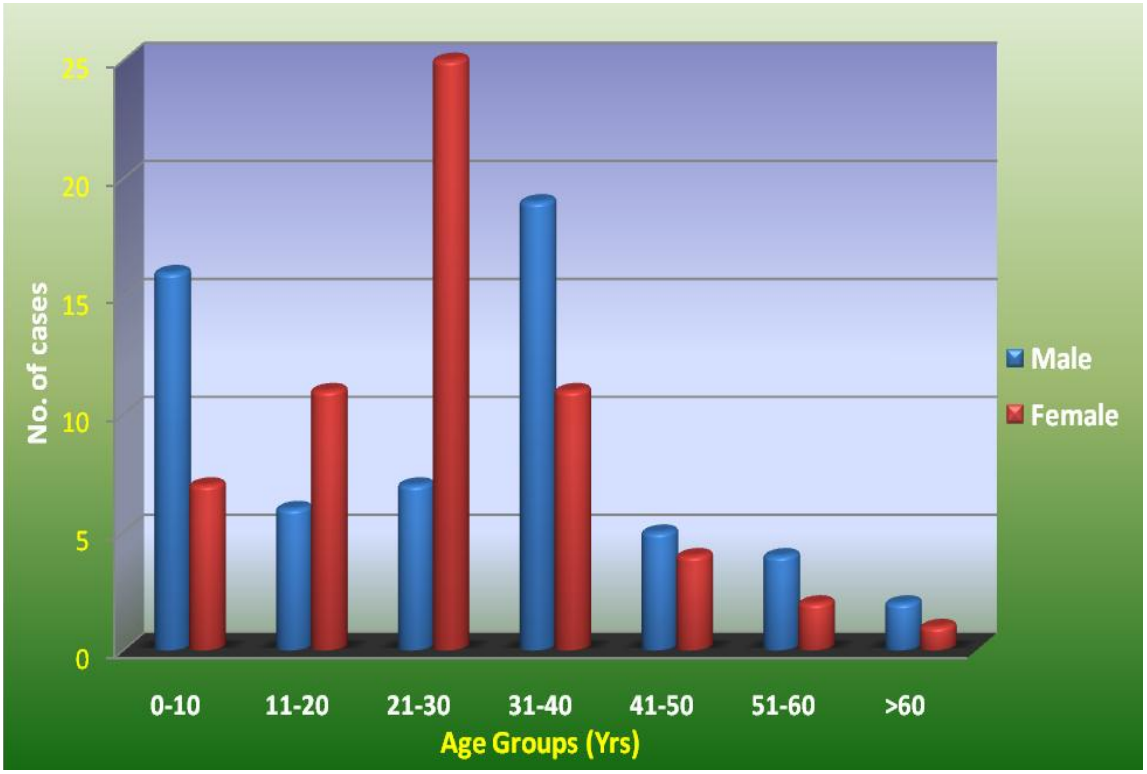


Table 3: Distribution of Species of CONS in Clinical Specimens

Species	Pus/ Wound Swab	Blood	Cervical swab	Endo tracheal tube	Urine	Pleural fluid	Amniotic fluid	CSF	Total
S.epidermidis	44 (56.41%)	25 (32.05%)	04 (5.13%)	02 (2.56%)	01 (1.28%)	01 (1.28%)	01 (1.28%)	-	78 (65.0%)
S.saprophyticus	-	-	-	-	18 (100%)	-	-	-	18 (15.0%)
S.hominis	02 (100%)	-	-	-	-	-	-	-	02 (1.67%)
S.simulans	01 (100%)	-	-	-	-	-		-	01 (0.83%)
S.xylosus	01 (50%)	-	-	-	01 (50%)	-	-	-	02 (1.67%)
S.sciuri	03 (75%)	01 (25%)	-	-	-	-	-	-	04 (3.33%)
S.hemolyticus	06 (46.15%)	01 (7.69%)	-	03 (23.08%)	03 (23.08%)	-	-	-	13 (10.83)
S.cohnii	01 (50%)	-	-	-	-	-	-	01 (50%)	02 (1.67%)

Graph 3: Distribution of Species of CONS

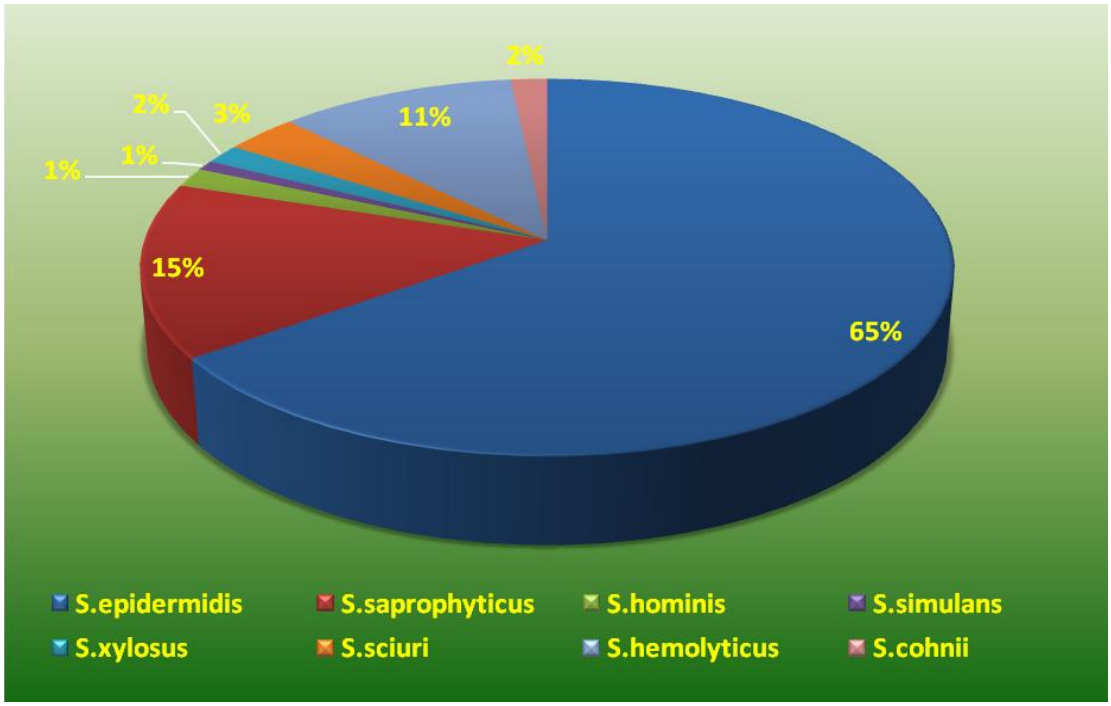


Table 4: Predisposing factors of CONS species

Species (total)	Factor	Diseases
***S.epidermidis* (78)**	IVD (15) Shunts (05) Catheter (18)	DM (40)
***S.saprophyticus* (18)**	Catheters (16)	Renal stones (4)
***S.sciuri* (04)**	IVD (02)	DM (02)
***S.cohnii* (02)**	Ventricular Shunt (01)	DM + ICSOL (01)

Table 5: Production of slime with regards to CONS species & Clinical specimens

Species	Pus/ Wound	Blood	Urine	Pleural fluid	Amniotic fluid	Endo Tracheal Swab	Cervical swab	CSF	Total
S.epidermidis	29	18	01	01	01	01	04	-	**55 (70.51%)**
S.saprophyticus	-	-	-	-	-	-	-	-	-
S.hemolyticus	03	01	-	-	-	02	-	-	**06 (46.15%)**
S.sciuri	02	01	-	-	-	-	-	-	**03 (75%)**
S.xylosus	-	-	-	-	-	-	-	-	-
S.hominis	01	-	-	-	-	-	-	-	**01 (50%)**
S.cohnii	-	-	-	-	-	-	-	01	**01 (50%)**
S.simulans	01	-	-	-	-	-	-	-	**01 (100%)**
Total	**36**	**20**	**01**	**01**	**01**	**03**	**04**	**01**	**67**

Table 6: Production of slime with regards to CONS species

Species	Slime Positive	Slime Negative	Total
S.epidermidis	55 (70.51%)	23 (29.49%)	78
S.saprophyticus	-	18 (100%)	18
S.hemolyticus	06 (46.15%)	07 (53.85%)	13
S.sciuri	03 (75%)	01 (25%)	04
S.xylosus	-	02 (100%)	02
S.hominis	01 (50%)	01 (50%)	02
S.cohnii	01 (50%)	01 (50%)	02
S.simulans	01 (100%)	-	01
Total	**67 (55.83%)**	**53 (44.17%)**	**120**

Graph 4: Slime production by CONS species

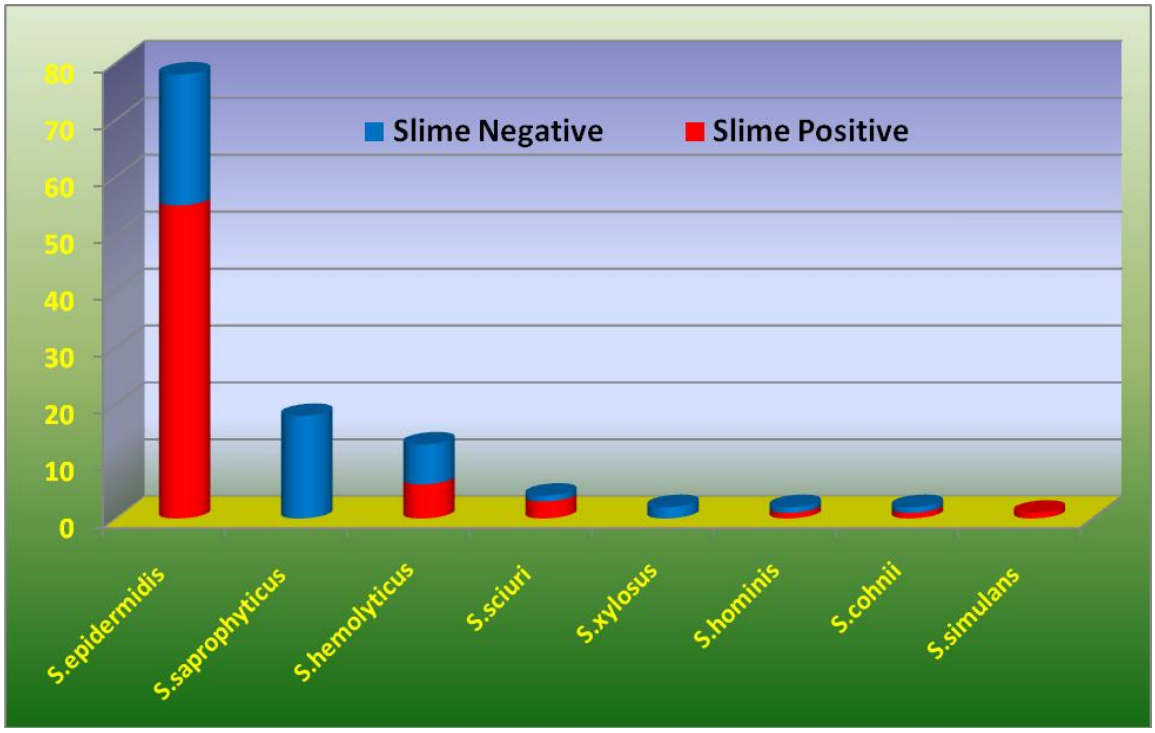


Table 7: Production of β-Lactamase with regards to CONS species

Species	β-Lactamase Positive	β-Lactamase Negative	Total
S.epidermidis	39 (50%)	39 (50%)	**78**
S.saprophyticus	-	18 (100%)	**18**
S.hemolyticus	03 (23.08%)	10 (76.92%)	**13**
S.sciuri	-	04 (100%)	**04**
S.xylosus	02 (100%)	-	**02**
S.hominis	01 (50%)	01 (50%)	**02**
S.cohnii	-	02 (100%)	**02**
S.simulans	-	01	**01**
Total	**45 (37.5%)**	**75 (62.5%)**	**120**

Graph 5: β-Lactamase production by CONS species

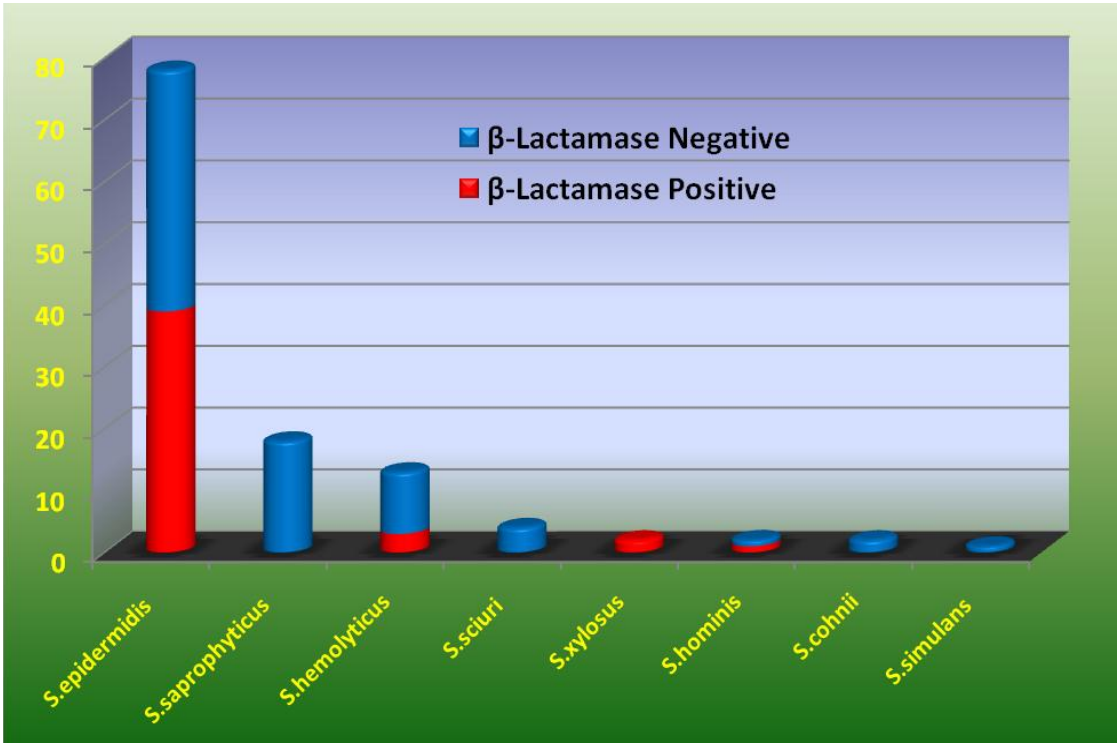


Table 8: Biochemical Characters of CONS

Parametrs	Coagulase	Clumping Factor	V-P Test	Nitrate Red	Urease	Phosphatase	Novabiocin	Maltose	Sucrose	Lactose	Fructose	Manitol	Arabinose	Xylose	Trehalose	Mod. Oxidasee
S.epidermidis	-	-	+	+	+	+	**S**	+	+	±	+	-	-	-	-	-
S.saprophyticus	-	-	+	-	+	+	**R**	+	+	±	+	±	-	-	+	-
S.hemolyticus	-	-	+	+	-	-	**S**	+	+	±	±	±	-	-	+	-
S.sciuri	-	-	-	+	-	-	**R**	+	+	+	-	+	+	+	+	+
S.xylosus	-	-	±	±	+	+	**R**	+	+	±	+	+	±	+	+	-
S.hominis	-	-	±	±	+	-	**S**	+	+	±	+	-	-	-	±	-
S.cohnii	-	-	±	-	-	-	**R**	+	-	-	+	±	-	-	+	-
S.simulans	-	-	±	+	-	±	**S**	+	+	+	+	+	-	-	±	-

Table 9: Antibiotic Sensitivity and Resistance Pattern of CONS Species

Antibiotics	Sensitive (%)	Resistant (%)
Penicillin	30 (25%)	90 (75%)
Ampicillin	33 (27.5%)	87 (72.5%)
Gentamycin	52 (43.33%)	68 (56.67%)
Erthromycin	64 (53%)	56 (47%)
Tetracyclin	62 (51%)	58 (49%)
Co-triamoxazole	68 (56.67%)	52 (43.33%)
Cephalexin	86 (71%)	34 (29%)
Oxacillin	72 (60%)	48 (40%)

Graph 6: Antibiotic Sensitivity and Resistance Pattern of CONS Species

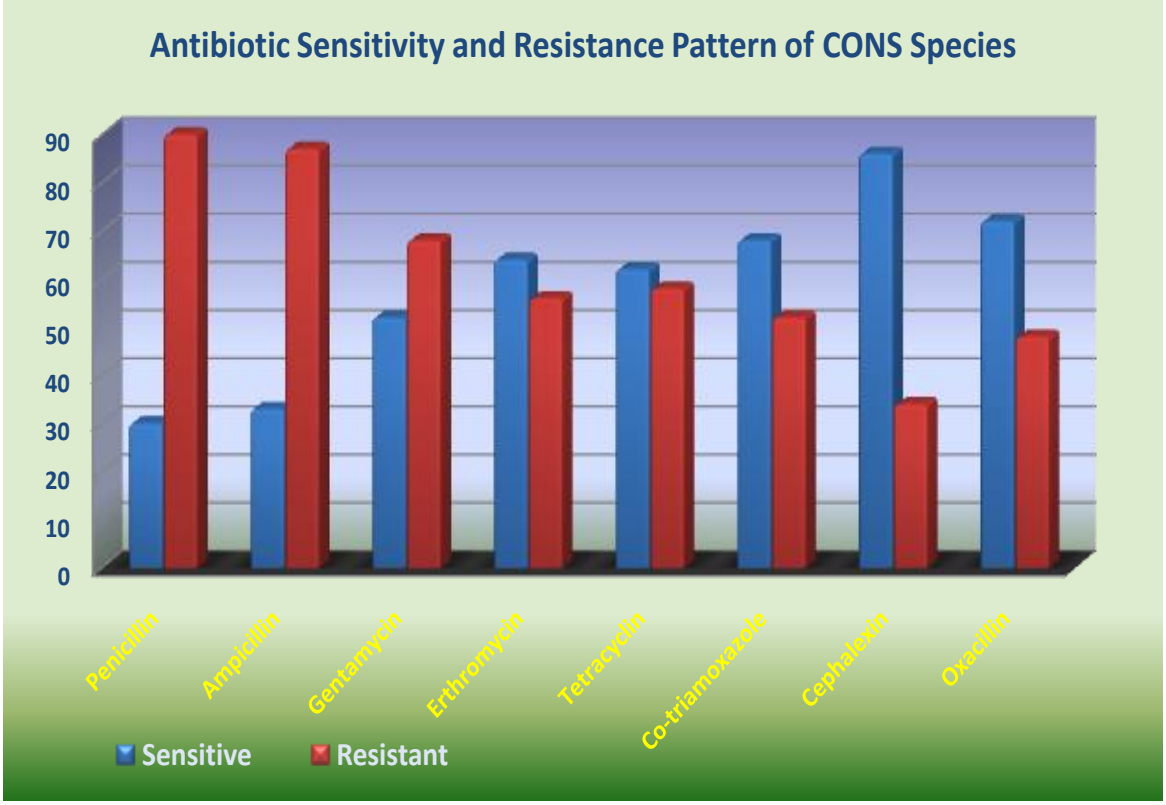


Table 10: Methicillin resistant pattern of CONS species

Species	Sensitive	Resistance	Total
S.epidermidis	41(52.56%)	37(47.44%)	**78**
S.saprophyticus	17(94.44%)	01(5.56%)	**18**
S.hemolyticus	10(76.92%)	03(13.08%)	**13**
S.sciuri	01(25%)	03(75%)	**04**
S.xylosus	-	02(100%)	**02**
S.hominis	01(50%)	01(50%)	**02**
S.cohnii	02(100%)	-	**02**
S.simulans	-	01(100%)	**01**
Total	**72**	**48**	**120**

Table 11: Susceptibility pattern of Methicillin Resistance CONS (MRCONS)

	MRCONS		MSCONS	
Antibiotic	**Sensitive**	**Resistant**	**Sensitive**	**Resistant**
Pencillin	-	**48**	30	**42**
Ampicillin	-	**48**	33	**39**
Gentamycin	2	**46**	50	**22**
Erthromycin	4	**44**	60	**12**
Tetracyclin	5	**43**	57	**15**
Co-triamoxazole	4	**44**	64	**8**
Cephalexin	16	**32**	70	**2**
Oxacillin	-	**48**	72	**-**

DISCUSSION

Coagulase negative staphylococci (CONS) form part of the residential flora of healthy skin and were considered in the past to be non-pathogenic, with the increasing use of interventional procedures, prosthetic valves, endotracheal tubes, urinary catheters and increase in incidence of immunocompromised cases. CONS have emerged as one of the important cause of nosocomial infections. During the past 10 years CONS have been recognized as major cause of septicemia in patients with various implanted medical devices[2].

Most developed countries have reported an increase in colonization and infections in hospitalized patients by CONS, which are resistance to methicillin and other antibiotics. However only few data are available on CONS infections with methicillin resistance in developing countries[31].

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

CLINICAL SAMPLES:

In this study, a total of 120 pathogenic CONS isolated from various clinical samples were included, most of the isolates were from pus samples 58(48.39%) followed by blood 27(22.50%), urine 23(19.17%), endotracheal tube 05(4.17%), cervical swab 04(3.34%) and one sample (0.83%) each from pleural fluid, amniotic fluid and CSF.

The present study when compared with previous published studies[13,19,22,37,62](Table 12) clearly shows that CONS were commonly isolated from pus, urine, blood and indwelling catheters and uncommonly from body fluids.

Table 12: Comparison of CONS species isolated among clinical samples

Species	Pal and Ayyageri[13]. 1989	Goel MM et al[19]. 1991	Adriana N etal[22]. 2003	Boynukara B et al[37]. 2007	Choudhari, Arora and Sharma[62]. 2008	Present Study 2010
Pus/ wound swab	7 (9.3%)	13 (13.5%)	30 (15.0%)	6 (9.2%)	25 (25.0%)	**58 (48.33%)**
Blood	28 (37.3%)	8 (8.3%)	26 (13.9%)	33 (50.8%)	45 (45.0%)	**27 (22.50%)**
Urine	17 (22.7%)	41 (42.7%)	72 (36.0%)	5 (7.7%)	20 (20.0%)	**23 (19.17%)**
Endotracheal tube/ catheter	-	1 (1.1%)	22 (11.0%)	13 (20.0%)	-	**5 (4.17%)**
Cervical swab	2 (2.1%)	3 (3.1%)	-	-	5 (5.0%)	**4 (3.34%)**
CSF fluid	15 (15.7%)	8 (8.3%)	2 (1.0%)	2 (3.1%)	-	**1 (0.83%)**
Amniotic fluid	-	-	-	-	-	**1 (0.83%)**
Pleural fluid	-	-	-	-	-	**1 (0.83%)**
Peritoneal fluid	-	1 (1.1%)	19 (9.5%)	-	5 (5.0%)	**-**
Sputum	-	12 (12.5%)	-	-	-	**-**
Others	6 (8.0%)	9 (9.4%)	29 (14.5%)	6 (9.2%)	-	**-**
Total	75	96	200	65	100	**120**

PREDISPOSING FACTORS:

In the present study, with the available history of the patients, out of 120 CONS isolated, 43 strains were isolated form diabetics patients who either had the history of catheters in 18(41.86%) or intravenous devices in 17(39.53%) or shunts in 7(16.328%) cases. Thirty four cases had history of catheter which showed growth of *S.epidermidis* 18(52.94%) and *S.saprophyticus* 16(47.06%) species. Among the 18 *S.saprophyticus* isolated, 16(88.89%) had history of catheterization. Similar

predisposing factors have been discussed in study conducted by Singhal R et al[31] and Singh S et al[30].

CONS SPECIATION:

In the present study all the 120 CONS, isolated were speciated based on Kloss and Scheifer chart[20]. *S.epidermidis* was the most common species isolated 78(65%): majority of these were from pus 44(56.41%) followed by blood 25(32.5%), cervical swab 4(5.13%), endotracheal tube 2(2.56%) and one each (1.28%) from urine, pleural fluid and amniotic fluid.

S.saprophyticus was the second most common species isolated 18(15.0%), all were from urine sample. *S.haemolyticus* was the next common species 13(10.83%), majority were from pus 6(46.15%), endotracheal tube 3(23.08%) and blood 1(7.69%). We have isolated *S.sciuri* species in 4(3.33%) samples, 3(7.5%) were from pus and 1(25%) from blood.

We have also isolated *S.hominis* 2(1.67%) from pus, *S.xylosus* 2(1.6.7%) one each from pus and urine, *S.simulans* 1(0.83%) from pus sample and *S.cohnii* 2(1.67%) was isolated one each from pus and CSF sample.

In the present study as well as studies conducted by Singh S et al[30], Singhal R et al[31], Adriana N et al[22], Pal and Ayyageri[13] and Seetha K S et al[2]: *S.epidermidis* was the commonest species among CONS isolated (Table 13). *S.saprophyticus* and *S.haemolyticus* formed next common species in the present study as well as in the previous studies[13,22,30].

However, *S.haemolyticus* and *S.lugdunensis* formed the next common species in the study conducted by Singhal R et al[31].

Table 13: Comparison of distribution of CONS species

Species	Pal and Ayyageri[13]. 1989	Adriana N etal[22]. 2003	Singhal R et al[31]. 2006	Singh S et al[30]. 2008	Present Study 2010
S.epidermidis	60 (45.0%)	101 (50.5%)	28 (33.7%)	60 (40.0%)	**78 (65.0%)**
S.saprophyticus	3 (4.0%)	32 (16.0%)	-	21 (14.0%)	**18 (15.0%)**
S.hemolyticus	3 (4.0%)	37 (18.5%)	11 (13.3%)	18 (12.0%)	**13 (10.83%)**
S.scuri	--	2 (1.0%)	-	-	**04 (3.33%)**
S.xylosus	--	-	5 (6.0%)	-	**02 (1.67%)**
S.hominis	8 (10.7%)	1 (0.5%)	2 (2.4%)	9 (6.0%)	**02 (1.67%)**
S.cohnii	10 (13.3%)	1 (6.5%)	-	-	**02 (1.67%)**
S.simulans	1 (1.3%)	3 (1.5%)	-	-	**01 (0.83%)**
S.lugdunensis	--	12 (6.0%)	11 (13.3%)	9 (6.0%)	-
S.schleiferi	--	2 (1.0%)	10 (12.1%)	3 (2.0%)	-
S.capitis	4 (5.3%)	4 (2.0%)	8 (9.6%)	6 (4.0%)	-
S.warneri	1 (1.3%)	5 (2.5%)	1 (1.2%)	9 (6.0%)	-

Where as Boynukara B et al[37] observed that *S.hominis* was the commonest species (30.8%) isolated followed by *S.epidermidis* (27.7%) and *S.xylosus* (9.2%).

These differences in the relative incidence of individual species in various publications (Table 13) could be attributed to the clinical samples received from different types and section of the hospitals, like tertiary care hospital, NICU or general hospitals as well as different methodology applied by various authors for specialization.

SLIME PRODUCTION

CONS are characterized by an ability to colonize the surfaces of biomaterial by adhering in biofilm – structured communities of cells encased in a self-produced polymeric matrix called slime. Slime is believed to make micro organisms more resistance to administered antibiotic and to host defense mechanism[37].

Table 14: Comparison of slime production by CONS

Studies	**Cunha MLR et al[63] 2004**		**Boynukara B et al[37] 2007**		**Present study**	
Species	**No. of Strains**	**Slime Positive**	**No. of Strains**	**Slime Positive**	**No. of Strains**	**Slime Positive**
S.epidermidis	24	4 (16.7%)	18	11 (61.1%)	**78**	**55 (70.51%)**
S.saprophyticus	-	-	2	2 (100%)	**18**	**0**
S.hominis	5	0	20	11 (55%)	**2**	**1 (50.0%)**
S.simulans	1	0	2	2 (100%)	**1**	**1 (100%)**
S.xylosus	2	0	6	4 (66.7%)	**2**	**0**
S.sciuri	-	-	5	3 (60%)	**4**	**3 (75.0%)**
S.hemolyticus	11	2 (18.2%)	4	3 (75%)	**13**	**6 (46.15%)**
S.cohnii	2	0	-		**2**	**1 (50.0%)**
S.warneri	5	2 (40.0%)	6	3 (50%)	**-**	**-**
S.capitis	-	-	2	0	**-**	**-**
S.lugdunensis	1	1 (100%)	-	-	**-**	**-**
Total:	**51**	**9 (17.6%)**	**65**	**39 (60.0%)**	**120**	**67 (55.83%)**

In present study, out of 120 CONS isolated from various clinical specimens 67(55.83%) were slime positive (slime producer) and 53 (44.17%) were slime negative (slime non producer). *S.epidermidis* 55 (70.51%) was the common species in

producing slime followed by *S.scuri* 3(75%) and *S.haemolyticus* 6(46.15%). *S.saprophytic* didn't produce the slime

In agreement with our results Boynukara B et al[37] also reported that 39(60%) of the 65 clinical CONS isolates were slime producer strains. Similar studies by Seetha KS et al[2], Choudhary et al[62] and Pal and Ayyageri[13] has also shown the slime producing CONS in 56.11%, 88.0% and 100% respectively among the total CONS isolated from various clinical specimens.

On the other hand Cunha MLR et al[63] reported only 17.6% were slime positive among the total CONS isolated from recurring peritonitis in the patients on dialysis.

In the present study *S.epidermidis* 55(82.09%) was the most common species in producing slime among all CONS followed by *S.haemolyticus* 6(8.96%) and *S.scuri* 3(4.48%). *S.saprophyticus* did not produce the slime. Similarly Boynukara B et al[37] and Seetha KS et al[2] also reported the *S.epidermidis* was the most common slime producer among all CONS isolates that is 74.19% and 61.1% respectively.

In contrast Cunha MLR et al[63] reported 83.3% of *S.epidermidis* were slime non producer.

Also Seetha KS et al[2] and Boyunkara B et al[37] reported 16.07% and 100% of *S.saprophyticus* were slime producer, but, in present study none of *S.saprophyticus* were slime producer.

In the present study, majority of the slime positive *S.epidermidis* 55(70.51%) were isolated from pus 29(52.73%) followed by blood 18(32.73%), cervical swab 4(7.27%) and one each (1.81%) from urine, pleural fluid, amniotic fluid and endotracheal tube. In contrast Boynukara et al[37] has shown that out of 11 strains of

slime positive *S.epidermidis*, 6(54.6%) were isolated from blood and only 1(9.1%) strains was isolated from pus.

When our results are compared to those of others, some differences were observed. These variations can be explained by the variation in strain number as well as the assumption that the CONS species tested in some studies were isolated under different environmental conditions and that the nature of the strain was different. On the other hand, it should also be considered that slime production may be affected by various factors, such as medium composition, the presence of carbohydrate, iron and CO_2 and oxidation[43].

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF CONS

The emergence of antibiotic resistant organism and their spread in the community has been a subject of increasing concern in recent years. Methicillin resistance in *S.aureus* was first appeared in 1961[53]. CONS often considered, in past, only less pathogenic has proven to be significant pathogenic in recent years. Also methicillin resistance have been described among the CONS which were posing the problem in treating the patient. We have hence examined our isolates of CONS from various clinical specimens with special reference to methicillin resistance.

In the present study, 120 CONS strains were isolated and studied for antibiotic resistance: 90(75%) CONS strains were resistance to penicillin, 87(72.5%) to ampicillin, 68 (56.67%) to gentamycin, 56 (49.0%) to tetracycline, 52 (43.33%) to co-trimoxazole, 34 (29.0%) to cephalexin and 48 (40.0%) to oxacilllin. This correlated well with the report by Singh S et al, which stated that, CONS had maximum resistance to penicillin (80%) and ampicillin (80%) followed by erythromycin (54%),

oxacillin (38%) and gentamycin (28%). In contrast Singhal R et al* reported, CONS had maximum resistance to co-trimoxazole (74.7%) followed by penicillin (72.3%) and methicillin (62.7%). Also, Seetha et al[2] reported CONS were resistance to maximum to amoxicillin+clavilonic acid in 92.77% followed by ampicillin (82.77%), oxacillin (82.77%) and penicillin (73.33%), but all these strains of CONS were isolated from blood cultures.

METHICILLIN RESISTANCE CONS (MRCONS):

Incidence: Out of 120 CONS isolated in the present study, 48 strains (40%) were resistance of ocacillin. The incidence of MRCONS of various published report has been compared in Table 15, which clearly depicts that: there is a increasing incidence of methicillin resistance in CONS from 1990 onwards. Jesudason, Anandraj and Jegadeesan[53] cleared this fact by reporting increasing MRCONS incidence from 1.6% (1993) to 14.6% (1996) among the CONS isolated from blood cultures.

High incidence of MRCONS were reported by Seetha et al[2] (82.77%), Singhal R et al[31] (72.3%) and Jain, Agarwal and Bansal[64] (66.0%) among the CONS strains which were isolated from super-speciality, tertiary care hospitals and neonatal ICU setups in tertiary care hospital respectively. Where as MRCONS incidence varied from 14.6%[53] to 38.0%[30] among the CONS isolated from all the clinical specimens of general hospitals, well correlating with the 40% incidence of MRCONS in the present study.

Table 15: Comparison of Incidences of MRCONS

Study	Clinical Samples of CONS	Incidence of MRCONS
1977: Vijaylaxmi, Mohaputra and Bhujwala[1]. AIIMS, New Delhi.	All clinical samples	**0**
1989: Pal and Ayyageri[13]. PGIMER, Chandigarh	All clinical samples	**14.6 %**
1991: Goel MM et al[19]. KG Medical College, Lucknow.	All clinical samples	**10 %**
1993: Jesudason, Anandraj and Jegadeesan[13] CMC, Vellore.	Only blood culture specimens	**1.6 %**
1996: Jesudason, Anandraj and Jegadeesan[13]. CMC, Vellore.	Only blood culture specimens	**14.6 %**
2000: Seetha, Santosh and Shivananda[2]. KMC, Manipal.	Super-specialty units (cardiology, nephrology, burns, NICU)	**82.77 %**
2002: Mohan, Jindal and Aggarwal[21]. Govt. Medical College, Amristar.	All clinical samples	**20.8 %**
2004: Jain, Agarwal and Bansal[64]. KG Medical University, Lucknow.	Blood cultures from NICU (tertiary care hospital)	**66 %**
2006: Singhal R et al[31]. AIIMS, New Delhi	All clinical samples (tertiary hospital)	**72.3 %**
2008: Singh S et al[30]. CSM Medical University. Lucknow.	NICU (tertiary care hospital)	**38 %**
2008: Choudhary, Arora and Sharma[62]. Pt BD Sharma PG Institute, Rotak.	All clinical samples	**25 %**
2010: Present Study JNMC, KLE Dr Prabhakar Kore Hospital and MRC, Belgaum	**All clinical samples**	**40 %**

In Table 15, we can conclude that MRCONS incidences were high among the CONS isolated from the super-speciality hospitals and intensive care units of tertiary care hospitals.

Antibiotic susceptibility pattern of MRCONS:

Forty eight MRCONS were isolated in the present study. All the MRCONS (100%) were resistance to penicillin and ampicillin followed by gentamycin (95.83%), erythromycin and co-trimoxazole (91.67%), tertracyclin (89.58%) and least resistance to cephalexin (66.67%). Similarly, Choudhari, Arora and Shrama[62] reported MRCONS were resistance to erythromycin (68%), gentamycin (60%), co-trimoxazole (60%), ciprofloxacin (48%), teicoplanin (20%), vancomycin (12%) and rifampicin (12%). Also Jain, Agarwal and Bansal[64] reported MRCONS had maximum resistance to penicillin (100%) followed by cefotaxim (85.3%), ciprofloxacin (61.8%), gentamycin (54.4%) and amikacin (25%), but all were sensitive to vancomycin.

Variability in the antibiotic susceptibility patterns of CONS and MRCONS has been observed by various authors[19,62,64], which positively reflects the different protocols and panels of antibiotics being used in different hospitals and differences in the geographical locations from where these isolates have been obtained. It is therefore suggested that, to fully asses the importance of CONS, it may be essential to determine its particular species and antibiotic sensitivity pattern as no particular pattern can be predicted in any problematic situation, especially in MRCONS strains.

CONCLUSION

1. In the present study CONS were isolated from different clinical samples. *S.epidermidis* was the commonest isolate. Out of 120 CONS, 78(65.0%) were *S.epidermidis*, followed by *S.saprophyticus* 18(15%) and *S.haemolyticus* 13(10.83%).

2. The majority of the isolates were isolated from pus/wound discharge 58(48.33%) followed by blood 27(22.5%) and urine 23(19.17%).

3. Maximum number of isolates were patients of age group 21-30 years (26.66%). The least number of isolates from age group > 60 years (2.5%).

4. Females were commonly affected than males.

5. Forty eight (37.50%) were beta-lactamase producer.

6. Sixty seven (55.83%) were slime producer.

7. Most of the CONS were resistant to penicillin 90(75.1%) followed by ampicillin 87(72.5%) and gentamycin 68(56.66%).

SUMMARY

The present study was conducted between Jan 2008-Jan 2009 in the department of microbiology J N Medical College, Belgaum.

A total of 120 isolates from various clinical samples were taken and identified to the species level by using conventional tests, and also tested for slime and Beta-lactamse production.

Out of 120 isolates, 78 (65%) were *S.epidermidis*, 18(15%) *S.saprophyticus*, 13 (10.83%) *S.haemolyticus*, 04(3.33%) *S.sciuri*, 02 (1.67%) *S.hominis*, 02 (1.67%) *S.xylosus*, 02 (1.67%) *S.cohnii*.

The majority of the isolates were isolated from pus/wound discharge (58) followed by blood (27) urine (23), Endotracheal tube (05), Cervical Swab (04), Pleural fluid, Ascitic fluid, and CSF o1 each.

Maximum number of isolates were from patient with age group 21-30 years most of them were having history of indwelling catheters, intravascular devices. The least number of isolates were from age group >60. In the age group 0-10 years, all the samples collected were Blood, having history of low birth weight, premature birth, septicemia. CONS were more frequently isolates from females (61) as compared to females (59).

Slime production was done on 120 isolates out of which 67 (55.83%) were slime positive *S.epidermidis* was the commonest species producing slime 55 (82.09%) *S.saprophyticus* did not show any slime production many of the CONS isolated from blood culture showed slime production 20 (74.08%), from pus 36 (62.07%) 45 (37.5%) isolates produced beta-lactamase. Beta-lactamase production was seen most

commonly in *S.epidermidis* 39(49.37%). Most of the beta-lactamse powder and slime producer showed resistant to penicillin, ampicillin and to methicillin.

Out of 120 isolates, most of the species showed resistance to penicillin 90 (75%) followed by ampicillin 87 (72.5%), gentamycin 68 (56.66%). Most species showed least resistance to cephalexin 34 (29%). Forty eight (40%) of CONS showed resistance to methicillin.

BIBLIOGRAPHY:

1. Vijaylaxmi N, Mohapatra LN and Bhujwala RA. Biological characters and antimicrobial sensitivity of *S.epidermidis* isolated from human source. Indian J Med Res 1980;72:16-22.
2. Seetha KS, Santhosh PK, Shivanand PG. Study of coagulase negative Staphylococci isolated from blood and CSF culture. Indian J Pathol Microbiol. 2000;43(1):41-43.
3. Eiff C, Proctor RA, Peters G. In symposium coagulase-negative Staphylococci. Pathogens have major role in nosocomial infections. Postgrad Med 2001;110(4):63-64,69-70,73-76.
4. Koneman E, Allen S, Janda W, Schrcckenberger P, Winn W. Colour atlas and textbook of diagnostic microbiology. 6th edition, Lippincott Kaven Publisher, Philadelphia; 1997:624-671.
5. Collee JG, Doguid JP, Fraser AG and Marmion BP. Mackie and Mc Cartney. Practical medical microbiology: 14th ed. Churchill Livingstone, USA; 1996:245-261
6 Jain A, Agarwal A and Verma RK. Cefoxitin disc diffusion test for detection of methicillin-resistant Staphylococci. J Med Microbiol 2008;57:957-961.
7. Neihart RE, Fried JS and Hodges GR. Coagulase negative Staphylococci: Review article. Southern Med J 1988;81(4):491-500.
8. Hiekens E, Fleer A, Paauw A, Florijin A and Fluit AC. Comparisons of genotypic and phenotypic methods for species level identification of clinical isolates of coagulase negative Staphylococci. J Clin Microbiol 2005;43(5):2286-2290.
9. Forbes B, Sahm D, Weissfeld A. Baily and Scott's Diagnostic Mirobiology. 10th Edition, Mosby Elsevier, Missouri; 1998:257.

10. Murdoch DR, Events RJ, Chambers ST and Cowan IA. Vertebral osteomyelitis due to *Staphylococcus lugdunensis*. J Clin Microbiol 1996;24(4):993-994.

11. Couto I, Sanches IS, Saleao R and Lencastre H. Molecular characterization of *Staphylococcus sciurii* strains isolated from humans. J Clin Microbiol 2000;38(3):1136-1143.

12. Stepanovic S, Hauschild T, Dakic T, Al Doori Z, Svabic-Vlahovic M, Ranin L, Morison D. Evaluation of phenotypic and molecular methods for detection of oxacillin resistance in members of the *Staphylococcus sciurii* group. J Clin Microbiol 2006;44(3):934-937.

13. Pal N and Ayyageri A. Species identification and methicillin resistance of coagulase negative Staphylocci from clinical specimens. Indian J Med Res. 1989 Sept;89:300-3005

14 Christensen GD, Parisi JT, Bisno AL, Simpson WA and Beachey EH. Characterization of clinically significant strains of coagulase-negative Staphylococci. J Clin Microbiol 1983;258-269.

15. Vandenesch F, Eykyn S. Identification of ribotypes of *Staphylococcus caprae* isolated as human pathogens and from goat milk. J Clin Microbiol 1995;33:888-892.

16. Gill VG, Selepak ST, Williams EC. Species identification and antibiotic susceptibility of CONS isolated from clinical specimens. J Clin Microbiol 1983;18(6):1310-1319

17. John JF, Gramling PK and O'Dell NM. Species identification of coagulase negative Staphylococci from urinary tract infection. J Clin Microbiol 1978;8(4):435-437.

18. Wilkinson BJ, Maxwall S and Schaus SM. Classification and characteristics of coagulase negative, methicillin resistance staphylococci. J Clin Microbiol 1980:12(2):161-166.

19. Goel MM, Singh AV, Mathur SK, Singh M, Singhal R. Resistant coagulase negative Staphylococci from clinical samples. Indian J Med Res 1991;93:350-

20. Kloss WE, Schleifer KH. Simplified scheme for routine identification of human *Staphylococcus* species. J Clin Microbiol 1975;1:82-88.

21. Mohan U. Jindal N and Agarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative Staphylococci isolated from various clinical specimens. Indian Jr Med Microbiol. 2003;20(1):45-46.

22. Adriana N, Paulis D, Predari SC, Chazarreta CD, Santoianni JE. Five-Test simple scheme for species-level identification of clinically significant coagulase-negative Staphylococci. J Clin Microbiol 2003;1219-1224.

23. Goyal R, Singh NP, Kumar A, Kaur J, Singh M, Sunita N, Mathur M. Simple economical method for speciation and resistotyping of clinically significant coagulase negative Staphylococci. Indian J Med Microbiol. 2006;24(3):201-204.

24. Jordon PA, Irvani A, Richard GA. UTI caused by *S.saprophyticus*. J of Inf Dis. 1980;142(4):510-515.

25. Chiquet C, Pechionot, Creuzot–Garcher C. Acute post operative endophthalmitis caused by Staphylococcus lugdunensis. J Clin Microbiol 2007;45(6):1673-1678.

26. Kaabia N, Scauarda D, Lena G and Drancourt M, Molecular identification of *Staphylococcus lugdinensis* in a patient with meningitis. J Clin Microbiol 2002;40(5);1824-1825.

27. Giammarinaro P, Levwy S, Chacornac JP, Delmas J and Talon R. Development of a new oligonucleotide array to identify Staphylococcal strains at species level. J Clin Microbiol 2005;43(8):3673-3680.

28. Khatib R, Riederer KM, Clark JA, Khatib S, Briski LE and Wilson FM. Coagulase negative Staphylococci in multiple blood culture; Strain relatednesss and determinants of same strain bacteremia. J Clin Microbiol 1995;33(4):816-820.

29.` Carretto E, Barbarini D , Couto I, Vitis DD et al. Identification of Coagulase negative *Staphylococcus epidermidis* by automated ribotyping. Clin Microbiol Infect 2005;11:177-184.

30. Singh S, Banerjee G, Agarwal SK, Kumar M and Singh RK. Simple method for speciation of clinically significant coagulase negative Staphylococci and its antibiotic sensitivity/ resistance pattern in NICU of tertiary care centre. Biomedical Research 2008;19(2):97-101.

31. Singhal R, Dhawan S, Mohanty S et al. Species distribution and antimicrobial susceptibility of coagulase negative Staphylococci in a tertiary care hospital. Correspondence. Indian J Med Res 2006;123:569-570.

32. Stevens NT, Greene CM, O'Gara JP and Humphreys H. Biofilm characteristics of *Staphylococcus epidermidis* isolates associated with device related meningitis. J Med Microbiol 2009;58:855-862.

33. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surface. Infection and Immunity 1982 July;37(1):318-326.

34. Nayak N, Satpathy G, Vajpayee RB, Pande RM, A simple alternative method for rapid detection of slime produced by *S.epidermidis* isolates in bacterial keratitis. Ind J Med Res 2001;114:169-172.

35. Nayak N, Satpathy G, Vajpayee RB, Seti A, Ray M. Sinha P. Clinical significance of slime production by *S.epidermidis* in bacterial keratitis. Annaul Opthalmal 2002;34:204-210

36. Davenport DS, Massanari RM, Pfaller MA, Bale MJ, Streed SA, Hierholzer WJ. Usefullness of a test for slime production as a marker for clinically significant infections with coagulase negative Staphylococci. The Jr of Inf Dis 1986;153(2):332-339

37. Boyunkara B, Gulhan T, Gurtuk , Alisarli M and Ogun E. Evolution of slime production by coagulase negative Staphylococci and enterotoxigenic characteristics of *Staphylococcus aureus* strains isolated from various human clinical specimens. Jr of Med Microbiol. 2007;56:1296-1300.

38. Ratinama K, Shanmugam J and Rout D. Slime production by coagulase negative Staphylococci species from hospitalized patients, healthy carriers and environment. Indian J Med Microbiol. 1993;11(4):243-246.

39. Freeman DJ, Falkiner FR, Keane CT New method for detecting slime production by Coagulase –negative Staphylococcus. J Clin Patho.1989;42:872-874.

40 Kotilainen P et al. Association of CONS Slime production and adherence with the development and outcome of adult septicemia. J Clin Microbiol.1990;28(12):2779-2785.

41. Koksal F, Yasar H and Samasti M. Antibiotic resistance pattern of coagulase negative Staphylococcus strains isolated from blood cultures of septicemic patients of Turkey. Microbiol Res. 2009;164(4):404-10

42. Donlan RM, Closterton JW. Biofilms: Survival mechanism of clinically relevant microorganisms: Reviews. Clin Microbiol Reviews 2002;15(2):167-193.

43. Gotz F. Staphylococcus and biofilms: MicroReview. Mol Microbiol 2002;43(6):1367-1378.

44. Forbes BA, Schaberg DR, Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugative exchange of resistance.J Bacteriol.1983;153:627-634.

45. John JF, Harven AM. History of evalution of antibiotic resistance in coagulase negative Staphylococci: Susceptibility profiles of new anti-staphylococcal agents: Review article. Therapeutics and Clin Risk Mang 2007:3(6):1143-1152

46. Hussain Z, Stoakes L, John MA, Garrow S. Fitzgerald V. Detection of methicillin resistance in primary blood culture isolates of coagulase negative Staphylococci by PCR, slide agglutination disk diffusion and a commercial method. J Clin Microbiol. 2002;40(6):2251-2253.

47. Totake K, Kobayashi N, Odaka T, Trimethoprime resistance and susceptibility genes in *S.epidermidis.* Microbial Immunol.1998;42:497-501.

48. Archer GL, Goughter JP, Johnston JL. Plasmid encoded trimethoprime resistance in Staphylococci. Antimicro Agents Chemother 1986;29:733-740.

49. Jacoby GA, Archer GC. New mechanism of bacterial resistance to antimicrobial agent. N Eng J Med. 1991;324(9):601-612

50. Oliveira AD, Azevedo PA, De Sousa LB et al. Laboratory detection methods for methicillin resistance in coagulase negative *Staphylococcus* isolated from ophthalmic infections. Arq Bras Ofthalmol. 2007;70(4):667-675.

51. Hackbarth CJ, Chambers HF. Methicillin resistant Staphylococci: Detection methods and treatment of infection. Antimicrob Agents Chemother 1986;33(7);995-999.

52. Leven M, Jensens H, Urs D, Verhoeven J, Goossens H. Rapid detection of methicillin resistance in CONS by commercially available flourscence test. J CLin Microbiol 1995;33(8):2183-2185.

53. Jesudason MV, Anandraj SW, Jegadeesan P. Incidence of methicillin resistance coagulase positive and negative Staphylocci in blood culture. Indian J Med Res 1997;105:155-157.

54. Phatak J, Udgoankar U, Kulkarni RD etal. Study of coagulase negative Staphylococcal and their incidence in human infections. Indian J Med Microbiol. 1994; 12(2):90-95.

55. Eric S, Sancak B, Hascelik G. A comparison of PCR detection of mecA with oxacillin disk susceptibility testing in different media and scepter automated system for both *Staphylococcus aureus* and coagulase negative Staphylococci isolates. Indian J Med Microbiol 2008;26(1):21-24.

56. Armstrong DG, Lanthier G, Leliever P and Edelson GW. Methicillin resistance coagulase negative staphylococcal osteomyelitis and its relationship to broad spectrum oral antibiotics in a predominantly diabetic patients.
The Journal of Foot and Ankle Surgery, 1995;34(6):563-566.

57. Dryden MS, Talsania HG, Martins et al. Evaluation of methods for typing coagulase negative Staphylococci. J Med Microbiol.1992;37:109-117.

58. Geary C, Jordens JZ, Richardsons JF, Haweroft DM, Mitchell CJ. Epidemiological typing of CONS from nosocomial infections. J .Med Microbiol.1997;46:195-203.

59. Dean BA, Williams RE, Hall F, Coarse J. Phage typing of CONS and micrococci. Jr Hyg (Lon). 1973;71:261-270.

60. Freeman R, Goodfellow A, Ward AC, Hurdson SJ, Goald FK, Lightfool NF. Epidemiological typing of CONS by pyrolysis mass spectrometry. J Med Microboil .1991;34:245-248.

61. Parsi JT, Coagulase negative Staphylococci and the epidemiological typing of *Staphylococcus epidermidis*. Microbiol Rev.1985;49:126-139.

62. Choudhary U, Arora B and Sharma M. The prevalence of methicillin resistance coagulase negative Staphylococcus in a tertiary care hospital in north India. J Infect Dis Antimicrob Agents. 2008;25(1);13-17.

63. Cunha MLR, Caramori JCT, Fibravante AM, Batalha JEN, Montelli AC and Baretti P. Significance of slime as virulence factor in coagulase negative Staphylococcus peritonitis in CAPN. (Short report). Peritoneal dialysis international 2004;24:191-193.

64. Jain A, Agarwal J, Bansal S. Prevalence of methicillin resistance coagulase negative staphylococci in neonatal intensive care units: findings from a tertiary care hospital in India. J Med Microbiol 2004;53:941-944.

PROFORMA

Name:	**Age/Sex:**
Hospital No:	**Lab No:**
Ref by:	**Date of Admission :**
Presenting complaints :	
History of presenting complaints :	
Past history :	
History of antibiotic administration :	
Diagnosis :	
Specimen collected :	
Gram stain:	
Colony morphology :-	
Nutrient agar –	
Blood agar –	
Microbiological investigations :-	
Tests:	
a. Bacitracin susceptibility -	
b. Furazolidine susceptibility -	
c. Novobiocin resistance -	
d. Oxidase -	
e. Catalase -	
f. Slide coagulase -	
g. Tube Coagulase -	
h. O/F Glucose :	
i. Urease -	
j. Phosphatase -	
k. Voges-proskauer -	

1. Nitrate reduction -
2. Test for sugars
Mannitol -
Sucrose -
Maltose -
Lactose -
Trehalose -
Xylose -
Arabinose -
Fructose -
3.Beta- lactamase production-
4.Slime production-
Antibiotic sensitivity :-
Ampicillin -
Pencillin –G -
Gentamycin -
Erthromycin -
Tetracyclin -
Co- triamazole -
Cephalexin -
Novobiocin -
Oxacillin/methicillin -

CONSENT FOR PARTICIPATION IN RESEARCH WORK

Mr/Mrs. ……., we are requesting you to enroll yourself in the study titled **"CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF COAGULASE NEGATIVE STAPHYLOCOCCI FROM PATIENTS ADMITTED IN DR.PRABHAKAR KORE HOSPITAL AND MRC WITH SPECIAL REFERENCE TO METHICILLIN RESISTANCE"** conducted by Dr. Rashmi, post-graduate student in Microbiology, under the guidance of Dr. Mahantesh B. Nagmoti at J.N.M.C, Belgaum under KLE University.

You have been requested to participate in research because you are into a study group. During the study you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with JNMC, Belgaum. If you decide to participate you are free to withdraw at any time.

The purpose of research is to isolate and identify the various organisms associated with bacterial vaginosis and to compare two non-cultural techniques for the diagnosis.

Procedure involved

Microbiological investigations are done to detect various organisms from the clinical sample.

Risks and benefits

There are no extra risks involved and benefits are to be evaluated.

Privacy and confidentiality

The only people to know that your are a research subject are members of the research team, no information will be disclosed to others without your written permission, except.

1. In emergency to protect your rights and welfare.
2. If required by law.

Authorization to publish results

When the results of the research are published or discussed in a conference no information will be displayed that would disclose your identity. Any information

obtained in connection with this study and that can be identified with you will remain confidential.

Financial incentives for participation

You will not be paid / offered gifts for participation in the research. You will not be reimbursed for expenses.

Consent statement

I undersigned _____ have been explained in my vernacular language about the study and my participation in the study is voluntary, I have been given enough time to clear my doubts and rights as study participant.

In case you have any questions related to the study you can contact Dr. Rashmi, Mobile No. 9480385894 in case you have any questions about your rights as a study participant you can contact Dr. V. D. Patil, 0831-2471350.

Signature or left thumb print of participants or legally authorized representative

Participant's name : _____ Sign : _____

Witness name : _____ Sign : _____

Experimenter's name _____ Sign : _____

Date : _____

Place :_____

S. No.	Age/ Sex	Hos No.	Lab No.	specimen	Species	Slime production	B-Lactamase Production	Antibiotic susceptibility pattern							
								PG	AMP	GEN	ER	TETRA	CO	CP	OX
1	22 y /F	301814	11	Pus	S.epidermidis	+ ve	+ ve	R	R	R	R	R	R	R	R
2	34y/M	299630	24	Wound Swab	S.epidermidis	+ ve	+ ve	R	R	R	R	R	R	R	R
3	38y/F	302211	47	Pus	S.epidermidis	-ve	+ ve	R	S	S	S	S	S	S	R
4	19y/F	NA	56	Urine	S.Saprophyticus	-ve	-ve	R	S	S	S	S	S	S	R
5	40y/F	302310	57	Splenic swab	S.simulans	-ve	+ ve	R	S	S	R	R	S	S	R
6	20y/F	303245	118	Cervical swab	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
7	28y/F	303475	214	Urine	S.saprophyticus	-ve	-ve	R	R	S	S	S	S	S	S
8	22y/F	304102	269	Urine	S.Saprophyticus	-ve	-ve	R	R	S	S	S	S	S	S
9	23y/F	303459	270	Pus	S.hominis	+ve	+ve	R	R	R	R	R	R	R	R
10	20y/F	304193	282	Cevical swab	S.epidermidis	+ ve	+ ve	R	R	R	R	R	R	R	R
11	30y/F	304821	300	Wound Swab	S.epidermidis	-ve	+ve	S	S	S	S	S	S	S	S
12	52y/M	305058	501	Wound Swab	S.epidermidis	+ve	+ve	R	R	R	S	R	S	S	S
13	20y/F	305607	533	Urine	S.saprophyticus	-ve	-ve	R	R	S	S	S	S	S	S
14	43y/F	364756	564	Pus	S.epidermidis	+ ve	+ ve	R	R	R	R	R	R	S	R
15	24y/F	306182	598	Cervical swab	S.epidermidis	-ve	+ve	S	S	S	S	S	S	S	S
16	24y/F	306244	599	Cervical swab	S.epidermidis	+ ve	+ ve	R	R	R	R	R	R	R	R
17	D4/M	366124	618	Blood	S.epidermidis	+ ve	+ ve	R	R	R	R	R	R	S	S
18	20y/F	306302	698	Pus	S.epidermidis	-ve	-ve	S	R	R	S	S	S	S	S
19	40y/M	307606	885	Pus	S.epidermidis	-ve	-ve	S	R	S	S	S	S	S	S
20	34y/F	308578	1146	Pus	S.epidermidis	+ve	+ ve	R	R	R	R	R	R	R	R
21	4m/M	309889	1169	Blood	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
22	3yr/M	311252	1525	Blood	S.epidermidis	-ve	+ ve	R	R	S	S	S	S	S	S
23	30yr/F	311258	1526	Blood	S.epidermidis	+ve	+ ve	R	R	R	R	R	R	R	R
24	32y/M	311207	1527	Pus	S.epidermidis	+ve	+ ve	R	R	R	R	R	R	R	R
25	20Y/M	313305	1842	Urine	S.saprophyticus	-ve	-ve	S	S	S	S	S	R	S	S
26	28yr/F	32476	1870	Urine	S.saprophyticus	-ve	-ve	R	R	S	s	S	S	S	S
27	35y/M	NA	1919	Urine	S.saprophyticus	-ve	-ve	S	S	S	s	S	S	S	S
28	3m/M	314171	1948	Blood	S.epidermidis	+ve	+ ve	R	R	S	S	R	R	S	R
29	30yr/M	314118	1967	Urine	S.xylosus	+ve	-ve	R	R	S	R	S	R	S	R
30	D2/F	NA	2197	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	S	R
31	62y/M	315975	2258	Urine	S.saprophyticus	-ve	-ve	S	S	S	S	S	S	S	S
32	D4/M	316259	2343	Blood	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
33	31y/F	316823	2455	Wound Swab	S.xylosus	+ve	-ve	R	R	R	R	R	R	R	R
34	D4/F	317091	2471	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
35	45/M	NA	2471	Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	S	R
36	1m/M	315601	2478	ET tip	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
37	43y/M	316876	2500	Pus	S.epidermidis	-ve	-ve	S	R	R	R	R	R	R	R
38	23y/F	317862	2479	Wound Swab	S.epidermidis	+ve	-ve	R	R	R	R	S	R	R	R
39	22y/F	317144	2610	Wound Swab	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
40	42/M	319708	2868	Pus	S.epidermidis	+ve	+ ve	R	R	R	R	R	R	S	R
41	20y/F	NA	2872	Wound Swab	S.cohnii	-ve	-ve	S	R	S	S	S	S	S	S
42	3y/M	319815	2879	Blood	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S

43	28yr/M	315930	2898	Wound Swab	S.epidermidis	+ve	+ ve	R	R	R	R	R	R	R	R
44	35yr/M	NA	3009	Pus	S.epidermidis	-ve	-ve	R	R	S	S	S	S	S	S
45	55y/M	32478	3158	Urine	S.saprophyticus	-ve	-ve	S	S	S	S	S	S	S	S
46	21/F	323469	3374	Amniotic fluid	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
47	37/M	323603	3441	Pleural fluid	S.epidermidis	+ve	+ve	R	R	R	R	R	S	S	R
48	61/F	323764	3472	Urine	S.saprophyticus	-ve	-ve	S	S	S	S	S	S	S	S
49	34/M	323783	3520	Urine	S.saprophyticus	-ve	-ve	R	S	S	S	S	R	S	S
50	/M	324845	3618	Wound Swab	S.epidermidis	-ve	-ve	R	S	S	S	S	R	S	S
51	20/F	325097	3654	Urine	S saprophyticus	-ve	-ve	R	R	R	R	R	R	R	S
52	22y/F	324992	3661	Urine	S.saprophytics	-ve	-ve	R	R	S	S	S	S	S	S
53	24yr/F	326324	3839	Urine	S.saprophytics	-ve	-ve	R	R	R	S	R	R	R	S
54	D1/M	322266		Blood	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
55	60y/M	32811	4800	Pus	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
56	19y/M	333294	4964	Pus	S.epidermidis	-ve	-ve	R	R	R	S	S	R	S	S
57	57y/M	333682	5074	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
58	25y/F	334452	5106	Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
59	65y/M	33292	5111	Pus	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
60	23y/F	335218		Urine	S.saprophyticus	-ve	-ve	S	S	S	S	S	S	S	S
61	40yr/M	336789		Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
62	33y/F	334562		Pus	S.epidermidis	-ve	-ve	S	S	S	S	R	S	S	S
63	1m/M	341099	7844	Pus	S.hominis	-ve	-ve	S	S	S	S	S	S	S	S
64	40y/F	341191	7938	Blood	S.epidermidis	-ve	-ve	S	R	R	S	S	S	S	S
65	40y/F	341591	7939	Pus	S. haemolyticus	-ve	-ve	S	S	S	S	S	S	S	S
66	38y/M	341708	7952	Pus	S.sciuri	-ve	+ve	R	R	R	R	S	S	S	S
67	30/F	342193	6421	Pus	S.epidermidis	-ve	+ve	R	R	R	R	S	S	S	S
68	2y/F	337693	7160	Pus	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
69	21y/F	1182879	7625	Blood	S.sciuri	-ve	+ ve	R	R	R	S	S	S`	S	R
70	28y/F	341793	7961	Blood	S.epidermidis	-ve	+ ve	R	R	R	S	S	S	S	S
71	34y/M	340085	7741	Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
72	40y/M	340859	7800	Pus	S.epidermidis	-ve	+ ve	R	R	S	R	R	S	R	S
73	38y/M	340888	7808	CSF	S.cohnii	-ve	+ ve	S	S	S	S	S	S	S	S
74	3m/M	333987	6577	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
75	18y/M	335550	6749	Pus	S.sciuri	-ve	-ve	R	R	R	R	R	S	S	S
76	20y/M	343415	8224	Pus	S.epidermidis	-ve	-ve	R	R	R	R	R	S	S	S
77	30y/M	343454	8245	Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
78	10y/M	343652	8360	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
79	47y/M	343154	8340	Blood	S.epidermidis	+ve	+ve	R	R	S	S	S	S	S	S
80	40y/M	393915	8466	Pus	S.epidermidis	-ve	-ve	R	S	S	S	R	S	S	S
81	22y/M	345881	6982	Blood	S.epidermidis	-ve	+ve	R	S	S	S	S	S	S	S
82	45y/F	345502	8568	Pus	S.epidermidis	+ve	-ve	R	R	R	R	R	R	R	R
83	50y/M	345169	85910	Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
84	30yr/M	345618	8601	Urine	S.haemolyticus	-ve	-ve	S	S	S	S	S	S	R	S
85	4y/F	1228815	7006	Pus	S.epidermidis	-ve	+ve	R	R	R	R	R	R	R	R
86	40y/M	344432	8722	Blood	S.epidermidis	-ve	-ve	R	R	S	s	S	S	S	S
87	26D/M	346283	7044	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R

88	D2/F	342695	8727	Pus	S.haemolyticus	+ve	+ve	R	R	R	R	R	R	R	R
89	42/M	345408	8794	Pus	S.haemolyticus	+ve	-ve	S	S	S	S	S	S	S	S
90	30yr/F	346761	8820	Urine	S.Saprophyticus	-ve	-ve	S	S	S	S	S	S	S	S
91	32yr/F	345921	8653	Pus	S.epidermidis	-ve	+ve	R	R	R	R	R	R	S	R
92	29yr/F	120444	8944	Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
93	D/4/F	346531	9077	IV line	S.epidermidis	-ve	+ve	R	R	R	S	R	R	S	S
94	60y/M	347359	90726	pus	S.epidermidis	-ve	-ve	R	R	R	S	S	S	S	S
95	D3/m	347618	9064	Et tip	S.epidermidis	-ve	-ve	R	R	R	S	S	S	S	S
96	3m/M	345100	8903	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
97	30y/M	347979	205	Pus	S.epidermidis	+ve	-ve	R	R	S	S	S	S	S	S
98	45y/F	346928	45	ET Tip	S.haemolyticus	+ve	+ve	R	R	R	R	R	R	S	R
99	7m/F	348439	52	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
100	60y/M	347359	62	Pus	S.epidermidis	+ve	-ve	R	R	R	S	S	S	S	S
101	D10/M	347685		Blood	S.epidermidis	-ve	-ve	S	S	S	S	S	S	S	S
102	11y/F	347660	60	Et tip	S.haemolyticus	-ve	-ve	R	R	R	R	S	S	S	S
103	22y/M	349027	312	ET Tip	S.haemolyticus	+ve	-ve	R	R	S	S	S	S	S	S
104	20y/F	347492	387	Wound Swab	S.haemolyticus	+ve	-ve	R	R	R	S	S	S	S	S
105	2D/M	350244	426	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
106	34/M	349841	503	Urine	S.haemolyticus	-ve	-ve	S	S	S	S	S	S	S	S
107	20/F	350872	530	Urine	S. Saprophyticus	-ve	-ve	R	R	R	R	R	R	R	R
108	40/M	349040	544	Pus	S.Saprophyticus	-ve	-ve	R	R	R	R	S	S	S	S
109	38/F	1128559	771	Urine	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	S
110	40/F	1254456	772	Urine	S .Saprophyticus	-ve	-ve	R	R	R	S	R	S	S	S
111	34y/M	1257281	774	Pus	S.sciuri	+ve	-ve	R	R	R	R	R	R	R	R
112	11y/F	927089	966	Pus	S.epidermidis	+ve	-ve	R	R	R	R	R	S	S	S
113	40y/M	351389	1053	Pus	S.haemolyticus	-ve	+ve	S	R	R	S	R	S	S	S
114	14y/M	353878	1105	Pus	S.epidermidis	-ve	-ve	R	R	S	S	S	S	S	S
115	2D/F	352849	1188	Pus	S.epidermidis	+ve	-ve	S	S	S	S	S	S	S	S
116	18y/M	353934	1288	Urine	S.haemolyticus	+ve	-ve	R	R	R	R	R	S	S	S
117	40y/M	354966	1303	Blood	S.haemolyticus	+ve	-ve	R	R	R	R	R	S	R	R
118	33y/M	355205	1340	Blood	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R
119	38 y / F	125885	857	Pus	S.epidermidis	+ve	-ve	S	R	R	R	S	S	S	S
120	24/F	351789	696	Pus	S.epidermidis	+ve	+ve	R	R	R	R	R	R	R	R

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KEY TO MASTER CHART

+ve	:	Positive
A	:	Ampicillin
Co	:	Cotrimoxazole
Cp	:	Cephalexin
D	:	Days
F	:	Female
Gen	:	Gentamycin
M	:	Male
Ox	:	Oxacillin
Pen	:	Penicillin
S.epidermidis	:	Staphylococcus epidermidis
S.haemolyticus	:	Staphylococcus haemolyticus
S.saprophyticus	:	Staphylococcus saprophyticus
S.sciuri	:	Staphylococcus scuiri
S.simulans	:	Staphylococcus simulans
S.xylosus	:	Staphylococcus xylosus
Tetra	:	Tetracyclin
-ve	:	Negative
Y	:	Years