“Prevalence and Distribution of Staphylococcus from Health care settings and Genotypic Identification of Antibiotic Resistant Determinants”

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BY

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UNDER THE GUIDANCE

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DECLARARTION

I hereby declare that the thesis entitled **"PREVALENCE AND DISTRIBUTION OF STAPHYLOOCCUS FROM HEALTHCARE SETTINGS AND GENOTYPIC IDENTIFICATION OF ANTIBIOTIC RESISTANT DETERMMINANTS"** submitted for the award of degree of ***Doctor of Philosophy*** *in* ***Medical Microbiology*** *under the* ***Faculty of Medicine*** *of D.Y. Patil Education Society (Deemed to be University), Kolhapur* is completed and written by me, has not previously formed the basis for the award of any Degree or Diploma or other similar title of this or any other University in India or any other country or examining body to the best of my knowledge. Further, I declare that I have not violated any of the provisions under copyright and privacy/cyber/IPR Act amended by UGC from time to time

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List of Abbreviations

AACs-	Aminoglycoside Acetyltransferases	IR	Inverted Repeats
agr	*accessory* gene regulator	LLMR	Low level Mupirocin resistance
AMR	Antimicrobial Resistance	LMC	Low and Middle income countries
ANTs	Aminoglycosides Nucleotidyltransferases	LZ	Linezolid
APHs	Aminoglycosides Phosphotransferase	MHA	Muller Hinton Agar
AST	Antibiotic susceptibility testing	MHB	Muller Hinton Broth
BHIB	Brain Heart Infusion Broth	MIC	Minimum Inhibitory Concentration
C	Chloramphenicol	$MLSB_i$	Inducible Macrolid Lincosamide Streptogram B Resistant
CA-MRSA	Community Acquired MRSA	MRSA	Methicillin Resistant *Staphylococcus aureus*
Ccr	Cassette Chromosome Recombinase	MSA	Mannitol Salt Agar
CDC	Center for Disease Control	*msrA*	Macrolide Specific Resistance gene
Cfr	Chloramphenicol-Florfenicol resistance	Mup	Mupirocin
Cfu	Colony Forming Unit	NAG	N-actylglucosamine
Cipro	Ciprofloxacin	NAM	N-actylmuramic acid
CLSI	Clinical and Laboratory Standard Institute	ORF	Open Reading Frame
CoNS	Coagulase Negative Staphylococcus	*Otr*	Oxytetracycline resistance gene
CoPS	Coagulase Positive Staphylococcus	PABA	Para-Amino Benzoic Acid
Cot	Co-trimoxazole	PBP	Penicillin Binding Protein
CSF	Cerebrospinal Fluid	PCR	Polymerase Chain Reaction
CX	Cefoxitin	PPL	Priority Pathogen List
DHFR	Dihydrofolate Reductase	PTC	Peptidyltransferase center
DHPS	Dihydropeteroate synthetase	PVL	Panton Valentine Leukocidin
dMTP	deoxythymididylate Monophosphate	*rot*	repressor of toxins
DNA	Deoxyribose Nucleic Acid	SCC	Staphylococcal Cassette Chromosome
dNTPs	Deoxynucleotide triphosphates	Tet	Tetracycline
DR	Direct Repeats	TMP	Trimethoprim
E	Erythromycin	Tn	Transposons
EF	Elongation factor	VISA	Vancomycin Intermediate Staphylococcus aureus
erm	Erythromycin ribosome methylation	VRSA	Vancomycin Resistant *Staphylococcus aureus*
FDA	Food and Drug Administration	WHO	World Health Organization
Fus	Fusidic acid		
Gen	Gentamicin		
HA-MRSA	Hospital Acquired MRSA		
HCWs	Health Care Workers		
HLMR	High level Mupirocin Resistance		
hVISA	Heterogeneous Vancomycin Intermediate Staphylococcus aureus		
ICMR	Indian Council of Medical Research		
IEC	Institutional Ethical Committee		

Introduction

Chapter-1 Introduction

Staphylococci are gram positive spherical shape bacterium with diameter of 0.5-1 µm in diameter which belongs to the family micrococcaceae [1]. They are catalase positive, non-motile, non-sporing, facultative anaerobes and also differentiated based on the ability to form coagulase enzyme [2]. In the database list of prokaryotic names with standing in nomenclature, more than 50 species of Staphylococci are listed [3]. Skin and Mucous membrane are the natural inhabitants of many *Staphylococcus* species However, the prevalence of *Staphylococcus* species varies depending on the host.

1.1.Classification of Staphylococcus:-

The ability of Staphylococcus to clot plasma is one of the key diagnostic characteristics used to distinguish between Coagulase Negative Staphylococci (CoNS) and Coagulase Positive Staphylococci (CoPS) [4]. In addition, Coagulase is an important virulence factor; the main function is breakdown of soluble fibrinogen into fibrin on the surface of bacteria. Coagulase helps the bacteria by protecting from phagocytosis and host defenses [5].

1.2 Coagulase Positive Staphylococcus (*S.aureus*)

S.aureus is a highly significant member of the Coagulase Positive Staphylococcus (CoPS), because of its inherent virulence factor, involvement of tissue adhesion, immune evasion, and host cell damage, it is considered to be more pathogenic than CoNS. In addition, a wide variety of toxins are secreted to evade the immune the host defense mechanisms, causing variety of life threatening conditions (such as skin and soft tissue infections to systemic infections)[6]. *S.aureus* is commensal bacteria and it colonizes the anterior nares, skin and mucous membrane. It acts as a reservoir of future infections [7]. 20-80% of the healthy human populations carry *S.aureus* in anterior nares[8], Staphylococcus can establish the solid interaction with epithelial cells of nasal cavity by various protein molecules and cell surface components thus transforming into persistent carrier [9].

1.3 Coagulase Negative Staphylococcus:-

Coagulase Negative *Staphylococcus* (CoNS) is a large heterogeneous group among the Staphylococcus species and it is considered less pathogenic or non-

pathogenic species [10]. Today, CoNS are typical opportunistic pathogen and are recognized as one of the major nosocomial pathogen in hospitals with considerable increasing the impact on human health and life [4]. CoNS can give rise to a variety of infectious diseases in deep organs, Central nervous system, Heart, Joints, Immunocompromised individuals, Patients with medical indwelling devices, Oncological diseases, and Neonates [11]. As of 2018, 41 CoNS species are described validly. Of these, only few species are regularly associated with causing infections to human begins [12]. CoNS are the normal commensal component of skin and mucus membrane of animals and human beings. However, *Staphylococc*i prefer moist areas to colonize such as axillae, the gluteal and inguinal regions, the umbilicus, the antecubital and poplital spaces and the plantar foot region [13]. CoNS are the major source of endogenous infection especially which colonize the skin and mucous membrane of the host. CoNS are transmitted by crossing the physical barriers (eg. Skin) during the medical procedures (Invasive devices) [14]

Infections caused by the CoNS are subacute and chronic courses of infection with subtle clinical syndromes. However, it is more severe and leads to lethal outcomes in patients with improper management of chronic foreign body related infections. This leads to significant increase in duration hospital stay, higher mortality rate and increased cost of the hospital stay [16]. In addition, CoNS are involved in native value destruction, infective endocarditis, and septic thrombophlebitis in blood stream infections [15].

1.4 Antibiotic Resistance to Staphylococcus:-

Staphylococcus is a potential infectious organism and acquires antibiotic resistance quickly. Antibiotic resistance is a major emerging problem and offers a global public threat in modern medical world. Based on epidemiological features, *S.aureus* is categorized into nosocomial, community and livestock associated but, the pathogen core genome is highly distinct and variable in terms of mobile genetic element [16]. *S.aureus* acquires antibiotic resistance by horizontal gene transfer method (eg. Conjugation) of mobile genetic element [17]. About 15% of *S.aureus* genome is made up of mobile genetic elements namely Transposons, Integrons, Staphylococcal Cassette Chromosomes (SCCs), Plasmids, Pathogenicity Island and Bacteriophages [16]. Plasmids play a major role to transfer antibiotic resistant determinants. *S.aureus* posses

a plasmid with a size ranging from 1 kb to 60 kb, small ranges of plasmids carry Tetracycline, Chloramphenicol and Erythromycin resistant determinants. The larger plasmids of *S.aureus* contain resistant genes of Aminoglycosides, Beta-lactum and Macrolides. Moreover, larger plasmids of S.aureus can combine with other mobile genetic elements such as transposons and exhibit resistance to erythromycin, trimethoprim, spectinomycin, vancomycin, and beta-lactams. [18]

Staphylococcus resistance can also develop as a result of mutations that alter the drug-binding sites on a variety of molecular targets and enhance the expression of efflux pump proteins [19]. Hospital acquired infection resulting in death and morbidity is a significant pathogen for causing hospital acquired infection. MRSA is resistant to all beta-lactum antibiotics except ceftaroline and shows resistance to other antibiotics such as vancomycin, chloramphenicol, tetracycline, and macrolides. ([20] MRSA is an emerging infection in the Indian sub-continent with an incidence rate of 25 to 50% in a multicenter study reported by the Indian Council of Medical Research with Global Antimicrobial Surveillance Network in 2015 [21]. Nasal colonisation by MRSA is a high risk of infection and an important source for person-to-person transmission.

1.5 Importance of Coagulase Negative Staphylococcus:-

Coagulase Negative Staphylococcus (CoNS) is a typical opportunistic pathogen and considered as less pathogenic but antibiotic resistance of these pathogens is an important risk factor for contributing to transfer of antibiotic resistant determinants to other pathogens. This is due to the false finding of CoNS Which is treated with unnecessary antibiotics and increases the selection pressure of antibiotics.[22] Hence, the present study was designed to know the prevalence of Staphylococcal infections in various clinical specimens, Nasal colonization of *Staphylococcus* (including CoNS) among healthcare workers, Patient visitors and its antibiotic resistant determinants.

1.6 Research Problem

In 2017, the WHO published an antibiotic resistance priority pathogens list into three categories, Critical, High, and Medium. In these, 12 families of antibiotic resistant bacteria are included, and these bacteria pose a serious risk to human health. Moreover, characterization of these pathogens has to stimulate research and develop new antimicrobials. Staphylococcus aureus (resistant to vancomycin and methicillin) is a high priority [23]. Antimicrobial resistance in India has been raised as an important public health concern. The Indian Council of Medical Research (ICMR) focused drug resistance on six pathogens namely, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, *and Enterobacter* species generally called ESKAPE pathogens [24].

For several years, coagulase negative staphylococci were thought to be non-pathogenic or contaminates. It has been rarely reported to cause severe infections. By the end of the 1980s, CoNS' had become accepted and recognised as pathogenic bacteria, but most of the underlying molecular mechanisms still awaited discovery. A few clinical studies reported S.epidermidis, S.haemolyticus, S.warneri, and S.hominis, to be more prevalent causes of infections than other CoNS. Recently, S.chromogens and S.cohnii have been identified as emerging clinical nosocomial pathogens [25].

Various clinical and diagnostic laboratories have proposed their own guidelines to determine pathogenic vs. non-pathogenic contaminants and their clinical relevance. CDC guidelines define how to identify CoNS as causing an infection by requiring

1. Clinical evidence of an infection and appropriate antibiotic therapy
2. At least two Specimen cultures from same site or
3. Detection of biofilm formation [26].

Resistance to antimicrobials is a major problem nowadays in the treatment of *staphylococcal* infections (S.aureus and CoNS). The majority of Staphylococci are resistant to gram-positive antibiotics such as Macrolides, Tetracyclines, Vancomycin, Linezolid, Chloramphenicol, and β-lactam antibiotics. [27]

This development of multidrug and pan-drug resistance is due to the rapid production of biofilm, genetic modification, irrational use of antibiotics, etc. Detection of appropriate resistance by using a phenotypic method does not provide accurate

results. Molecular methods provide more accurate information, such as the site of resistance genes, whether they are intrinsic or extrinsic, what type of resistance mechanism, etc.

The introduction of molecular methods in diagnostic laboratories for the identification of accurate resistance mechanisms is difficult, but very essential to identify and avoid false negative reports, which will lead to better clinical outcomes.

Nasal colonization of Staphylococcus and its antimicrobial resistance is a major risk factor in hospitalized patient's with underlying diseased conditions. Screening for antimicrobial resistant determinants among HCWs, patient visitors, and Staphylococcal Cassette Chromosome mec (SSCmec) types is an important epidemiological factor in monitoring multidrug resistance determinants and their spread.

Knowing the accurate antibiotic resistance prevalence in developing countries, especially in India, is difficult as only limited data is available. Accurate statistical analysis of antibiotic resistance in developing countries is still lacking, and we are not adopting any proper antibiotic policy. Identification of antibiotic resistant genes and their SCCmec types is an important epidemiological tool to investigate multidrug resistance in the community as well as hospitals. There is no adequate data about community studies on SCCmec type's and their antibiotic resistance. Hence, the present study is designed to investigate the SCCmec types and their antibiotic resistance determinants in the community as well as hospital personnel.

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OPEN ACCESS PEER-REVIEWED CHAPTER

Antibiotic Resistant *Staphylococcus aureus*

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Review Of Literature

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Chapter-2 Review of Literature

Staphylococcus species are gram positive Cocci occur singly, in pairs, short chains, and are commonly seen in grape like clusters [1]. *Staphylococci* are non-motile, non-sporing bacteria that are catalase positive, facultative anaerobic bacteria that can tolerate high salt concentrations and are heat resistant [2]. They belong to the family Micrococcaceae and the phylum Firmicutes [3]. CoNS are commensal bacteria in the skin and mucous membranes of humans and other mammals'. They can also be found in water, dust, and the air. Depending on the humid condition of the environment, the bacterial colonization density varies. In moist locations (such as the anterior nares, axillae, and perianal areas), 10^3 to 106/cm2 of bacteria are seen, whereas in dry skin areas, the count may reach 10^1 to 10^3 colony forming units/cm2 [4].

There are 54 species and 28 sub-species in the genus *Staphylococcus*. The genus Staphylococcus is classified into 2 groups based on the enzyme Coagulase, namely, 1. Coagulase Positive *Staphylococcus* (CoPS) 2. Coagulation negative Staphylococcus (CoNS)[5] Furthermore, new Staphylococcus spp. are being validated and identified. Among 54 *Staphylococcus* spp, a few species are medically important and can cause human infections. Other species are relevant to veterinary medicine, causing infections in animals and birds [6]. Coagulase positive *Staphylococci*, primarily *S.aureus* are more important and known to cause a variety of infections than Coagulase negative *Staphylococci.*

Staphylococcus aureus colonizes the anterior nares (*vestibulum nasi),* which accounts for 20 to 80% of the human population, which is the most frequent carriage site and acts as a reservoir for the spread of the pathogen [7]. *Staphylococcus aureus* and nasal epithelial cells show great interactions, and this association transforms into persistent carriage through a variety of bacterial cell membrane components [8]. Nasal carriage of *S.aureus* plays a major role in the pathogenesis of infection in patients undergoing surgery, dialysis and intensive care unit, where patients have been shown with high risk of infection [9]. *S.aureus* causes infective endocarditis, pleuropulmonary, bacteremia, skin and soft tissue infections, osteoarticular, and device- related infections.

2. 1 Coagulase Negative *Staphylococcus* (CoNS):

As of 2019, 38 recognized CoNS species are available. Of these, *S.saprophyticus, S.haemolyticus, S.hominis, S.warneri, S.xylosus, S.epidermidis, S.schlefieri, and S.lugdunensis* are the ones that tend to colonise and are most often found in clinical samples [1]. More recently, *S.pettenkoferi, S.petrasii,* and *S.massiliensis* have been isolated from clinical specimens [10]. Among the CoNS spp., S. epidermidis is often found with bio-material associated with prosthetic devices, *S. haemolyticus* can cause a variety of diseases, including infections of prosthetic joints and bacteremia. In sexually active women, *S.saprophyticus* is often identified by urinary tract infections [11].

2.2 Ecological niches of CoNS associated with Human beings:-

- ***S.epidermidis*** colonizes the body surface especially on moist areas such as inguinal perianal areas, Conjunctiva, axillae, web toes and anterior nares [12].
- ***S.haemolyticus*** is frequently isolated from pubic areas, aprocrine glands and axillae[4]
- ***S.saprophyticus*** is frequently seen in genito-urinary tract and rectum [12]
- ***S.capitis*** colonizes the sebaceous gland of forehead and Scalp [13]
- ***S.lugdunensis*** is an internal part of human skin flora particularly the perineal area and pelvic. It is less frequently seen in anterior nasal cavity [14].
- ***Other CoNS*** are seen in animals, birds and environmental surfaces such as dust, air and fermented food products as contaminants[15]

2.3 Importance of CoNS in clinical settings:-

In the modern medical world, the increasing use of medical indwelling devices and implanted foreign bodies has been recognized as one of the important sources of colonizing and causing nosocomial infections caused by CoNS [16]. In addition, demographic and hospital-related factors (elderly, pre-term babies, transfer of patients between hospitals, etc.) also contribute to the pathogenicity of CoNS which are considered opportunistic infections [17].

2.4 Bloodstream infection due to CoNS:-

Blood Stream Infection (BSI) of CoNS is associated with indwelling medical devices (Central Venous Catheters, pacemaker leads, prosthetic valves, etc.) [18]. CoNS are the most common cause of hospital acquired blood stream infection and account for 30-60% [19] of the infections. S.epidermidis and S.haemolyticus are frequently isolated pathogens in laboratories and are involved in late-onset sepsis [20]. CoNS are frequent contaminants of blood culture and it is difficult to investigate whether it is Commensals of a Skin or true pathogen of CoNS causing bacteremia [21]. Neonates, cancer patients, and immunosupressed patients, especially those who have neutopenia, are at high risk of CoNS related blood stream infections [22].

2.5 CoNS in Surgical wound infection:-

Surgical wound infections (SWI) are more common in hospital acquired infections, accounting for 20% of total cases [23]. CoNS are frequently seen in superficial incisional infections, causing varying complications in surgeries, and the risk of SWI is 1.9 per 100 surgeries [24]. The major risk factors for causing CoNS SWI include duration of surgery, host factors, surgeons and nursing staff experience and handling of different surgeries [22].

2.6 Endocarditis:-

CoNS are major pathogens affecting cardiac devices, coronary stents, prosthetic heart valves, prosthetic vascular valves, etc. CoNS are important pathogens and the second leading cause of prosthetic valve endocarditis globally [25]. In hospitalized patients, CoNS is the most common cause of early endocarditis (37–47%) and late endocarditis (25%); *S.aureus* infection is the second most common cause of infective endocarditis [26]. A database on heart failure from an international partnership on endocarditis revealed that CoNS (54%) are the pathogens most frequently encountered, followed by other gram-positive cocci [27]. Among CoNS causing endocarditis, *S.epidermidis* is the most common, followed by *S.lugdunensis*, *S.hominis*, *S.capitis*, and *S.haemolyticus* [28].

2.7 Endopthalmitis:-

CoNS are often found in people who have endopthamitis after penetrating eye surgery, post-operative cases after virectomy or cataract surgery, which make up 15% to 73% of all cases. epidermidis is the most commonly encountered pathogen in endopthamitis cases [29].

2.8 Central Nervous System Shunt Infections:-

CoNS are the common cause of CVS-shunt infections. It happens within 30 days of surgery [30]. CVS-shunt infections mostly occur due to normal skin commensals like Diptheroides, Propionibacterium acne, *S.aureus*, CoNS etc. Shunt infections are potentially influenced by a variety of variables, including the length of the hospital stay, the number of revisions per patient, the experience of the surgeon, the surgical technique, the length of the procedure, the use of indwelling devices during the procedure, etc [31]. *S.epidermidis* is the most common pathogen responsible for CVS-shunt infections [32].

2.9 Vascular Graft Infections:-

An incidence of vascular graft infections ranging from 1% to 6% is seen within 30 days after the procedure, but it is more common after a month or year of implementation with the highest rate of mortality [33]. Vascular grafts are divided into 2 categories, namely, 1. intracavitary vascular grafts, which are located in the groin, and 2. extracavitary vascular grafts, which are located in the abdomen and the thorax region. CoNS are a common cause of vascular graft infections [34].

2.10 Prosthetic Graft infections:-

Joint replacement is one of the most common operations in orthopaedic surgery in the modern medical world. Periprosthetic joint infection is associated with a higher economic burden and has an increased rate of mortality and morbidity than other aseptic complications [35]. Periprosthetic joint infections occur, ranging from 1 % to 2% in primary cases and 4% in repeated arthoplastic surgery cases [36]. Rheumatoid arthritis, surgery duration, previous surgical history, other bacterial infections, and so on are all major risk factors for periprosthetic graft infections [37]. The most common is *S.epidermidis*, followed by *S.lugdunensis* and other CoNS spp [33].

2.11 Urinary Tract Infections (UTI):-

CoNS causing UTI are categorized into 2 types in humans.

1. Indwelling medical devices such as catheterized urine or supra-pubic aspiration of urine is the most complicated nosocomial urinary tract infection in hospital caused by *S.epidermidis*
2. Lower UTI in sexually active young women is mostly caused by *S.saprophyticus*, and it is a true uro-pathogen causing UTI [37].

2.12 CoNS in neonatal sepsis:-

Infectious disease management of neonates, divided neonatal sepsis into 2 types:

1. Early onset (less than 72 hours of life)
2. Late onset (between 72 hours and 30 days of life)[38]

   CoNS are mostly seen in late onset neonatal sepsis especially in very low birth weight infants with increased risk of neonatal morbidity and mortality. This leads to long duration of hospitalization [39]. Colonization of CoNS on the surface of human body is initiated in the first few days or weeks [40]. *S.epidermidis, S.warneri*, *S.haemolyticus* and *S.capitis* are the commonly encountered pathogens in neonatal sepsis [41].

2.13 Antibiotic Resistance [42]:-

Development of antibiotic resistance can be categorized into 2 forms;

1. **Acquired resistance** – Acquiring resistance by the transfer of the genes encoding antimicrobial resistance
2. **Intrinsic resistance** – Bacteria resist to antibiotic agents due to their inherent structural or functional characteristics. For Ex. Gram negative bacteria are resistant to Vancomycin. It is not transferable.

2.14 Mechanisms of Antibiotic Resistance:-

2. 14 β-Lactum Antibiotics:-

Penicillin resistance in Staphylococcus has historical importance. Penicillin resistance is primarily caused by Penicillinase, an extracellular enzyme of Class A beta-lactamase that cleaves the ring of Penicillin and Ampicillin antibiotic -lactam rings. Novick discovered Penicillinase in 1962. discovered the genes for penicillinase synthesis and its control in Staphylococcus extra-chromosomal genetic material and also showed that plasmids carry these genes [42]. Penicillinase production is encoded by the structural gene blaZ under the control of regulatory genes *blaI* and *bla* [43]. The plasmid-encoded penicillinase enzyme also contains resistance genes for fusidic acid, erythromycin, aminoglycosides, heavy metals such as cadmium, mercury, and lead, dyes such as acriflavine, quaternary ammonium components, and ethidium bromide [44].

2.15 Methicillin resistance:-

Methicillin, a semi-synthetic penicillin, was first used in clinical settings in 1959, but due to its toxicity, it is not commonly used today. It was replaced by penicillin-stable antibiotics such as Oxacillin, Flucloxacillin, and Dicloxacillin. It shows sufficient anti-bacterial activity against penicillin resistant Staphylococcus aureus. Although British scientist Jevons discovered penicillin-stable resistant *S.aureus,* the term Methicillin-resistant *S.aureus* (MRSA) is still used today [45]. Acquired resistance via the insertion of the mecA gene into the staphylococcal chromosome at a precise site [46]. The MecA gene encodes a Penicillin Binding Protein (PBP2a or PBP2') and shows less affinity for most of the cephalosporin-like agents and semi-synthetic penicillin [47]. The mecA gene makes bacteria resistant to almost all β-lactam antibiotics except ceftaroline and ceftobiprole [48].

Mechanisms of Methicillin Resistant:-

β-lactam antibiotics prevent the synthesis of bacterial cell wall peptidoglycan, the main structural component of Staphylococcus. It consists of glycan strands made up of disaccharide repeating units such as N-actylmuramic acid (NAM) and N-actylglucosamine (NAG) linked by cross-linkages of peptides between NAG moieties on adjacent strands [49]. In *S.aureus,* bifunctional transglycolylase-transpeptidase (also known as Penicillin binding protein 'a' or *PBPa*) is a crucial target of β-lactam

antibiotics. The Transglycolylase domain is responsible for transferring the pentapeptide disaccharide (L-alanine, D-glutamine, Lysine, and 2 D-alanines) from membrane lipids to elongating chains of polysaccharide at reducing ends. Cross links of Glycine Bridge from the domain of Transpeptidase (TP) and link the D-alanine at the 4th position to the nearby growing chain of the peptidoglycan layer, making the cell-wall sturdy. Blockage of the active site of Transpeptidase (TP) serine (PBP2a) is caused by structural analogous (changing from D-Ala4 to D-Ala5). This is followed by cleavage of β- lactam ring and a penicilloyl-O-serine intermediate is produced [50]. mecA gene is encoded by PBP2a. The mecA gene is transferred to other *S.aureus* species that are methicillin sensitive via horizontal gene transfer mechanisms [51].

Staphylococcal Cassette Chromosome mec (SCC mec):-

SSC mec is a mobile genetic component with a size range of 21–60 kb. It is inserted inside orfX (RNA methyltransferase gene of *S.aureus*). Other genetic elements found in SSCmec include insertion sequences, transposons (Tn554), and integrated plasmids (pI258, pT181, pUB110) [52].

1. pUB110 codes for Tobramycin, Bleomycin and Kanamycin resistance.
2. pI258 codes for heavy metals and penicillin resistance
3. pT181 encodes resistance to Tetracycline
4. Tn554 carries Erythromycin resistance and is responsible for Macrolid-induced Clindamycin resistance (MLSi)[53]

SSCmec contains three basic structural/genetic elements namely [54]

1. mec gene complex (*mecA, mec B, mec C and mec D*) and its regulatory structures to control the expression of genes such as
    - *mecR1*- encoding a signal transducer protein
    - *mecI*-encoding a repressor protein
2. The *ccr* (Cassette Chromosome Recombinase) complex, which is made up of the *ccrA, ccrB, and ccrC* genes, encodes site-specific recombinases that mediate integration and excision.
3. Joining (J) region.

***mec* gene complex [55] :-**

mec gene complex mostly Consists of *mec* gene, *mecR1* and *mecI* (regulatory elements) and linked insertion sequences (IS). Five different classes of *mec* gene complex (*mec A, mecB, mec, mecD* and *mecE)* are classified based on the difference in IS and *mec* gene complex upstream and downstream regulatory elements.

Class A mec complex consists of *mecA* gene and the regulatory components (*mecR1 and mecI*) is complete in upstream orientation of *mecA*. Downstream position of *mecA* involves IS431 and hyper variable region and.

Class B mec complex contain truncated *mecR1 (ΔmecR1), mecA*, IS431, hyper variable region and. IS272.

Class C mec is composed of *mecA, ΔmecR1,* IS431, hyper variable region and IS431. Based on the orientation of IS431, *mec C* has 2 distinct versions.

- Class C1, IS431upstream and downstream of *mecA* seen in same positions.
- Class C2, IS431 are seen in opposite direction and reversed.

Class D mec complex consists of *mecA, ΔmecR1*, IS431 but no IS in downstream position of *ΔmecR1*

Class E mec complex contains *mecR1, blaZ, mec,* and *mecI*.

***ccr* (cassette chromosome recombinase) gene complex [56,57]:-**

The Cassette Chromosome Recombinase genes and ORFs of surrounding regions contain the *ccr* gene complex. Many of the ORFs in *Staphylococcus* have unknown functions. Integration and excision of SCCmec into the Staphylococcus chromosome is mediated by the *ccrA, ccrB,* and *ccrC* genes. The recombinase function of the *ccr* gene complex is similar to that of Bacteriophage integrates. It breaks down nucleic acids, swaps genetic DNA strands, and allows recombination. site of the SCCmec element (*attSCC*) in the bacterial chromosome is *attB*. Inverted repeats (IR) of both sides of the SCCmec play a significant role in excision but not integration. The rate and speed of the insertion of SSCmec elements are determined by 100-200 bp sequences in the upstream and downstream orientation of *attB*. There are 9 different combinations of *ccr* gene complex allotypes, namely 1 (A1B1), 2 (A2B2), 3 (A3, B3) , 4 (A4, B4), 5 (C), 6 (A5B3), 7 (A1B6), 8 (A1B3), and 9 (C2). Type 6 *ccr* complexes are seen in CONS and types 7 and 8 are seen only in MRSA. ccrA1B4 is seen in

S.saprophyticus and ccrA7B3 is found in *S.sciuri*. The *latter* two combination allotypes have been identified recently.

J regions [58]:-

J (Joining) regions of cassette chromosome are non-essential and it contains additional resistant genes. J regions are classified into three types based on the location within the SCCmec namely,

1. **J1 region** (formerly called L-C region) situated between the right chromosomal region and ccr gene (in upstream orientation). J1 region contains ORFs and regulatory genes.
2. **J2 region** (formerly called C-M region) situated between *ccr* gene complex and *mec* gene complex and it Consists of integrases gene and transposons Tn554
3. **J3 region** (formerly called L-R region) situated between the mec gene complex and the left chromosomal junction (downstream orientation of *mec* gene complex) and it also carries plasmid encoded antibiotic resistance genes (Tetracycline, Aminoglycosides etc)

Nomenclature [59]:-

International Working Group on Staphylococcal Cassette Chromosome, SCCmec types is designed in Roman numerals followed by *ccr* gene complex and *mec* gene complex For example. Type 1(1B) indicates SCCmec harbors type 1 *ccr* and class B *mec* gene complex. Till date 13 SCCmec types have been discovered in Methicillin resistant Staphylococcal strains. Type I, II, III and IV widely distributed among *S.epidermidis, S.haemolyticus, S.capitis, S.sciuri, and S.warneri*. SCCmec type I is seen in multidrug resistant Staphylococcus strain.

Types:-

Methicillin Resistant *Staphylococcus* is classified into two types 1. HA-MRSA (Hospital acquired) and CA-MRSA (Community acquired). MRSA acquired in hospital or healthcare setting are called as HA-MRSA. HA-MRSA is a major cause of multi-drug resistant nosocomial infection showing higher risk and is difficult to treat due to multiple antibiotic class resistance. Epidemic of HA-MRSA is due to the

transmission of clones that have been recorded in particular geographic locations. HA-MRSA strains carry SCCmec types I to III [60].

Community acquired MRSA (CA-MRSA) are phenotypically and genetically different from HA-MRSA. It is sensitive to most of the non-β-lactam antibiotics and they produce PVL (Panton-Valentine leukocidin) exo-toxin that destroys the leukocytes and express it as super antigen. PVL genes are largely seen in CA-MRSA and absent in HA-MRSA. PVL toxin is encoded by *lukS-PV, lukF-PV*, it is a component of the phage genome and is inserted in bacterial chromosome. CA-MRSA is predominantly found in skin and soft tissue infections and a CA-MRSA strain carries SCCmec type IV and V [61].

2.16 Tetracycline Resistance:

Tetracycline resistances have been described by three different mechanisms based on the Tetracycline resistant determinants acquired by bacteria, which are disseminated among bacterial population [62].

1.The most common resistance mechanism is Ribosomal protection,

2. Active antibiotic efflux

3. Enzymatic drug inactivation.

Mutations in the rRNA, permeability barriers, Multidrug transporter system lead to development of resistance to Tetracycline [63].

Active efflux of the antibiotic:-

Through the export proteins from the main facilitator super family, the antibiotic is effluxed. Tetracycline is effluxed from the bacterial cell via membrane-associated proteins and export proteins encoded by *tet efflux* genes. Tetracycline efflux from the bacterial cell lowers the intracellular drug concentration, protecting the bacterial cell ribosome [63].

Ribosomal Protection Proteins:-

The ribosomal protection protein shields bacterial ribosomes from the cytoplasmic proteins and tetracycline antibiotic's effects. When the elongation factors EF-Tu and EF-G attach to ribosomes, modifications take place in ribosome conformation. This inhibits protein synthesis or prevents tetracycline from attaching to

bacterial ribosomes. This occurs via ribosome-dependent GTPase activity. This mostly confers resistance to minocycline and doxycycline [64].

Tetracycline Resistance Genes:

38 different Tetracycline resistance (*tet*) genes and Three Oxytetracycline resistance genes (*otr*) are identified till date [65]. Among these, 23 genes codes for efflux proteins, 11 responsible for ribosomal protection, three for enzyme inhibition and one gene with unknown Tetracycline resistance mechanism.

Environmental *tet* genes encoding export proteins, which export Tetracycline out of the bacterial cell and lowers the intracellular drug concentrations to allow the bacterial ribosomes function optimally [66]. The most common Tetracycline resistance genes which are disseminated among *Staphylococcus* are *tet(K), tet(L), tet(M), tet(O)*

tet (K) gene in *S.aureus* carries the plasmid of pT181 family. pT181 plasmid is a small 4.45 kb in size and consists of 459 amino acids. pT181 plasmid belonging to incompatibility group inc3 and also found in the larger plasmids of *S.aureus* or in bacterial chromosomes. They are associated by insertion sequence (IS257) of directed repeats [67, 68, 69]

tet(L) gene: Plasmid pSTE1 carries the *tet (L)* gene. In 1992, it was discovered for the first time in *Staphylococcus hyicus*. t*et(L)* was also discovered on the *Staphylococcus epidermidis* plasmid pSTS7 in 1996 [70]. In *Streptococci and Enterococci*, it is the second most prevalent Tetracycline resistant gene [71]. There are 458 amino acids in it.

tet (M) :- The tetracycline resistance gene most frequently found in gram-positive bacteria is the *tet (M)* gene [72]. It was initially discovered in *Streptococcus* species. Additionally, it was discovered in both gram-positive and gram-negative bacteria, including *Ureoplasmas and Mycoplasmas* [72]. The conjugative transposons of the Tn916-Tn1545 family, which also contain additional antibiotic resistance genes, are usually linked to the *tet(M)* gene [73,74]. The most common kind of Tetracycline resistance seen in MRSA is *tet(M)* (Methicillin Resistant *Staphylococcus aureus*). The majority of *S.aureus* isolates have *tet(M)* as well as *tet(K)* determinants. As a result, *tet (M) or tet (K,M)* genotypes are commonly seen in MRSA isolates [75,76].

tet (O) genes : In Staphylococci *tet(O)* gene s are seen less frequently.

2.17 Macrolid Resistant:-

Macrolides prevents the synthesis of bacterial proteins by promoting the peptidyl-tRNA molecule's separation from the bacterial ribosomes during the elongation process of translation,. As a result, the polypeptide chain is broken, and protein production is reversibly stopped. The adenine -N6 methyltransferase of the 23S rRNA underwent post-transcriptional alteration, which resulted in Macrolid resistance. The 23S rRNA molecule's A2058 receives one or two additional methyl groups from N6 methyltransferase. The methyltransferase genes are numerous (erythromycin ribosome methylation). Genes encoding for methyltransferase are *erm* (erythromycin ribosome methylation) [65].

Macrolid Resistant determinants:-

Antibiotic resistance to Macrolides, Lincosamide, and Streptogramin is being studied simultaneously. Macrolide resistance genes *(erm)* encode resistance to antibiotic of two or all three antibiotic families' in Macrolides. A total of 60 distinct genes have been identified, including those linked to rRNA methylation, efflux, and inactivation, have been identified as causing resistance to MLS antibiotics.

The ***erm (A) gene*** carries the transposon Tn554 which is incorporated into SCCmec type II component. It is a conjugative or non-conjugative transposon and is mostly seen in Methicillin Resistance Staphylococcus [76].

The ***erm (B) gene :-*** Transposons Tn917/Tn551 contain the *erm (B)* gene. Its sizes are 2.3 and 4.4 kb, and it lacks any extra resistance genes**.** [76].

The ***erm (C) gene*** is situated at small plasmids and mostly seen in Methicillin susceptible strains [77].

The ***msr (A)*** gene is efflux- pump proteins which are mediated by ABC transporters system and is plasmid-borne. *msr (A)* genes codes for 488 amino acids. [77] The 14-membered ABC transporters system is an ATP-binding transport protein which mediates active efflux to antibiotics and confers resistance to Macrolides, Lincosamide as well as Streptogramin B-compounds.

2.18 Fusidic acid Resistant:-

A steroid-like antibiotic called Fusidic acid was extracted from *Fusidium coccineum*. It acts as bacteriostatic but could turn bactericidal at higher concentrations of Fusidic acid. It binds to elongation factor G (EF-G). Translocase enzyme is required for bacterial translocation on ribosome after peptide bond has been formed during protein synthesis. This mechanism of action explains there is no intrinsic cross resistance between other antibiotics and Fusidic acid. Fusidic acid activity is limited and acts mainly against Gram positive bacteria i.e. *S.epidermidis Clostridium spp, Staphylococcus aureus, and Corynebacterium*. *Streptococci* showed moderate susceptibility. Most Gram Negative Bacteria show resistance to Fusidic acid [78].

Mechanism of Resistance:-

Two resistance mechanisms of Fusidic acid were identified in S*taphylococcus* namely:

1. Changing the drug's target site as a result of *fusA* gene (which codes for the elongation factor G, or EF-G), *rplF, or fusE* (encoding ribosome protein L6) mutations
2. *fusA gene* Point mutation takes place in domain III of FF-G

Some other Fusidic acid resistant mechanisms are:

i. Small colony variant (SCV) Fusidic acid resistant, referred to as *fusA-SCV* class. Which occurs in domain V of EF-G due to mutations

ii. Acquired Fusidic acid resistance (horizontal gene transfer method) of *Staphylococcus* spp is mediated by *fusB*, fusC, and *fusD*. The *fusB gene found in plasmid pUB101 in S.aureus* and *fusC* were found in *S.aureus* and coagulase-negative *Staphylococci*

iii. Intrinsic factor of *fusD* causes resistance to Fusidic acid in *Staphylococcus saprophyticus* [79].

2.19 Mupirocin:-

It is a combination of pseudomonic acids that binds to the isoleucyl-tRNA synthetase's enzyme target site and prevents protein synthesis. It does not bind to the equivalent mammalian enzymes, rendering it non toxic to humans. Bacterial isoleucine tRNA synthesis is decreased which leads to stop the synthesis of bacterial protein and RNA synthesis. Mupirocin is bacteriostatic at concentrations close to the Minimum

Inhibitory Concentration (MIC), and it turns bactericidal at greater concentrations. It mostly combats gram positive bacteria[80].

Mechanism of Resistance:-

In United Kingdom, the first instance of Mupirocin-resistant S. aureus was discovered in 1987.

Mupirocin resistance is classified as follows;

1. **Low Level MUP resistance-** 8-64mcg/ml of MIC value is due to point mutations on bacterial chromosome in the wild *ileS1* gene. This causes changes in amino acid from Val-to-Phe in the MUP- binding site.

2. **High Level MUP resistance-** MIC value of 128- 256µg/ml, mediated by plasmid, by two different mechanisms:

1. Acquiring an alternate isoleucine - tRNA synthetase i.e. by acquisition of a plasmid mediated *mupA or isleS2* gene.
2. Acquisition of *mupB gene* [81,82].

2.20 Linezolid Resistance:-

It belongs to Oxazolidinone group, effective against the resistant gram positive cocci and bacillary infection. It primarily acts an bacteriostatic and also acts as bactericidal against some *Pneumococci , B. fragilis and Streptococci,*[83,84].

Linezolid inhibits the protein synthesis by acting at an intial stage of translation process in bacteria. Linezolid binds to the domain V of central loop in the 23S fraction (P site) of the 50S ribosome and prevents the formation of tertiary N-formylmethionine- tRNA- 70S initiation complex. Thus, it inhibits protein synthesis at early stage [84].

Mechanism of Resistance [85]:-

Linezolid is a synthetic drug therefore, inherent resistance to Linezolid does not exist; hence mutations are typically acquired.

Changes in the peptidyltransferase centre (PTC), where the conserved sections of the ribosome directly interact with linezolid, are caused by mutations in the 23srRNA

subunit domain V area of ribosomes. Gram positive bacterium contains 4 to 6 allelic copies of 23S rRNA; Therefore, Linezolid resistance requires more than one copy of 23SrRNA allele to be mutated.

1. Ribosomal proteins genes mutation i.e., *L3 (rplC gene), L4 (rplD gene), and L22 (rplV gene)* are seen in some gram positive bacteria.
2. The plasmid mediated Extrinsic resistance of *cfr* (Chloramphenicol – Florfenicol Resistance) gene from Chloramphenicol resistant bacteria, encodes a protein to catalyze the post transcriptional methylation of the C-8 atom (A2503) in the 23S rRNA. Methylation of the *cfr* confers the multidrug resistance to Streptomycin, Lincosamide and Linezolid [85].

.Genes encoding for Ribosomal proteins have been analyzed by polymerase chain reaction and whole genome sequencing. [86].

2.21 Aminoglycosides Resistance:-

Aminoglycosides are inhibitors of protein synthesis and broad spectrum antibiotics. Initially Aminoglycosides were isolated specifically from *Streptomyces* griseus which is a species of Acintomycetes, and was first used in clinical trials in 1944. Because of their lower toxicity and broader coverage than Fluoroquinolones, Carbapenems and Cephalosporins, have replaced Aminoglycosides as first-line antibiotics worldwide [87]. Tobramycin, Amikacin, Gentamicin, Neomycin, Kanamycin, Netilmicin are classical examples. Arbekacin and Plazomicin, two recently developed Aminoglycosides, were designed to overcome Aminoglycoside resistance mechanisms [88]. According to clinical studies, aminoglycosides have a greater rate of nephrotoxicity. As a result, screening patients for serum urea and creatinine levels after Aminoglycoside injection is critical for monitoring the severity of the toxic effects. Aminoglycosides have significant activity against infections caused by *S.aureus* namely, MRSA, VISA, and VRSA[88].

Aminoglycoside entry into bacteria is typically divided into three stages. [89]:-

1. **Enhancement of bacterial cell membrane permeability:** When polycationic Aminoglycoside antibiotics bind to the bacterial cell membrane, which contains negatively charged components such as phospholipids and teichoic acids,

electrostatic attraction occurs. This causes the bacterial cells' outer membrane to rupture.

2. **Energy dependent:** Aminoglycosides enter the cytoplasm via slow, energy-dependent, and electron-transport mechanisms.
3. **Protein synthesis mistranslation and inhibition:** Mistranslation causes damage to cytoplasm and allows rapid uptake of more Aminoglycosides within the bacterial cell. Aminoglycoside resistance mechanism mostly occurs by [90,91]

1. Modification of Enzyme
2. Modification of target site
3. Active Efflux pumps proteins present on bacterial cell.

Enzymatic methylation of rRNA: Methylation of guanine residues in the 16s rRNA at position N7 results in high levels of Aminoglycoside resistance

The enzymatic modification of the amino or hydroxyl group of Aminoglycosides is the main mechanism of resistance among clinically important gram negative and gram positive isolates. Three enzyme families are responsible for co-factor dependent drug modification:.

1. Aminoglycoside -Acetyltransferases (AACs)
2. .Aminoglycoside- Nucleotidyltransferases (ANTs)
3. Aminoglycoside -Phosphotransferases (APHs)

These were further classified into numerous types (depicted by Roman numerals'). AAC (6')-I enzymes are acetyltransferases that modify aminoglycosides at position 6' [90, 91].

The acquisition of cytoplasmic Aminoglycoside Modifying Enzyme (AME) by plasmids causes aminoglycoside resistance in clinical strains of *S.aureus*. For eg. Resistance to Neomycin and is mediated by the dually functioning Acetyl Transferase-Phosphotransferase (aac-aphD) which are encoded by Transposon Tn4001. [91].

Neomycin resistance is mediated by adenyl transferase encoded by *aphA* gene which is carried by Plasmid PUB 110 or Transposon Tn5405. This is seen in SSC II mec [92].

Target site Modification: - Changes in the target site may include mutations in ribosomal proteins or 16S rRNA. Streptomycin has the most mutational changes [87].

Efflux pump protein on bacterial cell membrane: is a mechanism of intrinsic aminoglycoside resistance in various pathogens. This is an efflux pump protein on

bacterial cells. The expression of the multiple efflux (Mex) XY-OprM system in the opportunistic pathogen of *P. aeruginosa* mediates intrinsic low-level resistance to aminoglycosides, Tetracycline, and Erythromycin. There are no efflux pump proteins in *S.aureus* that cause resistance to aminoglycosides. [87].

2.22 Vancomycin Resistance:-

Vancomycin was discovered in a soil sample in 1953 in a strain of *Amycolatopsis orientalis* (initially referred as *Nocardia orientalis*) [93]. It was first approved for clinical use by the Food and Drug Administration (FDA) in 1955 to treat *S.aureus* infections which show resistant to penicillin antibiotics [94]. Vancomycin is a glycopeptide antibiotic that prevents the formation of the peptidoglycan precursor lipid II. Furthermore, the D-Ala-D-Ala terminus of the Staphylococcus cell-wall is highly conserved and active against a wide range of gram positive pathogens. Vancomycin is an antibiotic used as a last resort to treat serious infections caused by *Enterococci, Penicillin-Resistant Streptococcus pneumonia and MRSA*, in admitted patients. [95].

In 1997, Japan reported the first case of Vancomycin decreased susceptibility in MRSA isolates with Minimum-Inhibitory Concentration (MIC) range of 3-8µg/ml. In 2002, first case of Vancomycin-Resistant Staph*ylococcus aureus* (VRSA) was reported in United States with the MIC range greater than 100µg/ml. In same year 52 *S.aureus* isolates showed decreased susceptibility to Vancomycin and carrying *vanA* gene were identified in India, Iran, Pakistan, Brazil and Portugal [96].

According to CLSI (Clinical and Laboratory Standard Institute) classified Vancomycin reduced susceptibility into three groups based on the MIC value namely [97]

1. MIC with 2µg/ml – Vancomycin Susceptibility *Staphylococcus aureus* (VSSA)

2. MIC with 4-8µg/ml- Vancomycin Intermediate *Staphylococcus aureus* (VISA)

3. MIC with ≥ 16µg/ml – Vancomycin Resistant *Staphylococcus aureus* (VRSA)

Vancomycin Intermediate *S.aureus*:-

VISA Strains are derived from heterogeneous Vancomycin intermediate *S.aureus* (hVISA) strain. The precursor of VISA is hVISA and is made up of sub-populations of bacterial cell which shows varying degrees of Vancomycin resistance. Vancomycin-intermediate *S. aureus* (VISA) bacteria shows a MIC value ranging from 4 to 8 mg/l. hVISA isolates found to be sensitive to Vancomycin-Sensitive with MIC range from 1–2 mg/l, but sub-population of bacteria on same colony of bacteria contains daughter cells of Vancomycin-intermediate with MIC ≥ 4 µg/ml[98]. This means that in the same culture plate, some bacteria shown sensitivity to Vancomycin and others displayed intermediate resistance, potentially leading to failure of treatment [99]. The mechanism behind is not yet understood. Scientists have made some efforts, however, to find the source of genetic determinants of VISA using various molecular identification methods such as transcriptomics, proteomics, and comparative genomics and so on. This resulted in the identification of VISA-causing genes such as VraSR GraSR and WalKR. [100]. The prime characteristics of VISA phenotype as follows; [101].

1. Thickening of the cell wall
2. Reduced peptidoglycan cross linking
3. Reduced bacterial autolytic activity
4. Variations in the profile of bacterial surface- proteins
5. Malfunction of the agr system (accessory gene regulator) of *S. aureus* is a common regulator that releases surface proteins, virulence factors and changing the bacterial growth-profile

The GraRs gene regulates cell wall biosynthesis transcription and up-regulates the genes involved in the operon of capsule biosynthesis. This increases the expression of the *dlt* operon and the mprF/fmtC genes, which are involved in teichoic acid alanylation and varies the cell-wall charge. Furthermore, the GraRS mutation can alter the expression of rot (toxin repressor) and *agr*. This has a knock-on effect on *agr* regulators. [101].

Vancomycin Resistant Staphylococcus:-

The *van* gene clusters are responsible for vancomycin resistance. The DNA sequence of the ligase *van* gene homogenous is used to classify *van* gene clusters. It is encoded by the key enzyme responsible for the synthesis of D-alanyl-D-serine (D-ala-D-ser) and D-alanyl-D-lactate (D-ala-D-lactate) (D-ala-D-lac). Vancomycin resistance is mediated by 11 *van* gene clusters: *vanA, vanB, vanD, vanF, vanI, vanM, vanC, vanE, vanG, vanL, and vanN***.** [102].

1. *vanA, vanB, vanD, vanF,vanI* and *vanM*- encodes D-ala-D-Lac ligase and confers high level vancomycin resistance with MIC value ≥ 256 mg/ml.

2. *vanC, vanE, vanG, vanL* and *vanN*- encodes D-ala-D-ser ligase and generally confers low level resistance with MIC of 8-16 mg/ml [103].

Of these 11 van gene clusters, only the *vanA* gene clusters confers Vancomycin resistance to *S.aureus* [99] *s. vanA* gene clusters are encoded by five proteins namely [104]

1. In the presence of vancomycin antibiotic, *vanS* and *vanR are* two component systems that up regulate the expression of vanA gene clusters.

2 *vanH, vanA, and VanX* convert D-ala-D-native ala's precursor to D-ala-D-lac, which mediates antibiotic resistance.

3. *vanH* functions as a dehydrogenase, reducing pyruvate to form D-lac.

4. *vanX* functions as a D, D dipeptidase, hydrolyzing D-ala-D-ala to prevent the synthesis of peptidoglycan in bacterial cell walls.

5. Vancomycin antibiotic resistance is created when *vanA* connects the D-ala to D-lac, replacing the original D-ala-D-ala.

A transposon-carried VanA operon (Tn1546) is a mobile genetic element. Co-infections with Vancomycin Resistant *Enterococcus fecalis* result in the acquisition of the *VanA* operon [104]. The horizontal gene transfer method of bacterial conjugation is used to spread vancomycin resistance from *Enterococcus* spp to other bacterial species. The Inc18 incompatibility conjugative plasmid is found naturally in Enterococcus but not in Staphylococci spp. Inc18 contains pSK41-like multi-resistant conjugative

plasmids. These plasmids are in charge of transferring resistant determinants from *E. faecalis to S. aureus*. [105]

2.23 Co-trimoxazole resistance:-

Sulfonamides are the first antimicrobial group discovered in 1932 and were introduced into clinical use for treating microbial infections in 1935 [106]. Sulfamethoxazole and sulfadiazine are medium and long acting Sulfonamides. They are still the most useful members of the antimicrobial family for using different clinical indications. However, Sulphonamides cause serious side effects, including hypersensitivity, toxic drug reactions, and blood dyscrasias [107].

In 1962, Trimethoprim (TMP) was registered with the FDA and introduced into clinical use with sulphonamides [108]. In 1972, sulphonamides were administered alone for the prophylaxis of urinary tract infection (UTI) in Finland. In 1979, TMP was used for the treatment of acute urinary tract infection. Trimethoprim also has side effects that aren't as bad as those of sulfonamides. For example, rashes and hypersensitivity reactions have been reported in people with weakened immune systems (like those with HIV, diabetes, etc.) and are rarely seen in aseptic meningitis [109]. non-allergic systemic infections (CNS irritation) have been seen in patients with Sjogren's syndrome, rheumatic disorders, and HIV infected patients [110]. Nowadays, a combination of Sulfamethoxazole with TMP in a formulation called Co-trimoxazole is used. Also, Sliver-Sulfadiazine is a combination used for the prophylaxis of wounds and burns dressings [110].

Both TMP and Sulphonamides affect the bacterial folic acid biosynthesis. Sulfonamide inhibits the synthesis of dihydropteroate synthetase (DHPS), which catalyzes the formation of dihydrofolate from p-aminobenzoic acid (PABA) to form dihydropeteroate, a precursor of folic acid. The sulfonamide binds at the active site of the enzyme and competes with PABA. In addition, Sulfonamide acts as an alternative substrate to form a petroate- sulfonamide product. This leads to the shut down of forming bacterial folic acid precursor (dihydropeteroate) and shows a bacteriostatic effect [111].

TMP (2,4 diaminopyridine trimethoprim) is a target for dihydrofolate reductase (DHFR). The biosynthetic pathway of folate, delivers the biologically inactive oxidized form of product dihydrofolate, which converts dihydrofolate to tetrahydrofolate by the action of the enzyme DHFR. Folic acid acts as a carbon donor during the deoxythymididylate monophosphate (dMTP) synthesis. It is catalyzed by the enzyme thymidylate synthase. Furthermore, N5-N10 methylene tetrahydrofolate is required for the production of serine transhydroxymethylase. For every mole of dMTP made, 1 mole of reduced, inactive folate is needed to change it into dihydrofolate [111].

Mechanism of Resistance [54]:-

Emergence of Co-trimoxazole resistance is occurs by mutations in the chromosomal genes of bacteria which encodes the DHPS and DHFR. TMP Resistance in clinical isolates is confers by change in substitutions of amino acids in the chromosomally encoded DHFR or by horizontal gene transfer that encodes the DHFR which allows the blockage of the chromosomal DHFR.

The most common resistance in TMP is a single amino acid substitution F98Y in the *dfrB* resistance phenotype, which demonstrates intermediate resistance with a MIC less than 256 mg/ml. Horizontal gene transfer resistance may be classified into three types which show high level of TMP resistance with an MIC value of more than 512 mg/ml.

1. *dfrA* resistant determinants. They are carried by Tn4001
2. *dfrK* determinants are found in Live stock associated -Staphylococci (LA-Staphylococci) and are rarely seen in clinical isolates
3. *dfrG* determinants are mainly seen in LA-Staphylococci[54]

Resistance to Co-trimoxazole is rare. However, recent studies reported that Co-trimoxazole resistance is distributed widely in MSSA and MRSA in sub-Saharan Africa and in Asia [54].

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Aim

&

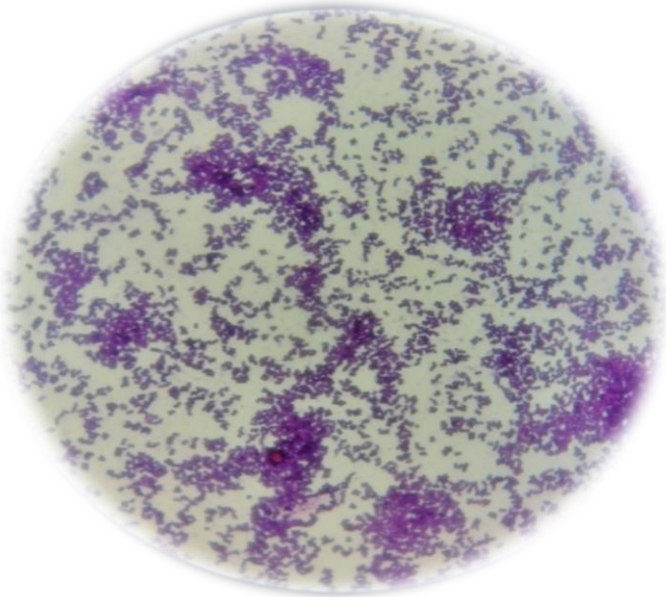
Objective

Aim & Objectives

To study the prevalence and distribution of *Staphylococcus* from healthcare settings and genotypic identification of antibiotic resistance determinants

Objective:-

1. To study the prevalence and distribution of *Staphylococcus* from Clinical specimen and nasal colonization of Healthcare workers and Patient visitors.
2. To screen the antibiotic resistance phenotypically by Kirby-Bauer Disc Diffusion method and Minimum Inhibitory Concentration.
3. To study the *mecA* gene distribution and its associated SCCmec types among Clinical specimens and Nasal colonization of HCWs and Patient visitors
4. To determine the antibiotic resistant determinants of *Staphylococcus* by using simplex and multiplex PCR



Prevalence and Distribution of Staphylococcus among Clinical Isolates, Nasal carriage of Health care workers and Patient visitors

Chapter-3

Prevalence and distribution *of CoNS and S.aureus* among Clinical Isolates, Nasal carriage of Health care workers and Patient Visitors

3.1. Introduction:-

Gram positive cocci are generally found in the upper respiratory tract of human begins as a normal micro flora. However, some factors like age, immunity, socio-economical factors, recent treatment of infections, climate conditions etc may turn these opportunistic colonizer into pathogens causing human infections leading to serious illness [1]. *Staphylococcus spp* are opportunistic pathogens commonly seen in the nasopharynx and skin of the vertebrate animals. Among the *Staphylococcus spp, Staphylococcus aureus* is a significant pathogen causing variety of infections to human begins from superficial skin infections and deep seated infections [2]. In addition, *S.aureus* is carried asymptomatically with varying rates in normal populations [3].

Coagulase Negative Staphylococcus (CoNS) is the normal commensals in the humans, animals and birds. However, the CoNS have emerged as the most common opportunistic pathogens responsible for Hospital Acquired Infections (HAIs). Risk factors of CoNS causing infection include patients with post-surgical, wound infections, intravascular catheters or other foreign medical indwelling devices, immunocompromised host such as premature newborns, leukemia patients and other malignant diseases [4]. Heterogeneous group of the CoNS contain approximately 40 species of which the most common CoNS species isolated from human clinical specimens are *S.epidermidis, S.haemolyticus, S.saprophyticus, S.hominis, S.warneri, S.lugdunensis, S.capitis, S.simulans, S.cohnii, S.Xyl*osus. It has been identified as important opportunistic pathogens causing nosocomial infection [5].

Anterior nares, skin and mucosal membranes are the important reservoirs which colonize the Staphylococcus permanently and transiently. It acts as an important source in causing blood stream infection, skin infections etc in humans. 60% of the population carries transiently, 20% of the population carries *S.aureus* permanently [6]. Among the CoNS, *S. hominis, S. haemolyticus* and *S. epidermidis* are common colonizers in nasal cavity and skin [7]. Screening of *Staphylococcus* in nasal carriage is important factor to reduce the risk of infections in hospitalized patients and prevent the spread of antibiotic

resistance between them. This chapter focusses on the prevalence of Staphylococcus among clinical isolates, Health care workers and Patient visitors.

3.2 Study plan:-

Type of Study: - Observational Cross sectional study

Ethical: - Samples were collected with proper informed consent **a**fter getting approval from Institutional Ethical Committee (IEC), D.Y Patil Medical College, and Kolhapur. No.DYPMCK/209/2019/IEC.

Inclusion Criteria:-

1. Healthcare workers and Patient Visitors without any clinical symptoms were included in the study
2. Patient visitors like Friends, Caregivers, Relatives those who came with patient without any respiratory infections
3. Healthcare workers like Nurses, Resident Doctors and Housekeeping workers those who were on duty without any symptoms were included in this study.
4. Clinical specimens from patients and Nasal swabs of HCWs , Patient visitors were from Kolhapur district were included in this study
5. In-Patient visitors, who visited hospital to visit patients continuously more than 2 days, were included in this study.
6. Out-Patient visitors, who visited hospital more than 2 days to hospital continuously without any break, were included in this study.
7. Clinical specimens from patient were included 16 -70 years of age group
8. HCWs, In-Patient Visitors and Out- Patient Visitors were included age group of 21-70 years

Exclusion Criteria:-

1. Patient visitors and Healthcare workers with any respiratory tract infections, skin and soft tissue infections up to 4 weeks before nasal sample collection.
2. Patient visitors and Healthcare Workers having treatment with anti-MRSA ointments and other antibiotics in the last 14 days.

3. Clinical specimens without requisition forms, incomplete patient clinical history and inappropriate specimens were excluded from the study.
4. Clinical specimens and Nasal swabs of HCWs and Patient visitors from out of Kolhapur district were not taken from this study.

3.3 Materials and Methods:-

Sample collection:-

Clinical specimens: - Clinical samples received to Microbiology department for diagnosis were taken (Pus, Sputum, Urine, Tips/suction, tissues) for analysis. Specimens were received in laboratory as per microbiological sample collection procedures.

Duration of Sample Collection: - April 2019 to June 2020

Total sample collected: - 1800 clinical samples from infected patient

Anterior Nasal swabs from Healthcare workers (Nurses, Resident Doctors and House Keeping Workers) and Patient visitors (Relatives, Friends, Care givers) were collected by using Hi-Sterile cotton swab, immediately inoculated into 5% Salt (NaCl) BHIB (Hi-Media, Brain Heart Infusion Broth) and transported to the laboratory immediately without any delay[8].

Duration of Sample Collection: - September 2019 to December 2020

Total Nasal swabs collected: - 200 Nasal Swabs from Healthcare Workers

200 Nasal swabs from In-Patient Visitors

200 Nasal Swabs from Out-Patient Visitors

Procedure for Nasal sample collection:-

1. Sterile cotton swab was removed from the package without touching the soft end
2. Soft end of the sterile swab was inserted to one side of the nostril, more than ¾ inches i.e., 1.5cm
3. Swab was rotated gently by pressed against the inside of nostril for 4 times atleast 15 seconds.

4. Swab was gently removed and the step 3 was followed on other nostril by same swab.

3.4 Primary isolation of Staphylococcus:-

Swabs from 5%Salt BHIB broth were inoculated into Blood agar and MacConkey agar (Hi-Media) and plates were incubated at 37ºC for 24 hours. Clinical specimens were directly inoculated into Blood agar and MacConkey agar.

3.5 Identification of Staphylococcus:-

After 24 hours of incubation, based on colony morphology, catalase test, gram stain reaction (Gram positive Cocci in clusters), Furazolidone sensitivity test and Glucose fermentation test Staphylococcus were identified. Differentiation of **Coagulase Negative Staphylococci** (CoNS) and **Coagulase Positive Staphylococci** (CoNS) was done by tube coagulase test

Identification of *S.aureus* [9]:-

Identification of *Staphylococcus aureus* was done by Catalase test [Fig 3.2], Mannitol fermentation reaction, DNAse test [Fig 3.6], and Gelatinase test and pigment production on Mannitol Salt agar [Fig 3.5].

Identification of CoNS [10]:-

Identification of CoNS was done by catalase test, Novobiocin resistance Sugar fermentation test [Fig 3.9] (Maltose, Trehalsoe, Xylose, Mannitol, Sucrose, Mannose, Fructose, Ribose, Furanose, Lactose and Raffinose, Urease, Nitrate Reduction test etc).

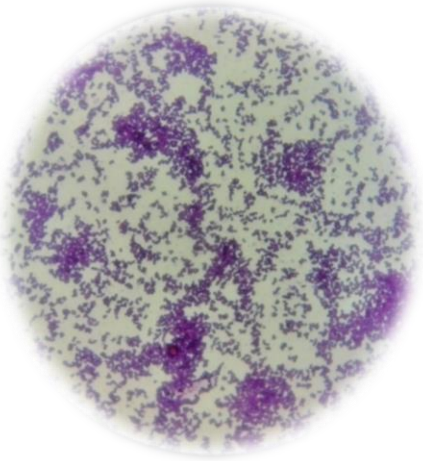


Fig 3.1 Gram Staining -Gram Positive Cocci in Clusters

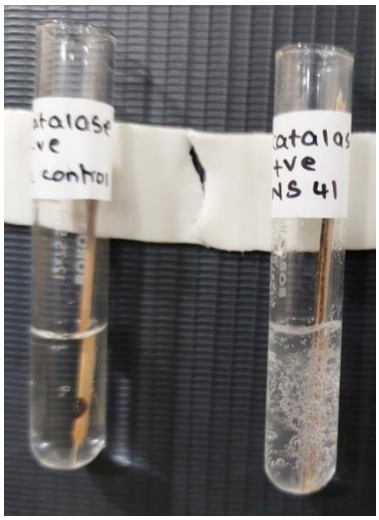


Fig 3.2 Tube Catalase test

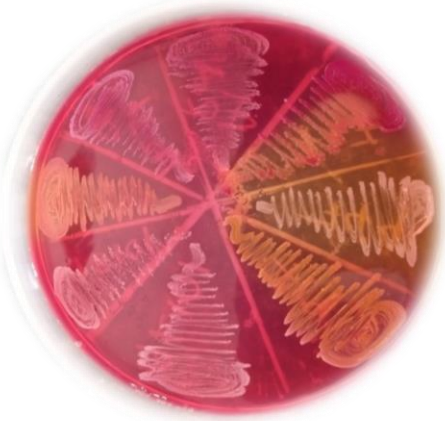


Fig 3.5 Growth on MacConkey agar

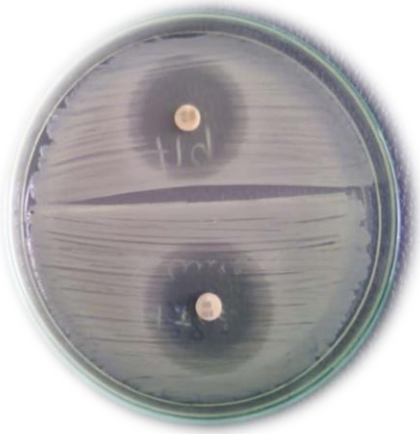


Fig 3.4Furazolidone susceptibility test

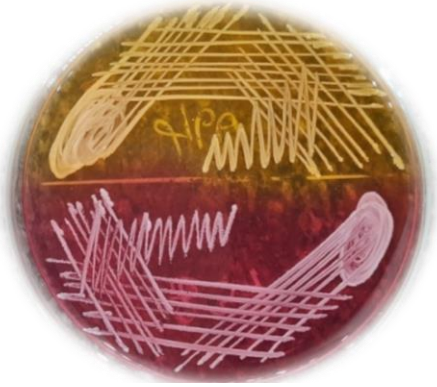


Fig 3.5 Growth on Mannitol Salt Agar

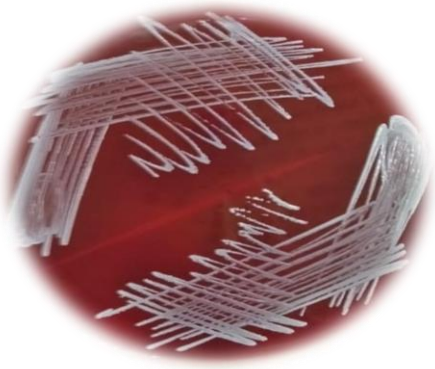


Fig 3.6 Growth Blood Agar

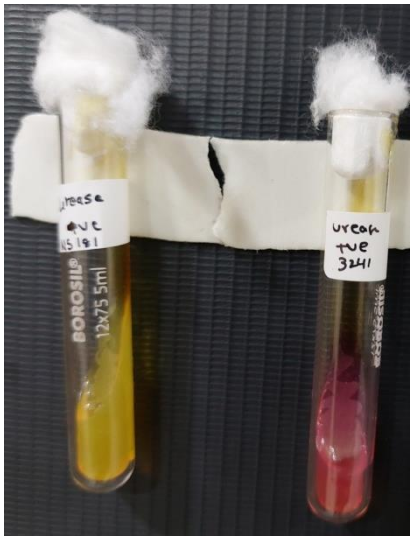


Fig 3.7 Urease test

Fig 3.8 DNAse test

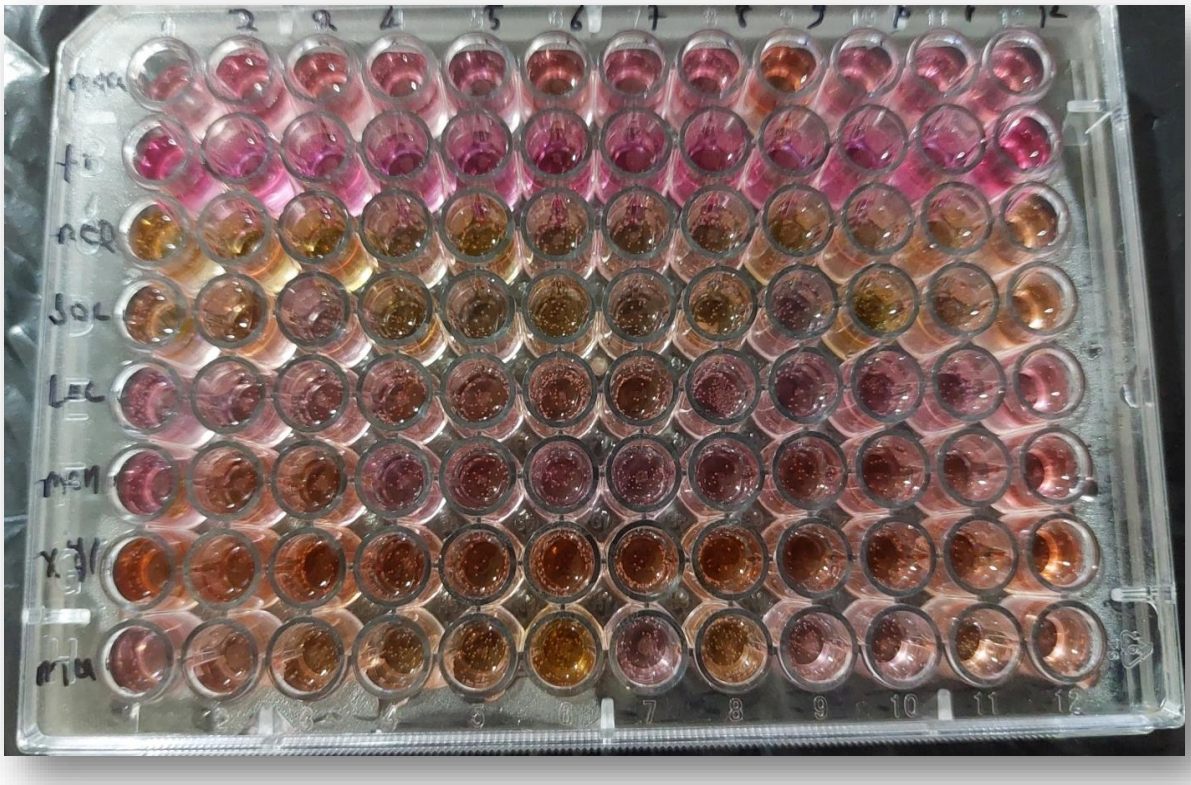


Fig 3.9 Carbohydrate fermentation test for identification of CoNS

3.6 Results:-

Total 1800 clinical specimens were collected. Of these, 800 *Staphylococcus* were isolated. Distribution of clinical specimens as follows [Table 3.1]

Table 3. 1: Distribution of Clinical Specimens and its isolated Staphylococci

Clinical Specimen	**Isolated Staphylococci (n=800)**
Aspirated Pus	86 (10.75%)
Wound Swab/Pus swab	186 (23.25%)
Urine Sample	163 (20.37%)
Blood culture	83 (10.37%)
Sputum	112 (14.0%)
ET secretion	97 (12.12%)
Catheter tips/Drain tips	25 (3.125%)
Sterile fluids	
1. Ascitic Fluid	20 (2.51%)
2. Pleural Fluid	15 (1.87%)
3. Cerebro-Spinal Fluid (CSF)	01 (0.125%)
4. Pericardial Fluid	12 (1.5%)
Total	**800 (44.44%)**

Out of 800 *Staphylococcus* isolated from clinical specimen, 350 were *S.aureus* and 450 were CoNS. Distribution of CoNs and *S.aureus* as follows [Table 3.2].

Table 3.2:- Distribution of *S.aureus* and CoNS among clinical specimen

Clinical Specimen	**CoNS (n=450)**	***S.aureus* (n=350)**
Aspirated Pus	47 (10.44%)	39 (11.14%)
Wound Swab/Pus swab	112 (24.88%)	73 (20.85%)
Urine Sample	101 (22.44%)	62 (17.71%)
Blood culture	45 (10.00%)	38 (10.85%)
Sputum	55 (12.22%)	57 (16.28%)
ET secretion	56 (12.44%)	42 (12.00%)

Catheter tips/Drain tips	12 (2.66 %)	13 (3.71%)
Sterile fluids		
5. Ascitic Fluid	09 (2.00%)	11 (3.14%)
6. Pleural Fluid	07 (1.55%)	08 (2.28%)
7. CSF	00	01 (0.28%)
8. Pericardial Fluid	06 (1.33%)	06 (1.71%)
Total	**450 (56.25%)**	**350 (43.75%)**

CoNS were isolated maximum in number (56.25%) than *S.aureus (*43.75%), Most of the CoNS were isolated from Wound/Pus swab (24.88%) and Urine Sample (20.37%) Specimens [Table 3.2] Most of the CoNS were isolated from the age group of above 40 years it might [Table 3.3] and most of the wound specimens were collected from ICUs and IPDs. Since most of the patients are immunocompromised and associated with Co-morbidity.

Table 3.3Age and Sex Distribution of collected clinical specimens

Sex	**CoNS (n=450)**	***S.aureus (n=350)***
Male	241	208
Female	209	142
Age Group		
16-20	43	51
21-40	89	74
41-60	191	132
60-70	126	93

Table 3.4 Distribution of clinical isolates of *CoNS and S.aureus* by ward-wise

Clinical Specimen	**CoNS (n=450)**				***S.aureus* (n=350)**			
	SICU	**MICU**	**OPD**	**IPD**	**SICU**	**MICU**	**OPD**	**IPD**
Aspirated Pus	19	12	10	6	11	14	5	9
Wound Swab/Pus swab	37	17	39	19	14	17	22	20
Urine Sample	9	14	42	36	11	13	19	18
Blood culture	10	29	15	12	13	19	0	6
Sputum	7	28	0	21	7	24	9	17
ET secretion	5	7	0	0	5	26	0	11
Catheter tips/Drain tips	0	9	0	0	3	10	0	0
Sterile fluids								
1. Ascitic Fluid	0	9	0	0	4	7	0	0
2. Pleural Fluid	0	7	0	0	2	6	0	0
3. CSF	0	0	0	0	0	1	0	0
4. Pericardial Fluid	0	6	0	0	0	6	0	0
Total	**97** **21.55 %**	**147** **32.66 %**	**112** **24.88 %**	**94** **20.88 %**	**70** **20.00 %**	**143** **40.86 %**	**55** **15.72 %**	**81** **23.15 %**

OPD- out Patient, IPD-In-Patient (General wards), MICU-Medicine Intensive care Unit, SICU-Surgery ICU

Table 3.4 shows the highest number of Staphylococcus was isolated from ICUs and General Wards.

Table 3.5 Distribution of CoNS and *S.aureus* of wound infection

Types of Wound infection	**CoNS*(n=159)***	***S.aureus*(n=112)**
Surgical wound Infection	31 (19.49%)	16 (14.28%)
Diabetic Foot ulcers	23 (14.46%)	21 (18.75%)
Abscesses Drainage of skin infection	43 (27.04%)	36 (32.14%)
Closed wound on skin surface	28 (17.61%)	24 (21.42%)
Lung abscess	15 (9.43%)	9 (8.03%)
Liver abscess	14 (8.80%)	6 (5.35%)
Burn Patient	5 (3.14%)	0

CoNS from Diabetic foot ulcers, surgical wound infections, Burn patient, Abscesses drainage on Skin were detected more in number [Table 3.5]. This clearly states that CoNS are emerging pathogen in immunocompromised patients.

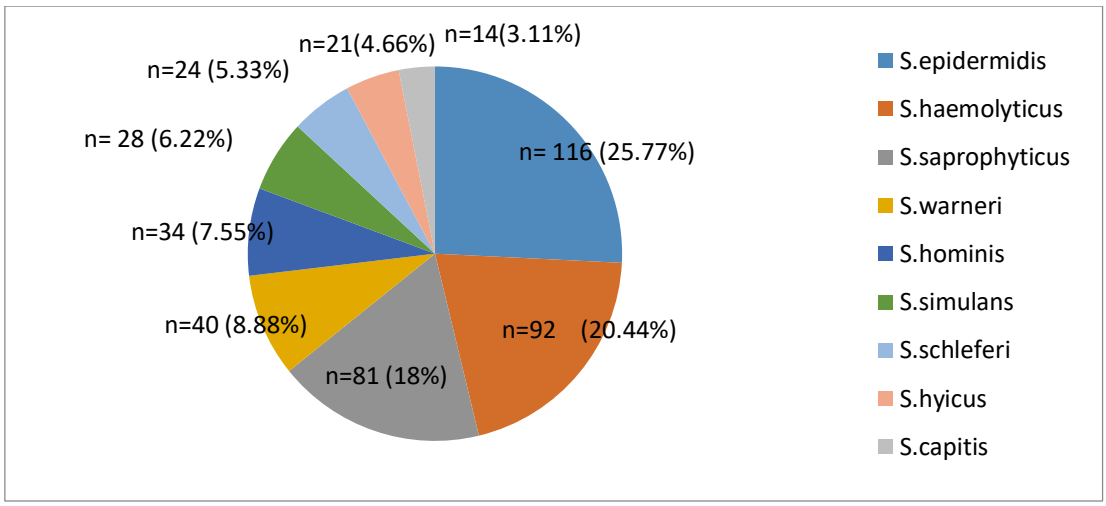
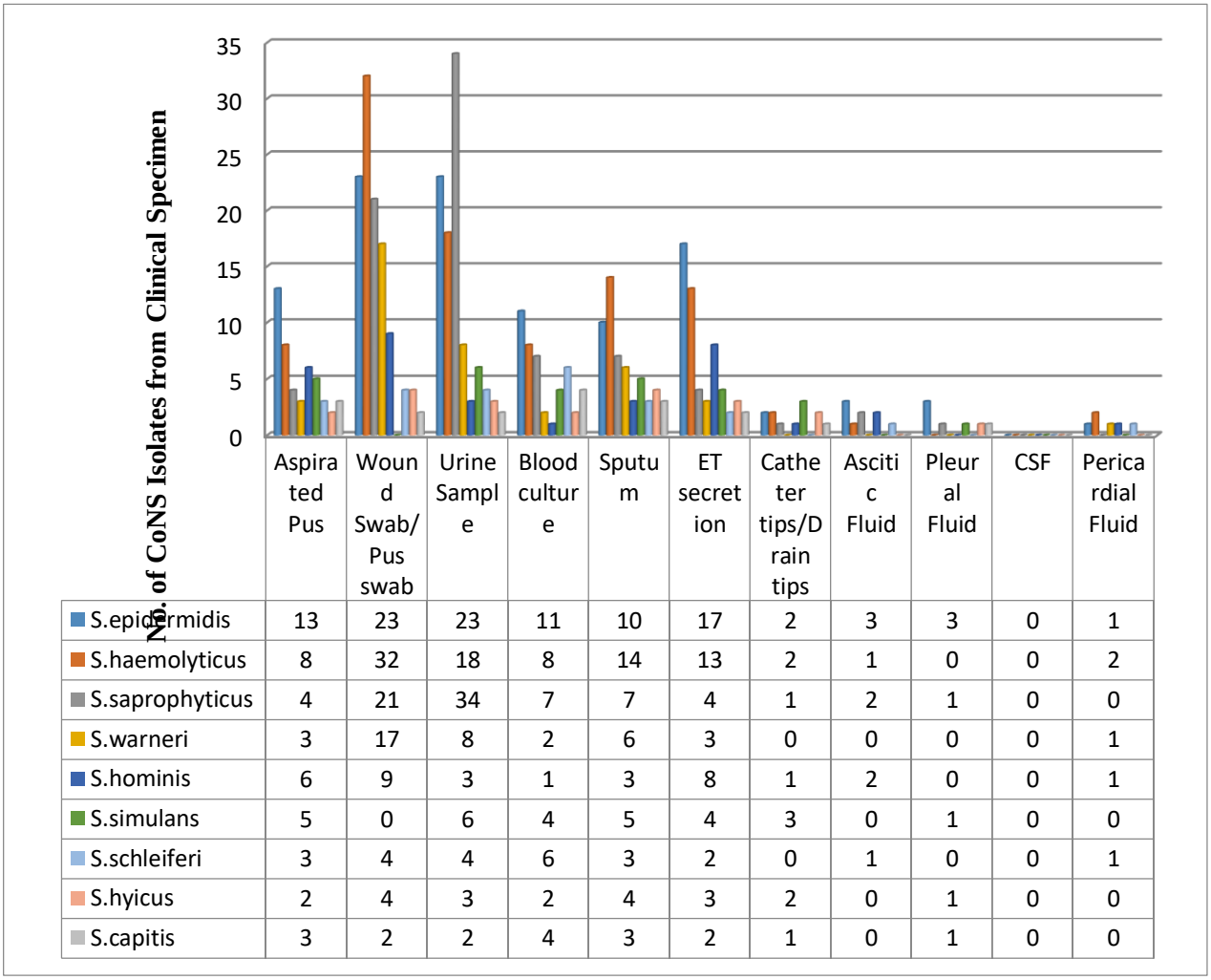


Figure 3.10 Distribution of CoNS species among Clinical specimen

(n=450) Out of 450 Coagulase Negative Staphylococcus (CONS), *S.epidermidis* (25.77%) was isolated maximum followed by *S.haemolyticus (20.44%), S.saprophyticus (18%), S.warneri (8.88%), S.hominis (7.55%), S.simulans (6.22%), S.schleferi (5.33%), S.hyicus (4.66%), and S.capitis (3.11%).* [Fig 3.10]



	Aspirated Pus	Wound Swab/ Pus swab	Urine Sample	Blood culture	Sputum	ET secretion	Catheter tips/Drain tips	Ascitic Fluid	Pleural Fluid	CSF	Pericardial Fluid
S.epidermidis	13	23	23	11	10	17	2	3	3	0	1
S.haemolyticus	8	32	18	8	14	13	2	1	0	0	2
S.saprophyticus	4	21	34	7	7	4	1	2	1	0	0
S.warneri	3	17	8	2	6	3	0	0	0	0	1
S.hominis	6	9	3	1	3	8	1	2	0	0	1
S.simulans	5	0	6	4	5	4	3	0	1	0	0
S.schleiferi	3	4	4	6	3	2	0	1	0	0	1
S.hyicus	2	4	3	2	4	3	2	0	1	0	0
S.capitis	3	2	2	4	3	2	1	0	1	0	0

Figure 3.11 Distribution of CoNS species among Clinical Isolates (n=450)

S. saprophyticus were isolated more in urine samples, *S.epidermidis and S.haemolyticus* were detected more in wound swab/Pus swab. Cerebro-Spinal Fluid (CSF) specimens was not detected CoNS [Fig 3.11]. In general, *S.saprophyticus* are frequently seen in urine samples among CoNS

Total 200 nasal swabs were collected from Healthcare Workers. Of these, 62% were *S.aureus* and 38% were CoNS [Figure 3.12]

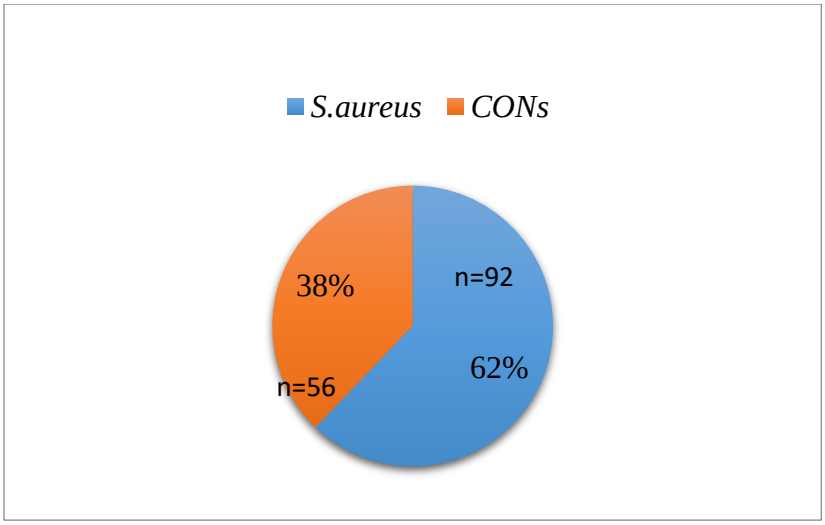


Figure 3.12 Distribution of Staphylococcus among Healthcare Workers (n=200)

S.aureus is frequent colonizer on anterior nasal cavity followed by CoNS. In present study 62% detected ***S.aureus*** and 38% detected CoNS

Table 3.6 Age and Sex distribution of HCWs

Sex	***S.aureus (n=92)***	**CoNS (n=56)**
Male	42	23
Female	54	33
Age Group		
21-40	31	11
41-60	42	19
≥61	19	26

Most of the Staphylococcus species were identified from HCWs were more than 40 years of age [Table 3.6].

Figure 2.13 Ward wise distributions of Staphylococcus among HCWs (n=148)

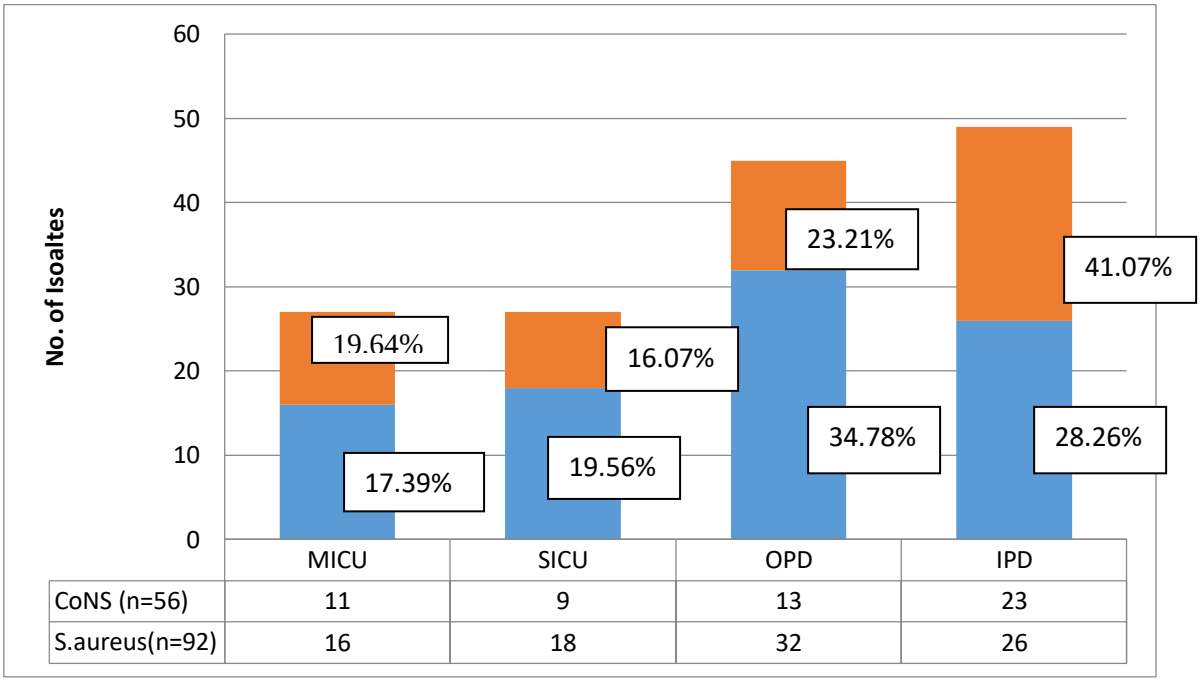


Figure 3.13 Ward wise distributions of Staphylococcus among HCWs (n=148)

Figure 3.13 shows the ward wise distribution of *S.aureus* and CoNS among HCWs. In-Patient department isolated Healthcare workers isolated more Staphylococcus i.e., 28.26% *S.aureus* and 41.07% CoNS.

Table 3.7:- Distribution of CoNs among HealthCare workers (HCWs)

Sr. No	CoNS	Isolated CoNS
1	*S.epidermidis*	20 (35.71%)
2	*S.haemolyticus*	14 (25%)
3	*S.saprophyticus*	08 (14.28%)
4	*S.warneri*	06(10.71%)
5	*S.hominis*	05(8.92%)
6	*S.hyicus*	03(5.35%)
Total		**56**

S.epidermidis was isolated highest i.e., 20 (35.71%) out of 56 isolated CoNs followed by others [Table 3.7]. *S.epidermidis* and *S.haemolyticus* are seen in moist area of the body than other CoNS.

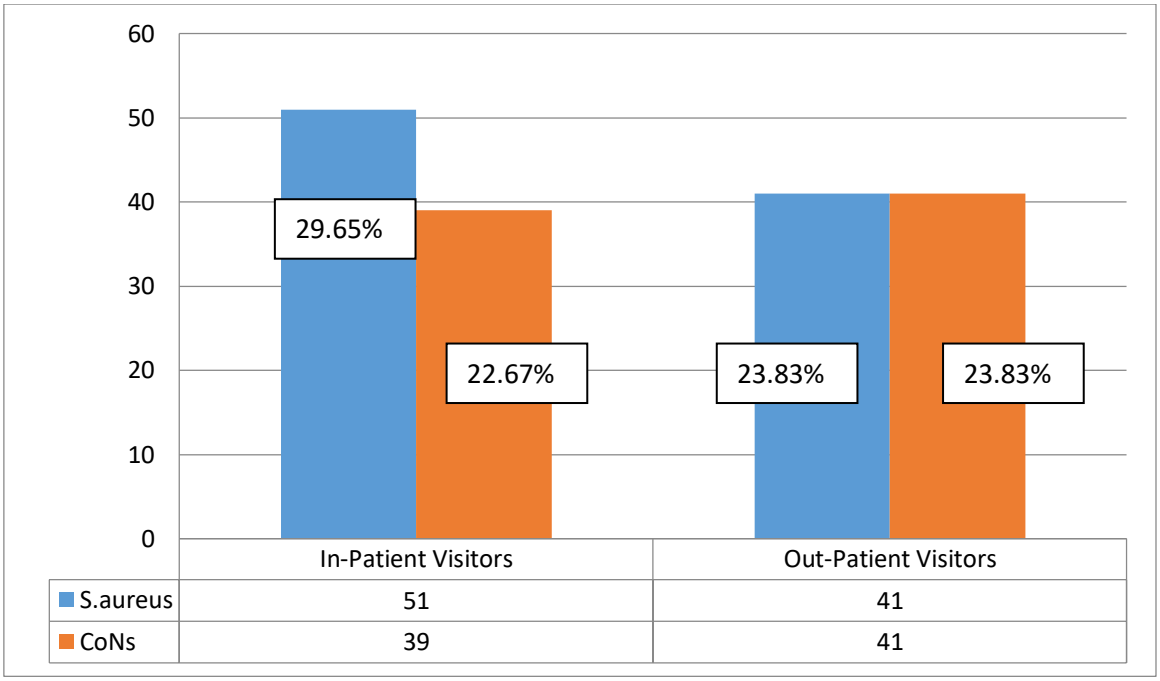


Figure 3.14 Distribution of Staphyloccus among Patient visitors

Total 400 nasal swabs were collected from patient visitors. Of these, 200 nasal swabs were collected from In-patient visitors and 200 swabs from out-patient visitors. Total 172 *Staphylococcus* spp were isolated. Out of these, 92 were *S.aureus* and 80 were CoNS [Figure 3].

Table 3.8 Distribution of Sex and Age group among Patient visitors

	In-Patient Visitors		**Out-Patient Visitors**	
Sex	***S.aureus* *(n=51)***	**CoNS (n=39)**	***S.aureus* *(n=41)***	**CoNS (n=41)**
Male	28	13	22	17
Female	23	26	19	24
Age Group				
21-40	19	18	19	12
41-60	21	21	15	21
≥61	11	39	7	8

Table 3.8 shows the sex and age group of the participant. Most of the Nasal carriage of Staphylococcus isolated above 40 years of age group

Table 3.9:- Distribution of CoNS among Patient visitors

Sr. No	Isolated CoNS	In-Patient visitors	Out-Patient Visitors
1	*S.epidermidis*	11 (28.20%)	13 (31.70%)
2	*S.haemolyticus*	12 (30.76%)	10 (24.39%)
3	*S.saprophyticus*	07 (17.94%)	08 (19.51%)
4	*S.warneri*	04 (10.25%)	06 (14.63%)
5	*S.homini*	03 (7.69%)	02 (4.87%)
6	*S. lugdunensis*	02 (5.12%)	02 (4.87%)
	Total	**39**	**41**

Table 3.9 shows distribution of CoNS species among Patient visitors. *S.epidermidis* was isolated more in number followed by others

3.7 Discussion:-

In present study, out of total 1800 clinical specimens 800 (44.44%) *Staphylococci were* isolated. Among these 450 (46.25%) were Coagulase negative *Staphylococci* (CoNS). Isolation and identification of CoNS among various clinical samples varies from different studies. Abishek et al isolated 42.94% of CoNS among various clinical specimens [11] where as Shiv Kumar et al isolated 15% of CoNS from clinical samples [12]. These variations might be due to availability of samples, type of clinical specimen used for analysis, type of study and consideration of CoNS as a pathogens in clinical specimens. Among 450 CoNS, *S.epidermidis* (25.77%) was isolated more followed by *S.haemolyticus* (20.44%), *S.saprophyticus* (18%), *S.warneri* (8.88%), *S.hominis* (7.55%), *S.simulans* (5.33%), S.*schleferi* (5.33%), *S.hyicus* (4.66%) and *S. capitis* (3.11%). Our result is consistent with other studies performed by Abhinaya et al, Sateesh K et al and Bora et al , who found *S.epidermidis* more followed by *S.saprophyticus* and *S.haemolyticus* [13-15]. While Singh et al found that *S.haemolyticus* was more than *S.epidermidis* [16]. This variable might be due to colonization of CoNS in skin and Mucous membrane of the individuals. *S.epidermidis* and *S.haemolyticus* are frequently found in humid areas of the body like axillae, inguinal and perianal area as normal flora. This

colonizer may turn to nosocomial pathogen and cause variety of nosocomial infection and capacity to form biofilm.

We found *Staphylococcus saprophyticus* more in urine samples than other clinical samples. *S.saprophyticus* is a common uropathogen among the CoNS species, and is mostly seen in sexually active young women and immunocompromised patients. This finding is similar to other studies conducted by Shiek and Mehdinejad et al , Usha et al [17, 18]. In the current study highest CoNS were isolated in wound swab (24.88%) followed by other clinical samples [Table 3.2]. This is in contrast to other studies conducted by Valli Punitha et al and Badampudi Vijayasri et al [19, 20]. Higher prevalence of CoNS in wound swab in our study might be because of maximum number of the samples were collected from wound swab (23.25%) followed by Urine sample (20.37%) and Sputum samples (14.0%). In general, CoNS are skin flora which may contaminate the wound due to poor personal hygiene, exposure of wound to environment. Furthermore, most of the people trend to treat wound infections on their own. Techniques used to collect wound swabs by healthcare workers may also vary [21].

In present study prevalence of *S.aureus* in clinical specimen was 43.75% (n=350). Prevalence of *S.aureus* varies from other studies. Shahi et al in Nepal found prevalence rate 14.4 % in clinical specimens at tertiary care teaching hospital [21], while Eyob Yohaness Garoy et al showed higher prevalence rate of 61.8% in a Multi center study at Asmara, Eritrea among various clinical specimens [22]. The variation of Staphylococcus aureus isolation among the clinical specimens in our study might be because ours is a single centric study and study was conducted in a tertiary care teaching hospital, most of the *S.aureus* isolated was above 40 age groups and most of specimens of *S.aureus* were from ICUs and IPDs. Most of the *S.aureus* were isolated from the wound swab/pus swab (20.85%) followed by Urine (17.71%) and Sputum (16.28%). This finding is similar to other studies, Jyotshna Sapkota et al 2018 who found more *S.aureus* in Pus /wound swab (78.95%)[23], Eyob Yohaness Garoy et al 2019 showed more numbers in discharging abscess (62.1%)[22]. In our study, maximum number of samples was collected from wound swab followed by urine and Sputum [Table 3.1] this may be the reason we got more isolates from these. In General, *S.aureus* is a common commensal of skin which may enter the host through cut, cracks, abrasions or minor injuries and cause pygenic infections [22].

Blood Stream Infection (BSI) by*S.aureus* and CoNS in present study was lower 10.85% in *S.aureus* and 10.00% in CoNS.In present study, most of the Blood specimens were collected from ICUs and IPDs patients with age group of above 40 years patients may be underlying diseased conditions. Among CoNS in BSI, *S.epidermidis* (n=11) was found more in current study. This was similar to BSIof other studies conducted by Worthington et al 2000, whofound high prevalence (96%) of *S.epidermidis* among catheter associated blood stream infection [24]. Ibrahim Ali et al 2015, found high rate of *S.epidermidis* (34%) among the blood specimens [5]. The results obtained from the above studies were similar and coinciding to our study, so above we conclude that *S.epidermidis* is the most common pathogen in BSI followed by *S.haemolyticus.*

Nasal colonization of *Staphylococcus aureus* is an major risk factor for invasive infections. In this study we had taken anterior nasal swabs from Healthcare workers and Patient visitors, the Prevalence of *S.aureus* and CoNS among HCWs and Patient visitors was found 62% and 23%.*S.aureus* colonizes the nasal cavity ranging from 20-80%. Rate of Nasal colonization of *S.aureus* varies from other studies; Khanal et al 2015 found less prevalence (15.7%) of *S.aureus* among HCWs [25], whereas Nipa Singh et al 2018 from Odhisa found 40.8% nasal colonization of *S.aureus* among health care workers [26]. Most of the nasal colonized *S.aureus* isolated in our study were above 40 years of age group which indicates that carriage of *S.aureus* varies in different age groups and Occupation. In general, HCWs are frequently in contact with hospital environment indirectly or directly, *S.aureus* may colonize the HCWs transiently. In the present study, HCWs from IPD and OPD were isolated maximum number of nasal colonization of Staphylococcus [Figure 3.13]

Nasal carriage of CoNS is an important and common reservoir with increased risk of causing nosocomial infections and antibiotic resistance. Epidemiology of CoNS in healthcare setting is much less than the *S.aureus*. The prevalence of CoNS in HCWs and Patient visitors seen in this study was 38% and 20%. *S.epidermidis* was the common species isolated followed by *S. haemolyticus, S. saprophyticus, S. warneri, S. hominis, S.hyicus* and *S.lugdunensis* [Table 3.3 and 3.4]. CoNS prevalence in nasal carriage in our study was less as compared to *S.aureus*. This finding differs in different studies, as prevalence of CoNS depends on the several factors like Occupation, habits, personal hygiene leading to variations in its prevalence rates.

3.8 Conclusion:-

Routine screening of *Staphylococcus* in nasal cavity among the healthcare workers, patient visitors will help to prevent the spread of *Staphylococcal* infection and its antibiotic resistance in hospital settings. It also helps to reduce the hospitalization, treatment cost of patient and prevent the unwanted high antibiotic pressure in hospitals. In Present study, 62% *S.aureus* and 38% CoNS were isolated among anterior nares of HCWs. Among Patient Visitors 23% *S.aureus* and 20% CoNS were detected. *S.aureus* is frequent colonizer than CoNS. Moreover, HCWs come directly or indirectly in contact with hospital environments and patients. Hence, the rate of isolation is more in HCWs than Patient visitors.

In present study, 43.75% *S.aureus* were isolated from clinical specimens most of the *S.aureus* seen in Wound swabs (20.85%). Present study maximum number of specimens was wound swab (23.25%) hence; the isolation rate is more in wound swabs. In general wound may get contaminate High number of *S.aureus* isolated in this study from ICUs and IPDs and age group of above 60 years.

56.25% of clinical specimens are identified as CoNS among these, *S.epidermidis* (25.77%) are higher prevalence than others. *S.epidermidis and S.haemolyticus* are frequently seen in moist surface of the body. In the present study, 54.22% clinical specimens from CoNS were from ICUs. ICUs patient may have medical implant devices, diabetes, surgical site infections, Burns, Lung abscess. Good knowledge on hospital infection practices and clinical follow up will help to eliminate this pathogen and minimize the horizontal transfer of antibiotic resistance to other pathogens.

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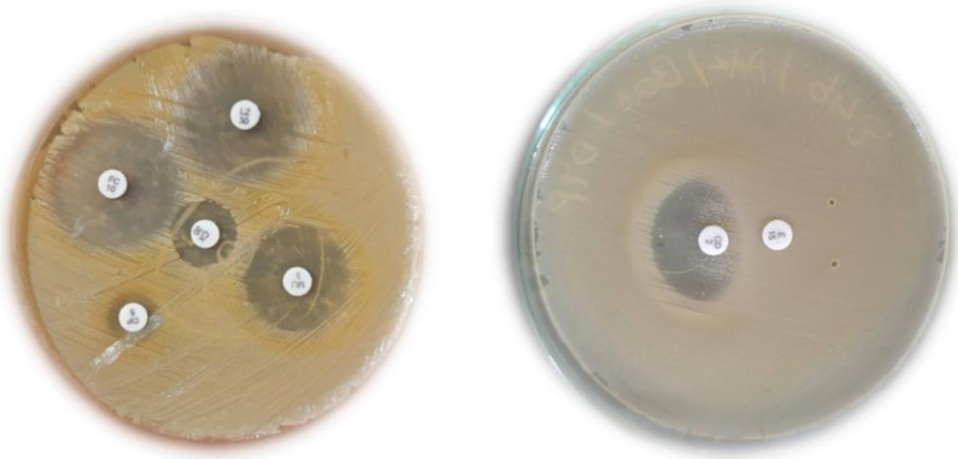


Prevalence of Antibiotic resistance by Kirby – Bauer Disc diffusion method and Minimum Inhibitory Concentration among Clinical isolates, Nasal carriage of Health care workers and Patient visitors

Chapter-4

Detection of antibiotic resistant by Kirby Bauer disc diffusion method and Minimum inhibitory concentration

4.1 Introduction: -

Antimicrobial resistance (AMR) is a major issue in the global health system [1]. According to the World Health Organization, AMR is caused by microorganism mutations, which result in the ineffectiveness of antimicrobials, the persistence of infections in the host body, and an increased risk of transmission of AMR pathogens to others [2]. One of the following factors may contribute to the emergence of AMR. [3]

- Over use and Over-prescription of Antimicrobials
- Use of antimicrobials in Agriculture like commercial application of antimicrobials in animal food production
- High antibiotic selection pressures in hospitals.

There are 12 drug-resistant bacteria families that pose the greatest threat to human health. As a result, WHO created the Priority Pathogen List (PPL) based on a multi-criteria decision analysis (MCDA). Priority pathogens are classified as critical, high, or medium priority. Staphylococcus aureus is a high priority pathogen that is methicillin-resistant, vancomycin-intermediate, and resistant [4].

The Indian Council of Medical Research (ICMR) has established a network for antimicrobial resistance surveillance and research (ICMR-AMRSN). AMRSN primarily focuses on six drug resistance pathogens: I Enterobacteriaceae causing sepsis, (ii) Gram-negative non-fermenters, (iii) Enteric fever pathogens, (iv) Diarrhoeagenic bacterial organisms, (v) Gram-positives: Staphylococci and Enterococci, and (vi) Fungal pathogens: yeasts (Candida and Cryptococcus). Antibiotics are widely used in India and other low and middle-income countries (LMICs).Monitoring of antibiotic resistance in LMIC is very difficult due to the unregulated sales of antimicrobials i.e., over the counter without any prescription and unwontedly, large number of antibiotics has been used in veterinary sector for increasing the meat production [6]. Till date, research and studies on antimicrobial resistance burden in LMICs is very limited and restricted hence the present chapter focuses on the antibiotic resistance of isolated *Staphylococcus* among the clinical

specimens and nasal colonization phenotypically by Kirby-Bauer Disc diffusion method and Minimum inhibitory concentration.

4.2 Materials and Methods: -

Antibiotic resistant detection was done by using Kirby- Bauer Disc Diffusion Method and Minimal inhibitory concentration by using following antibiotics as per the CLSI guidelines 2020 [7]

Table 4.1: List of Antibiotic discs used in current study

Sr. No	Antibiotic disc (Hi-Media, Mumbai, India)
1	Cefoxitin (30mcg)
2	Chloramphenicol (30 mcg)
3	Ciprofloxacin (5 mcg)
4	Erythromycin (15 mcg)
5	Clindamycin (2 mg)
6	Co-trimoxazole (25 mcg)
7	Fusidic acid (10 mcg)
8	Gentamicin (10 mcg)
9	Linezolid (30 mcg)
10	Tetracycline (30 mcg)
11	Mupirocin (5 mcg)

mcg- Microgram, Mg –Milligram

4.3 Kirby Bauer Disc Diffusion Method [7]:-

Using a sterile inoculation loop, four to five uniform isolated colonies from Clinical Specimens, HCWs and Patient visitors of *Staphylococcus* from 24 hour's bacterial culture plate were touched and transferred into test tube contained 2 ml of sterile saline. Saline tube was mixed properly with the help of vortex to create a uniform suspension. Turbidity of the bacterial suspension was adjusted to 0.5 McFarland standards by 0.5 McFarland control tube.

Sterile cotton swab was immersed into 0.5 McFarland adjusted suspension tube. Swab was gently rotated against the side of the test tube (above the fluid level) by gentle pressure to remove excess fluid. Swab was inoculated on the dried surface of Muller

Hinton agar (MHA) by streaking the swabs 3 times over the entire agar surface. The plate was rotated approximately 60 degrees each time to ensure an uniform distribution of the bacterial inoculum. Swab stick was discarded carefully in discarding jar containing 5% sodium hypochlorite solution.

Plates were allowed to stand at room temperature for 3 to 5 minutes for the agar surface to dry. After 5 minutes antibiotic discs (Hi-media, Table 4. 1) were dispensed by using sterile forceps on the agar surface carefully. Plates were kept at 35ºC in ambient incubator for 16 to 18 hours.

4.4 Minimum Inhibitory Concentration[8]:-

A. Stock solution preparation:-

Stock solution of antibiotics was prepared by using following formula

$$\frac{1000}{P} \times V \times C = W$$

Where, P = potency of antibiotic powder, given by the manufacturer (µg/mg)

V= Volume required (mL)

C= Final concentration of antibiotic solution (multiplies of 1000) (mg/L)

W= Weight of antibiotic (mg) powder to be dissolved in volume V (mL)

For example,

$$\frac{1000}{980} \times 20 \times 10 = 204.8\,mg$$

204.08 mg of antibiotic powder was dissolved in 20ml of solvent = 10,000 mg/L of antibiotic solution

Further stock solutions were prepared from initial 10,000 mg/L antibiotic solution as follows

1mL of 10,000mg/L antibiotic solution + 9 mL of diluents = 1000 mg/L of antibiotic solution

100µL of 10,000mg/L antibiotic solution + 9 mL of diluents= 100 mg/L of antibiotic solution

B. Preparation of antibiotic dilution range as per the CLSI guidelines 2020[7]

Dilution range was prepared from 0.25 to 128 mg/L

96 well flat bottom microtitre plate was labeled as follows 128, 64, 32, 16, 8, 4,2,1,0.5,0.25 and 0 mg/L

From the 10,000 mg/L stock solution of antibiotic was dispensed into 96 well microtitre plates as follows

256 µL was dispensed into the well labeled 128

128µL was dispensed into the well labeled 64

64 µL was dispensed into the well labeled 32

32µL was dispensed into the well labeled 16

From the 1000 mg/L stock solution of antibiotic was dispensed into 96 well microtitre plates as follows

16µL was dispensed into the well labeled 8

8µL was dispensed into the well labeled 4

4µL was dispensed into the well labeled 2

From the 100 mg/L stock solution of antibiotic was dispensed into 96 well microtitre plates as follows

2µL was dispensed into the well labeled 1

1µL was dispensed into the well labeled 0.5

0.5 µL was dispensed into the well labeled 0.25

No antibiotic solution was added in the well labeled as 0 mg/L (antibiotic free control)

C. Preparation of Inoculum

3-4 colonies with similar morphology were gently touched by a sterile inoculation loop and transferred to a 2ml test tube containing sterile Muller Hinton Broth. Suspension

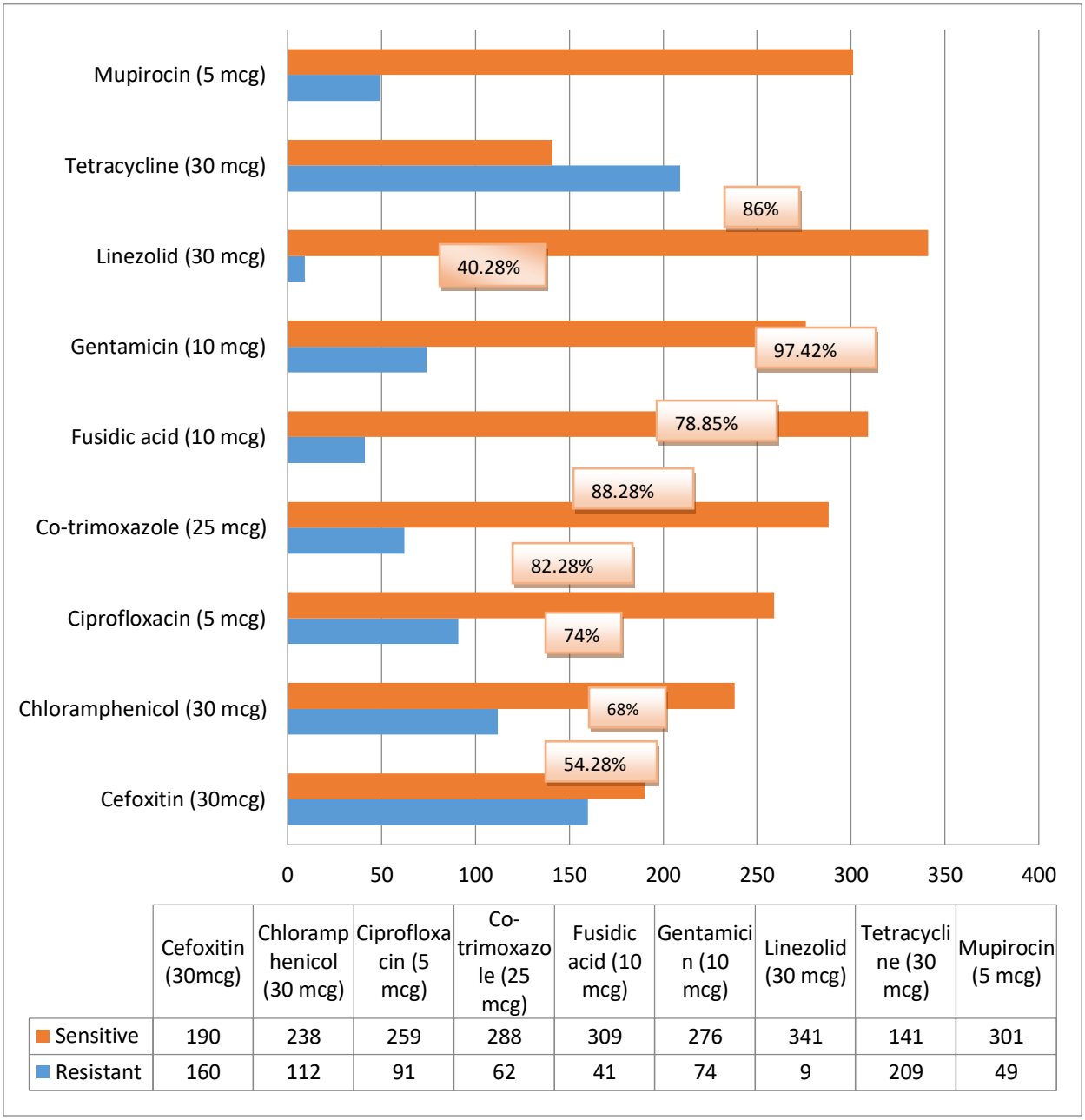
tube was gently mixed with the help of vortex to make a uniform microbial suspension. The suspension was adjusted to 0.5Mc Farland standard (10^7cfu/mL) with sterile distilled water. 75 µl of test organisms were dispensed to respective wells containing antibiotics.

75 µl of test organism suspension was inoculated into the well containing no antibiotic solution act as organism control. Plate lid was covered carefully and incubated at 35ºC for overnight.

Table 4.2: Potency and Diluents of Vancomycin Hydrochloride

Sr.No	**Antibiotic Powder**	**Potency (Given by Manufacturer) Hi-Media**	**Diluents**
1	Vancomycin Hydrochloride	≥950 µg/mg	Autoclaved distilled water

4.5 Results:-



	Cefoxitin (30mcg)	Chloramphenicol (30 mcg)	Ciprofloxacin (5 mcg)	Co-trimoxazole (25 mcg)	Fusidic acid (10 mcg)	Gentamicin (10 mcg)	Linezolid (30 mcg)	Tetracycline (30 mcg)	Mupirocin (5 mcg)
Sensitive	190	238	259	288	309	276	341	141	301
Resistant	160	112	91	62	41	74	9	209	49

Fig 4.1:- Antibiotic sensitivity Pattern of *S.aureus* among clinical isolates by Kirby Bauer-Diffusion Method (n=350)

Table 4.3:- Distribution of Antibiotic sensitivity pattern among clinical specimens of isolated *S.aureus (n=350)*

Types of Clinical Specimens	Cx		C			Cipro		Cot		Fus		Gen		LZ		Tet		Mup	
	R	**S**	**R**	**I**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Pus Aspirated (n=39)	19 (5.4%)	20 (5.71 %)	15 (4.28 %)	3 (0.85 %)	23 (6.57 %)	14 (4%)	25 (7.14 %)	07 (2.00 %)	32 (9.14 %)	4 (1.14 %)	35 (10%)	9 (2.57 %)	30 (8.57 %)	1 (0.28 %)	38 (10.85%)	22 (6.28 %)	17 (4.85 %)	35 (10%)	4 (1.14 %)
Pus/Wound Swab (n=73)	38 (10.85 %)	35 (10%)	20 (5.71 %)	1 (0.28 %)	42 (12.00%)	18 (5.14 %)	55 (15.71%)	15 (4.28 %)	58 (16.57%)	7 (2.00 %)	66 (18.85%)	17 (4.85 %)	56 (16%)	3 (0.85 %)	70 (20%)	35 (10%)	38 (10.85%	64 (18.28%	9 (2.57 %)
Urine (n=62)	32 (9.14 %)	30 (8.57 %)	23 (6.57 %)	2 (0.57 %)	39 (11.14%)	17 (4.85 %)	45 (12.85%)	10 (2.85 %)	52 (14.85%)	9 (2.57 %)	53 (15.14%)	15 (4.28 %)	47 (13.42%)	2 (0.57 %)	60 (17.14%)	33 (9.42 %)	29 (8.28 %)	49 (14%)	13 (3.71 %)
Sputum (n=57)	28 (8.00 %)	29 (8.28 %)	19 (5.42 %)	3 (0.85 %)	38 (10.85%)	14 (4%)	43 (12.28%)	09 (2.57 %)	48 (13.71%)	6 (1.71 %)	51 (14.57%)	12 (3.42 %)	45 (12.85%)	2 (0.57 %)	55 (15.71%)	36 (10.28%)	21 (6%)	48 (13.71%)	9 (2.57 %)
ET Secretion (n=42)	15 (4.28 %)	27 (7.71 %)	13 (3.71 %)	1 (0.28 %)	29 (8.28 %)	11 (3.14 %)	31 (8.85 %)	08 (2.28 %)	34 (9.71 %)	7 (2.00 %)	35 (10%)	08 (2.28 %)	34 (9.71 %)	1 (0.28 %)	41 (11.71%)	25 (7.14 %)	17 (4.85 %)	36 (10.28%	6 (1.71 %)
Blood culture (n=38)	09 (2.57 %)	29 (8.28 %)	07 (2.00 %)	1 (0.28 %)	31 (8.85 %)	07 (2.00 %)	31 (31.85%)	07 (2.00 %)	31 (8.85 %)	3 (0.85 %)	35 (10%)	05 (1.42 %)	33 (9.42 %)	-	38 (10.85%)	29 (8.28 %)	09 (2.57 %)	35 (10%)	3 (0.85 %)
Catheter tips (n=13)	7 (2.00 %)	6 (1.71 %)	4 (1.14 %)	--	9 (2.57 %)	04 (1.14 %)	9 (2.57 %)	02 (0.57 %)	11 (3.14 %)	1 (0.28 %)	12 (3.42 %)	02 (0.57 %)	11 (3.14 %)	-	13 (3.71 %)	9 (2.57 %)	04 (1.14 %)	12 (3.42 %)	1 (0.28 %)
Sterile fluids (n=26)	12 3.42%	14 4%	09 2.57 %	1 0.28 %	17 4.85 %	06 1.71 %	20 5.71 %	04 1.14 %	22 6.28 %	4 1.14 %	22 6.28 %	6 1.71 %	20 5.71 %	-	26 7.42 %	20 5.71 %	06 1.71 %	22 6.28 %	4 1.14 %
Total	**160 45.71 %**	**190 54.28%**	**110 31.42%**	**12 3.42 %**	**228 65.14 %**	**91 26%**	**259 74%**	**62 17.71%**	**288 82.28 %**	**41 11.71%**	**309 88.28 %**	**74 21.14%**	**276 78.85 %**	**9 2.57 %**	**341 97.42 %**	**209 59.71 %**	**141 40.28 %**	**301 86%**	**49 14%**

Cx-Cefoxitin, C-Chloramphenicol, Cipro-Ciprofloxacin, Cot-Cot-Trimoxazole, Fus-Fusidic acid, Gen-Gentamicin, LZ-Linezolid, Tet-Tetracycline, Mup-Mupirocin

Table 4.4 Distribution of Antibiotic sensitivity pattern from isolated *S.aureus* among Health care workers (n=92)

Sr. No	Antibiotics	Resistant	Intermediate	Sensitive
1	Cefoxitin	25 (27.17%)	--	67 (72.82%)
2	Chloramphenicol	7 (7.60%)	7 (7.60%)	78 (84.78%)
3	Ciprofloxacin	4 (4.34%)	5 (5.43%)	83 (90.21%)
4	Co-trimoxazole	7 (7.60%)	3 (3.26%)	82 (89.13%)
5	Fusidic acid	4 (4.34%)	--	88 (95.65%)
6	Gentamicin	5 (5.43%)	3 (3.26%)	84 (91.30%)
7	Linezolid	--	--	92 (100%)
8	Tetracycline	8 (8.69%)	4 (4.34%)	80 (86.95%)
9	Mupirocin	3 (3.26%)	-	89 (96.73%)

27.17% isolates showed resistant to Cefoxitin i.e., MRSA and Linezolid showed 100% sensitive

Table 4.5 Distribution of Antibiotic sensitivity pattern from isolated *S.aureus* among Patient visitors

Sr. No	Antibiotics	In-Patient Visitors (n=51)			Out-Patient Visitors (n=41)		
		R	I	S	R	I	S
1	Cefoxitin	14 (27.45%)	--	37 (72.54%)	12 (29.26%)	--	29 (70.73%)
2	Chloramphenicol	4 (7.84%)	4 (7.84%)	43 (84.31%)	4 (9.75%)	2 (4.87%)	35 (85.36%)
3	Ciprofloxacin	3 (5.88%)	5 (9.80%)	43 (84.31%)	2 (4.87%)	1	38 (92.68%)
4	Co-trimoxazole	4 (7.84%)	1 (1.96%)	46 (90.19%)	2 (4.87%)	3 (7.31%)	36 (87.80%)
5	Fusidic acid	2 (3.92%)	--	49 (96.07%)	1 (2.43%)	--	40 (97.56%)
6	Gentamicin	5 (9.80%)	3 (5.88%)	43 (84.31%)	2 (2.43%)	1 (2.43%)	38 (92.68%)
7	Linezolid	--	--	51 (100%)	--	--	41 (100%)
8	Tetracycline	7 (13.72%)	5 (9.80%)	39 (76.47%)	6 (14.63%)	1 (2.43%)	34 (82.92%)
9	Mupirocin	2 (3.92%)	--	49 (96.07%)	2 (4.87%)	--	39 (95.12%)

In –Patient visitors and Out-Patient visitors showed more or less same resistance pattern.

D-test (Erythromycin induced clindamycin resistant) from isolated *S.aureus among clinical isolates*:-

15 µg Erythromycin and 2 µg clindamycin discs (HiMedia) were placed on Mueller–Hinton plate that had been inoculated with a staphylococcal isolate to detect inducible Clindamycin resistance. The antibiotic discs were kept at a distance of 15–20 mm edge to edge from each other. Plates were incubated overnight at 37 °C [9]

Table 4.6: - D-test Prevalence from isolated *S.aureus among clinical isolates*

	Healthcare Workers (n=92)				**In-Patient Visitors (n=51)**				**Out-Patient Visitors (n=41)**				**Clinical specimens (n=350)**			
	D-test MLSBi	**C-MLSB**	**MS**	**E-S/C-S**	**D-test MLSBi**	**C-MLSB**	**MS**	**E-S/C-S**	**D-test MLSBi**	**C-MLSB**	**MS**	**E-S/C-S**	**D-test MLSBi**	**C-MLSB**	**MS**	**E-S/C-S**
S.aureus	9 (9.78 %)	12 (13.04 %)	14 (15.21 %)	38 57 (61.95 %)	7 (13.72 %)	8 (15.68 %)	11 (21.56 %)	25 (49.01 %)	5 (12.19 %)	8 (19.51 %)	11 (26.82 %)	17 (41.46 %)	67 (19.14 %)	48 (13.71 %)	71 (20.28 %)	164 (46.85 %)

C-MLSB: Constitutive MLSB, C-S,E-S: Clindamycin sensitive and Erythromycin sensitive

A positive D test resulted in flattening of the zone of inhibition surrounding the Clindamycin disc proximal to the Erythromycin disc (D shaped zone of inhibition) and was defined as inducible MLSBi (Macrolid, Lincosamide, Streptomycin B inducible) resistance.

Constitutive MLSB resistance was defined as isolates that were resistant to both Erythromycin and Clindamycin.

The MS phenotype consisted of isolates that were resistant to Erythromycin but sensitive to Clindamycin.

Table 4.7: Distribution of antibiotic sensitivity pattern among Clinical isolates of isolated CoNS (n=450)

	Cx		Chloram			Cipro			Cot-tri			Fus		Gen			LZ		Tet			Mup	
	R	**S**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**R**	**S**	**I**	**R**	**S**	**R**	**S**	**I**	**R**	**S**
S.epidermidis (n=116)	44 37.93%	72 62.06%	29 25%	85 73.27%	2 1.72%	14 12.06%	98 84.48%	4 3.44%	42 36.20%	68 58.62%	6 5.17%	24 20.68%	92 79.31%	16 13.79%	98 84.48%	2 1.72%	5 (4.31%)	111 95.68%	61 (52.58%)	50 43.10%	5 4.31%	12 10.34%	104 89.65%
S.haemolyticus (n=92)	29 31.52%	63 68.47%	19 20.65%	63 68.47%	4 4.34%	23 25%	65 70.65%	4 4.34%	20 21.73%	72 78.26%	-	29 31.52%	63 68.47%	21 22.82%	68 73.91%	3 3.26%	-	92 100%	39 42.39%	45 48.91%	8 8.69%	16 17.39%	76 82.60%
S.saprophyticus (n=81)	9 11.11%	72 88.88%	8 9.87%	70 86.41%	3 3.70%	12 14.81%	68 83.95%	1 1.23%	11 13.58%	70 86.41%	-	Intrinsic Resistant		11 13.58%	68 83.95%	2 2.46%	-	81 100%	24 29.62%	50 61.72%	7 8.64%	6 7.40%	75 92.59%
S.warneri (n=40)	2 5%	38 95%	6 15%	32 80%	2 5%	4 10%	36 90%	-	6 15%	33 82.5%	1 2.5%	5 12.5%	35 87.5%	3 7.5%	37 92.5%	-	-	40 100%	7 17.5%	32 80%	1 2.5%	1 2.5%	39 97.5%
S.hominis (n=34)	1 2.94%	33 97.05%	2 5.88%	32 94.11%	-	-	34 100%	-	1 2.94%	33 97.05%	-	2 5.88%	32 94.11%	2 5.88%	32 94.11%	-	-	34 100%	3 8.82%	30 88.23%	1 2.94%	-	34 100%
S.simulans (n=28)	1 3.57%	27 96.42%	1 3.57%	26 92.85%%	1 3.57%	-	28 100%	-	1 3.57%	27 96.42%	-	2 7.14%	26 92.85%	-	28 100%	-	-	28 100%	2 7.14%	26 92.85%	-	-	28 100%
S.schleiferi (n=24)	1 4.16%	23 95.83%	1 4.16%	23 95.83%	-	-	24 100%	-	2 8.33%	22 91.66%	-	2 8.33%	22 91.66%%	2 8.33%	22 91.66%	-	-	24 100%	3 12.5%	20 83.33%	1 4.16%	-	24 100%
S.hyicus (n=21)	-	21 100%	3 14.28%	17 80.95%	1 4.76%	1 4.76%	20 95.23%	-	-	21 100%	-	3 14.28%	18 85.71%	1 4.76%	20 95.23%	-	-	21 100%	2 9.52%	18 85.71%	1 4.76%	-	21 100%
S.capitis (n=14)	1 7.14%	13 92.85%	1 7.14%	13 92.85%	-	-	14 100%	-	-	14 100%	-	1 7.14%	13 92.85%	-	14 100%	-	-	14 100%	2 14.28%	12 85.71%	-	2 14.28%	12 85.71%
Total	88 19.55%	362 80.44%	70 15.55%	367 81.55%	13 2.88%	54 12%	387 86%	9 2%	83 18.44%	260 57.77%	7 1.55%	68 15.11%	301 66.88%	56 12.44%	387 86%	7 1.55%	5 1.11%	445 98.88%	143 31.77%	283 62.88%	24 5.33%	37 8.22%	413 91.77%

Table 4.8:- Distribution of antibiotic sensitivity pattern of isolated CoNS among Healthcare workers(n=56)

	Cx		Chloram			Cipro			Cot-tri			Fus		Gen			LZ		Tet			Mup	
	R	**S**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**R**	**S**	**I**	**R**	**S**	**R**	**S**	**I**	**R**	**S**
S.epidermidis (n=20)	-	20 (35.71%)	5 (8.92%)	15 (26.78%)	-	2 (3.57%)	18 (32.14%)	-	-	20 (35.71%)	-	3 (5.35%)	17 (30.35%)	2 (3.57%)	18 (32.14%)	-	-	20 (35.71%)	5 (8.92%)	14 (25%)	1 (1.78%)	-	20 (35.71%)
S.haemolyticus (n=14)	-	14 (25%)	2 (3.57%)	12 (21.42%)	-	-	14 (25%)	-	-	14 (25%)	-	1 (1.78%)	13 (23.21%)	1 (1.78%)	13 (23.21%)	-	-	14 (25%)	3 (5.35%)	11 (19.64%)	-	-	14 (25%)
S.saprophyticus (n=8)	-	8 (14.28%)	-	8 (14.28%)	-	1 (1.78%)	7 (12.5%)	-	-	8 (14.28%)	-	**IR**		-	8 (14.28%)	-	-	8 (14.28%)	1 (1.78%)	7 (12.5%)	-	-	8 (14.28%)
S.warneri (n=6)	-	6 (10.71%)	1 (1.78%)	5 (8.92%)	-	-	6 (10.71%)	-	-	6 (10.71%)	-	1 (1.78%)	5 (8.92%)	1 (1.78%)	5 (8.92%)	-	-	6 (10.71%)	1 (1.78%)	4 (7.14%)	1 (1.78%)	-	6 (10.71%)
S.hominis (n=5)	-	5 (8.92%)	1 (1.78%)	4 (7.14%)	-	-	5 (8.92%)	-	-	5 (8.92%)	-	-	5 (8.92%)	-	5 (8.92%)	-	-	5 (8.92%)	2 (3.57%)	3 (5.35%)	-	-	5 (8.92%)
S.hyicus (n=3)	-	3 (5.35%)	-	3 (5.35%)	-	-	3 (5.35%)	-	-	3 (5.35%)	-	-	3 (5.35%)	-	3 (5.35%)	-	-	3 (5.35%)	-	3 (5.35%)	-	-	3 (5.35%)
Total	-	56 (100%)	9 (16.07%)	47 (83.92%)	-	3 (5.35%)	53 (94.64%)	-	-	56 (100%)	-	5 (8.92%)	43 (76.78%)	4 (7.14%)	52 92.85%)	-	-	56 (100%)	12 (21.42%)	42 (75%)	2 (3.57%)	-	56 (100%)

Table 4.9:-Distribution of antibiotic sensitivity pattern of isolated CoNS among visitors

	Cx		Chloram			Cipro			Cot-tri			Fus		Gen			LZ		Tet			Mup	
	R	**S**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**R**	**S**	**I**	**R**	**S**	**R**	**S**	**I**	**R**	**S**
S.epidermidis (n=24)	3 (3.75 %)	21 (26.25%)	4 (5.00%)	18 (22.5 %)	2 (2.5 %)	3 (3.75%)	20 (25%)	1 (1.25%)	4 (5.00%)	20 (25%)	-	5 (6.25%)	19 (23.75%)	3 (3.75%)	21 (26.25%)	-	-	24 (30%)	7 (8.75 %)	16 (20%)	1 (1.25%)	2 (2.5 %)	22 (27.5 %)
S.haemolyticus (n=22)	1 (1.25 %%)	21 (26.25%)	2 (2.5 %)	19 (23.75%)	1 (1.25%)	1 (1.25%)	21 (26.25%)	-	2 (2.5 %)	19 (23.75%)	-	2 (2.5 %)	20 (25%)	2 (2.5 %)	19 (23.75%)	1 (1.25%)	-	22 (27.5 %)	4 (5.00 %)	17 (21.25%)	1 (1.25%)	1 (1.25%)	21 (26.25%)
S.saprophyticus (n=15)	2 (2.5%)	13 (16.25%)	3 (3.75%)	12 (15%)	-	2 (2.5 %)	13 (16.25%)	-	1 (1.25%)	14 (17.5 %)	-	IR		-	15 (18.75%)	-	-	15 (18.75%)	5 (6.25 %)	10 (12.5 %)	-	-	15 (18.75%)
S.warneri (n=10)	-	10 (12.5 %)	1 (1.25%)	9 (11.25%)	-	-	10 (12.5 %)	-	-	10 (12.5 %)	-	-	10 (12.5 %)	-	9 (11.25%)	1 (1.25%)	-	10 (12.5 %)	3 (3.75 %)	7 (8.75 %)	-	-	10 (12.5 %)
S.hominis (n=5)	-	5 (6.25 %)	-	5 (6.25 %)	-	-	5 (6.25 %)	-	-	5 (6.25 %)	-	-	5 (6.25 %)	-	5 (6.25 %)	-	-	5 (6.25 %)	2 (2.5 %)	3 (3.75 %)	-	-	5 (6.25 %)
S.lugdunensis (n=4)	-	4 (5.00 %)	-	4 (5.00 %)	-	-	4 (5.00 %)	-	-	4 (5.00 %)	-	-	4 (5.00 %)	-	4 (5.00 %)	-	-	4 (5.00 %)	-	4 (5.00 %)	-	-	4 5.00 %)
Total	6 (7.5%)	74 (92.5 %)	10 (12. 5%)	67 (83.75%)	3 (3.75%)	6 (7.5 %)	73 (91.25%)	1 (1.25%)	7 (8.75%)	72 (90.00%)	-	7 (8.75%)	58 (72.5 %)	5 (6.25%)	73 (91.25%)	2 (2.5 %)	-	80 (100 %)	21 (26.25%)	57 (71.25%)	2 (2.5 %)	3 (3.75%)	77 (96.25%)

Table 4.10: - D-test (Erythromycin induced Clindamycin resistance) from isolated *CoNS*:-

Healthcare workers (n=56)				In-Patient Visitors (n=39)				Out-patient visitors (n=41)				Clinical specimens (n-450)			
D-test MLSBi	**C-MLSB**	**MS**	**E-S/C-S**	**D-test MLSBi**	**C-MLSB**	**MS**	**E-S/C-S**	**D-test MLSBi**	**C-MLSB**	**MS**	**E-S/C-S**	**D-test MLSBi**	**C-MLSB**	**MS**	**E-S/C-S**
4 (7.14 %)	7 (12.5 %)	11 (19.64 %)	34 (60.71 %)	2 (5.12 %)	4 (70.25 %)	8 (20.51 %)	25 (64.10 %)	5 (12.19 %)	3 (7.31 %)	12 (29.26 %)	21 (51.21 %)	37 (8.22 %)	64 (14.22 %)	77 (17.11 %)	272 (60.44 %)

C-MLSB: Constitutive MLSB, C-S,E-S: Clindamycin sensitive and Erythromycin sensitive

A positive D test resulted in flattening of the zone of inhibition surrounding the Clindamycin disc proximal to the Erythromycin disc (D shaped zone of inhibition) and was defined as inducible MLSBi (Macrolid, Lincosamide, Streptomycin B inducible) resistance.

Constitutive MLSB resistance was defined as isolates that were resistant to both Erythromycin and Clindamycin.

The MS phenotype consisted of isolates that were resistant to Erythromycin but sensitive to Clindamycin.

Minimum Inhibitory Concentration (MIC) of Vancomycin:-

Disc diffusion method does not distinguish Vancomycin susceptibility of *S.aureus* from Vancomycin-Intermediate isolates, nor does the test differentiate between Vancomycin Intermediate, Resistant and susceptible of staphylococcal spp other than *S.aureus,* all of which give similar zone of inhibition [CLSI Guidelines 2020].

Table 4. 11:-Vancomycin Minimum Inhibitory Concentration (MIC) of *S.aureus*

	Sensitive			**Intermediate**		**Resistant**
MIC (µg/mL)	**0.5**	**1**	**2**	**4**	**8**	**> 16**
HCWS (n=92)	7 (7.60%)	16 (17.39%)	54 (58.69%)	6 (6.52%)	4 (4.34%)	5 (5.43%)
In-Patient Visitors (n=51)	3 (5.88%)	12 (23.52%)	18 (35.29%)	7 (13.72%)	5 (9.80%)	6 (11.76%)
Out-Patient visitors (n=41)	1 (2.43%)	8 (19.51%)	19 (46.34%)	6 (14.63%)	3 (7.31%)	4 (9.75%)
Clinical specimens (n=350)	47 (13.42%)	74(21.14%)	175(50%)	11 (3.14%)	16 (4.57%)	27 (7.71%)

Table 4.12 :-Vancomycin Minimum Inhibitory Concentration (MIC) of CoNS

	Clinical Specimens					In-Patient Visitors					Out-Patient visitors					Healthcare Workers				
	S		I		R	S		I		R	S		I		R	S		I		R
	≤ 3	4	8	16	> 32	≤ 3	4	8	16	> 32	≤ 3	4	8	16	> 32	≤ 3	4	8	16	> 32
S.epidermidis	34 7.55 %	62 13.77%	2 0.44 %	4 0.88%	4 0.88%	2 5.13%	7 17.95 %	-	1 2.56 %	1 2.56 %	3 7.31 %	9 19.51%	-	1 2.44 %	-	6 10.71 %	14 25%	-	-	-
S.haemolyticus	35 7.77 %	54 12.00%	2 0.44 %	5 1.11%	2 0.44%	1 2.56%	9 23.07 %	-	-	2 5.13 %	1 2.44 %	7 17.07%	-	1 2.44 %	1 2.44 %	3 5.35%	10 17.85%	-	1 1.78 %	-
S.saprophyticus	36 8.00 %	43 9.55%	-	2 0.44%	-	1 2.56%	6 15.38 %	-	-	-	-	8 19.51%	-	-	-	-	7 12.5%	-	1 1.78 %	-
S.warneri	15 3.33 %	23 55.11%	-	1 0.22%	1 0.22%	-	4 10.25 %	-	-	-	-	5 12.20%	-	-	-	-	6 10.71%	-	1 1.78 %	-
S.hominis	4 0.88 %	29 6.44%	-	-	1 0.22%	-	3 7.69	-	-	-	-	2 4.88%	-	-	-	-	4 7.14%	-	-	-
S.simulans	7 1.55 %	19 4.22%	-	-	2 0.44%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.schleiferi	3 0.66 %	20 4.44%	-	-	1 0.22%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.hyicus	3 0.66 %	16 3.55%	-	1 0.22%	1 0.22%	-	-	-	-	-	-	-	-	-	-	-	3 5.35%	-	-	
S.capitis	6 1.33 %	11 2.4%	-	1 0.22%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S.lugdunensis	-	-	-	-	-	-	2 5.13 %	-	-	-	-	2 2.44%	-	-	-	-	-	-	-	-
Total	**143 (31.77%)**	**277(61.55%)**	**4(0.88%)**	**14(3.11%)**	**12(2.66%)**	**4(10.25%)**	**31 (79.48%)**	**-**	**1(2.56%)**	**3(7.69%)**	**4(9.75%)**	**32(78.04%)**	**-**	**2(7.31%)**	**3(2.43%)**	**9(16.07%)**	**44(78.57%)**	**-**	**3 (5.35%)**	**-**

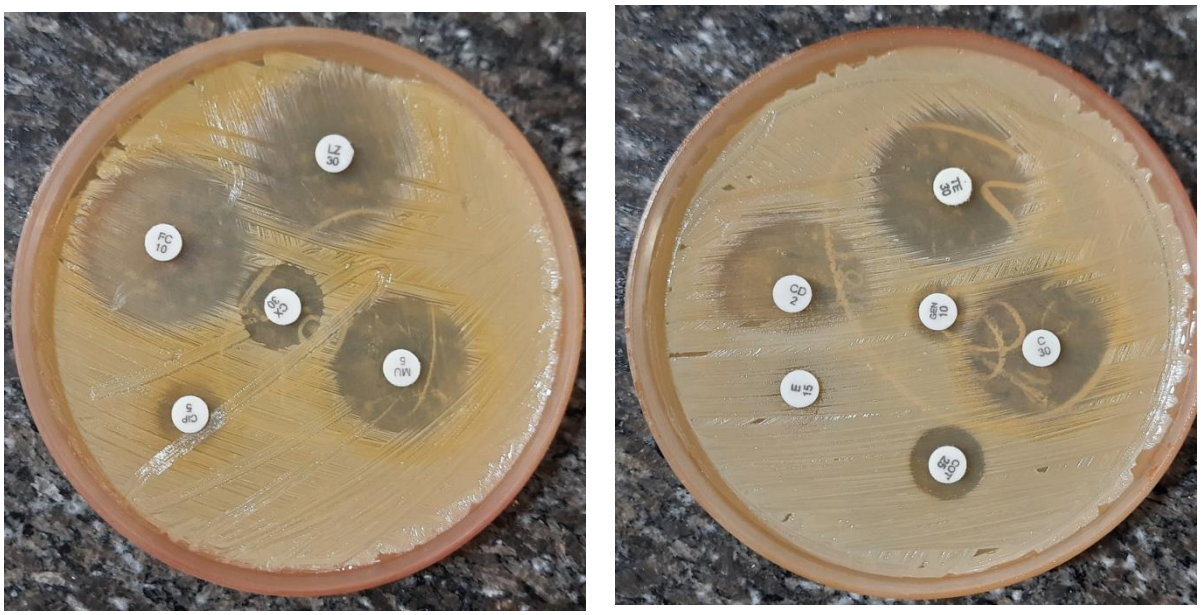


Fig 4.2 Antibiotic Susceptibility testing by Kirby Bauer Disc Diffusion method

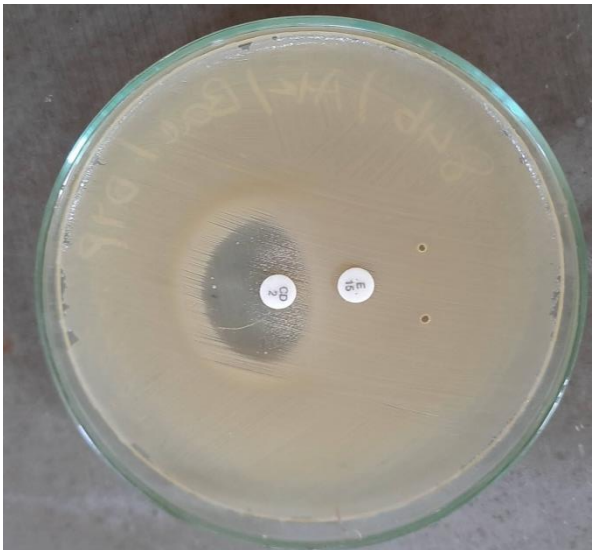


Fig 4.3 D-test (Erythromycin Induced Clindamycin Resistance)

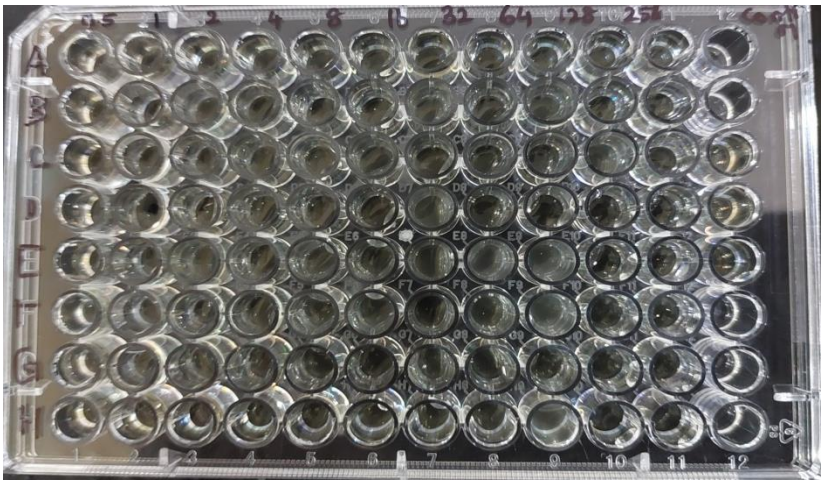


Fig 4.4 Micro broth dilution method of Minimum Inhibitory Concentration of Vancomycin

4.6 Discussion:-

S.aureus causes variety of infections and develops resistance among various types of antibiotics and limits the therapeutic options [10]. In the present study**,** we found more resistance to Tetracycline (59.71%) followed by others among the clinical isolates of *S.aureus*.

The prevalence rate of MRSA among the clinical isolates in the present study was 45.71% among the clinical isolates. Result of our study is in contrast with studies conducted by *Sweta* Shah et al 2021[11], from Mumbai showed 33.6% of MRSA among various clinical specimens, Kaur et al 2019[12] from Punjab who showed highest prevalence rate of MRSA (51.2%). Rate of MRSA is not uniform and it has more variation among the clinical isolates of S.*aureus.* Prevalence rate of MRSA varies from different parts of India ranging from 30-85% (Lowy et al)[13]. MRSA rate in our study is more or less similar to other countries, 55.9% Taiwan, 54.8% China, 41.9% Pakistan, 41% Japan, [13]. Factors responsible for variations of MRSA rates among the clinical samples from studies may be due to different geographical locations, sample size variations; type of study, specimen type's method used for analysis, antibiotic policies of hospital, infection control practices [14]. In present study, maximum of MRSA were isolated from pus or wound swabs (23.75%) followed by urine (20%) and sputum (17.5%) specimen. This may be because; *S.aureus* causes most of the skin infections, respiratory tract infections,

septicemia and urinary tract infections. This is comparable with studies conducted by Kulshrestha A et al 2017[15] and Pradeep Kumar et al 2021[16]

Vancomycin and Linezolid antibiotics should be used judiciously in MRSA cases and should be preserved for future use. Linezolid is a major antimicrobial agent targeted against *S.aureus* infection. Linezolid resistance has been increasing steadily since the first case identified [17]. Linezolid resistance in Europe is less than 2% whereas in India, Linezolid resistance has been reported ranging from 2-20% [18]. In present study the rate of Linezolid resistance was 2.57% among clinical specimens. The maximum number of Linezolid resistance was observed in pus swab/wound swab specimen (0.85%).

Wide spread use of Vancomycin in clinical practice leads to decrease in the susceptibility of Vancomycin. Disc diffusion test for Vancomycin susceptibility is not a reliable method as per CLSI guidelines 2020, hence the present study detection of vancomycin susceptibility by using broth dilution method (Minimum Inhibitory Concentration)[7]. In present study, we observed that 7.71% of *S.aureus* isolates from clinical specimens showed resistance to Vancomycin with MIC value of ≥ 16 µg/mL and 7.71% of *S.aureus* isolates showed intermediate resistance. Lower prevalence rates of VRSA were detected in other developing countries. However, they used the Kirby-Bauer disk diffusion method, which indicates that the actual prevalence rate is not accurate. Gohniem et al. 2014[19] showed higher resistance rate of VRSA and VISA (20.68% VISA and 20.68% VRSA) which indicates that resistance rates are increasing. However, Amr et al. 2017[20] showed lower incidence (8.8%) of VRSA among clinical isolates. This difference is probably due to the antibiotic policies in hospitals, geographical locations, use of antibiotic in other sectors without any prior knowledge about resistance etc.

Clindamycin is a drug of choice for the treatment of skin and soft tissue infections caused by MRSA and MSSA. In addition, clindamycin can be used for Penicillin allergic patients [21]. Overuse of this antibiotic leads to decrease in the efficacy of drug. In the present study, we showed 13.17% Clindamycin resistance among the clinical samples. This is comparable to other studies

conducted by Sebnem Ebru et al 2017[22] and Van et al 2011 [23] who showed prevalence rate of Clindamycin 6.2% and 11.1% among Clinical specimens. Maximum number of Clindamycin resistance was seen in wound swab (39.58%) followed by others. This might be due to the over use of Clindamycin in skin and soft tissue infection and transferable of resistance factor to other susceptible *S.aureus.*

Erythromycin resistance is mostly related with Clindamycin resistance. Detection of inducible Macrolid Lincosamide Streptrogram B (iMLSB) resistance phenotype in routine tests is difficult and isolates shows Clindamycin sensitive and Erythromycin resistance in laboratories in vitro [24]. This false identification of Clindamycin susceptibility in iMLSB isolates leads to treatment failure. In our study 67 (19.14%) isolates among the 350 isolates of *S.aureus* from clinical specimens showed iMLSB phenotype and this is comparable to other studies conducted by Addhikeri et al 2017[25], Mohapatra TM et al 2009 [26], Gade et al 2013 [27] found 11.48%, 18.2% and 24.3%. These wide variations may be due to the higher consumption of Lincosamide antibiotics and geographical variation of *S.aureus* clones circulation in particular region. Strains that are resistant to both Erythromycin and Clindamycin are generally termed as cMLSB (Constitutive Macrolid Lincosamide Streptogram B). The incidence of cMLSB phenotypes is varies from other studies. We detected 13.71% of cMLSB among the various clinical specimens. The Present study revealed that Inducible resistance of Macrolides was more (19.14%) compared to Constitutive resistance (13.71%). This is in contrast to a study conducted by Kishk et al 2020[9] who found Constitutive resistance is higher than the inducible resistance (38.6% and 13.6%). This discrepancy between other studies is due to the difference in bacterial susceptibility and method of analysis to detect resistance phenotypically.

Fusidic acid and Mupirocin are topical antibiotics used to treat Staphylococcal skin infections caused by MRSA strains [28]. As these antibiotics are increasingly used to treat skin infections, resistant strains of *S.aureus* emerge and spread. In the present study, we showed resistance rate of Fusidic acid and Mupirocin was 11.71% and 14%. Ebru Sebnem et al 2017[27] found the Fusidic acid resistance rate 12.4% among the clinical samples, which is

comparable to our study. Abdulgader et al 2020[29] found Mupirocin resistance 12% among clinical specimens of *S.aureus*. Maximum number of Fusidic acid and Mupirocin resistance was detected in Pus or Wound swab [Table] followed by other clinical specimens. Increasing resistance rate of Mupirocin might be the usage of Mupirocin and Fusidic acid as a topical agent more in skin infection and over the counter of antibiotics.

Resistance rate of Gentamicin among the clinical specimens of *S.aureus* showed 21.45% of resistance rate in present study it is lower than the study conducted by Dueran et al 2012[30] who found resistance rate 38.1% and higher than the study conducted by Calik et al 2015[31] who found the resistance rate of Gentamicin 6.4%. Co-trimoxazole showed 17.71% resistance rate in our study among the clinical specimens of *S.aureus* out of 350 isolates. Ozalkp and Baybek et al [32] found the rate of Co-trimoxazole resistance among the clinically isolated *S.aureus* was 29.8% and Aydin et al showed 15.8% [33]. Chloramphenicol and Ciprofloxacin showed resistance rate in present study was 32% and 26%. Aydin et al [33] showed rate of Ciprofloxacin resistance was 7.3%. Tetracycline resistance rate was found higher (40.28%) as compared to other antibiotics in present study. This might be due to overuse of tetracycline antibiotics in animals and birds for prophylaxis, treatment and growth promoters without any precautionary. Tetracycline resistant determinants from animal handler may be transferred to others. Apart from these, over and misuse of Tetracycline antibiotics in hospitals may be the reason.

Screening of nasal carriage of Staphylococci and its antibiotic resistance is a fundamental practice in nosocomial infection control practices [34]. Eradication of nasal colonization of Staphylococci will reduces the infection rates in patient. In present study, out of 92 S.*aureus* from health care workers, carriage rate of MRSA was 21.17%. Prevalence of MRSA among the healthcare workers is found to be variable among the different countries ranging from 0.4% in Sweden [35], 48.4% in Belgium [35]. In India, Nipa Singh et al 2018 [36] in Odhisa found 7.5% among HCWs. Gopala Krishanan et al [37] from Chennai who found 40% MRSA among HCWs. These variations of MRSA prevalence is due to the use of antibiotic disc for detection of MRSA, geographical locations, infection control practices in hospital, poor hygiene etc.

Mupirocin is an important antibiotic in eradication of MRSA from the nasal carriage and other body sites colonization. Increasing the Mupirocin resistance is major concern in public health [38]. In our study, 3.26% of Mupirocin resistance was detected among 92 *S.aureus* isolated from HCWs. 100% were sensitive to Linezolid antibiotic. None of the isolates detected Linezolid resistance among HCWs in our study. 4.34% of *S.aureus* isolates among HCWs detected Fusidic acid and Ciprofloxacin resistance in this study. 5.43% resistance was observed in Vancomycin and Gentamicin. In the present study, 8.69% of Tetracycline resistance followed by 7.60% of Co-trimoxazole resistance was found among HWs. We detected 23.91% *S.aureus* isolates resistance to Cefoxitin i.e., MRSA. Varying degree of antibiotic resistance towards different antibiotics among *S.aureus* of HCWs may be due to the spontaneous mutation in plasmid chromosome, high selective pressure of antibiotics in hospitals and constant exposure of HCWs to infected patients [39]. iMLSB resistance, cMLSB resistance of erythromycin was observed 9.78% and 13.04%. Constitutive resistance was more than the inducible resistance in this study. This indicates that Erythromycin induces resistance to Clindamycin.

Antibiotic resistance of *S.aureus* among patients visitors are categorized into in-patient visitors and out-patient visitors. Out-patient visitors showed more resistance towards Cefoxitin (29.26%) i.e. MRSA, Tetracycline (14.63%), Chloramphenicol (9.75%), Vancomycin (11.76%) and Mupirocin (4.87%) than In-patient visitors. None of isolates both In and Out-patient visitors detected any Linezolid resistance among *S.aureus*. In-patient visitors showed more resistance against Ciprofloxacin (5.88%), Co-trimoxazole (7.84%), Fusidic acid (3.92%) and Gentamicin (9.80%). Patient visitors can transfer antibiotic resistance bacteria from community setting to hospitals. This leads to problems in immunocompromised patients and patients having any underlying diseased condition. Screening of *S.aureus* in nasal carriage and its antibiotic profile among HCWs and In-Patient visitors is a fundamental hospital infection control practice.

MRSA is commonly resistant to beta-lactum antibiotics including Penicillin, Carbapenem, and Cephalosporin. The susceptibility of MRSA isolates in present study showed 5.65% Linezolid resistance among clinical specimens

and 100% sensitive among HCWs, and Patient visitors. 21.42% Vancomycin resistance was observed in In-patient visitors, followed by out-patient visitors (16.66%), HCWs (8%) and Clinical isolates (5%). Mupirocin is a topical antibiotic which can be used as alternative for MRSA isolates. In Present study, 16.55% Mupirocin resistance seen in out-patient visitors followed by HCWs (8%), In-patient visitors (7.14%) and clinical isolates (5.65%). Fusidic acid resistance was observed more in HCWs (16%) than other groups. Other antibiotics showed below 20% rate of resistance against MRSA isolates. This is comparable study conducted by Aila NAE et al 2017 [40].

Coagulase negative Staphylococcus (CoNS) is commensal flora of skin and mucosa which same the same ecological niche in anterior nares of human begins with *S.aureus* and other bacteria. CoNS can exchange the resistance genes by horizontal gene transfer method and CoNS have been identified as source of antibiotic resistance and reservoir to transfer antibiotic resistance across the entire family of Staphylococcaceae. In present study, 19.55% MRCoNS was detected among clinical isolates. Out of 88 MRCoNS, highest rate of MRCoNS was observed in *S.epidermidis* (50%) followed by *S.haemolyticus* (32.95%). *S.hyicus* did not detect any Methicillin resistance. The prevalence of MRCoNS is variable from other studies ranging from studies by Sateesh K et al 2017[41] from Mangalore who found 37%, Abinaya et al 2019 from Chennai [42] who found 26% MRCoNS among Clinical specimens. Higher incidence of MRCoNS is a common problem nowadays. Methicillin resistant in Staphylococci will make it necessary to use more glycopeptides antibiotics especially Vancomycin and Teicoplanin for the serious Staphylococcal infection. In most of the studies, *S.epidermidis* is frequently isolated bacteria among CoNS in the clinical isolates than others hence, the rate of Methicillin resistance is more in *S.epidermidis* among CoNS. 5 (1.11%) isolates of *S.epidermidis* showed resistance to Linezolid among the clinical isolates in present studies. Other clinically isolated CoNS showed 100% sensitivity. Vancomycin resistance detected only 2.66%. Among these, 33.33% of Vancomycin resistance was seen in *S.epidemidis*, 16.66% in *S.haemolyticus* and *S.simulans*, 8.33% in *S.warneri, S.hominis, S.schleiferi* and *S.hyicus.* 8.22% of CoNS detected resistance to Mupirocin among these, 43.24% of resistance seen

in *S.haemolyticus*, 32.43% in *S.epidermidis*, 16.21% in *S.saprophyticus*, 5.40% in *S.capitis* and 2.70% in *S.warneri*. 31.77% showed resistance to Tetracycline, 12.4% in Gentamicin, 18.44% in Co-trimoxazole, 12% in Ciprofloxacin15.11% in Fusidic acid and 15.33% in Chloramphenicol were observed. Development of antibiotic resistance among CoNS may be due to the selection of pre-existent resistant strain under high antibiotic pressure or from exogenous acquisition. 60.44% CoNS among the clinical specimens showed sensitivity to both Erythromycin and Clindamycin, 8.22% isolates were iMLSB, and 14.22% were cMLSB resistance. Methicillin resistance is a multidrug resistance and showed resistance to both beta-lactum and non-beta-lactum antibiotics. Among 88 (19.55%) MRCoNS in present study from the clinical isolates, 25% MRCoNS showed resistance to Linezolid, 13.63% in Chloramphenicol, 11.63% in Ciprofloxacin, 10.22% in Co-trimoxazole, Vancomycin, 9.09% in Fusidic acid and Tetracycline, 4.54% in Erythromycin and Clindamycin, 2.27% in Gentamicin.

Nasal colonization of CoNS is linked to an increased risk of nosocomial infection. In addition, transfer of antibiotic resistance genes among the Staphylococceae family this leads to development of unnecessary antimicrobial resistance. HCWs may carry opportunistic CoNS in their anterior nares they can acts as potential vector to transfer these opportunistic pathogen to patients who are in underlying condition and also transfer the antibiotic resistance in hospitalized pathogens [34]. This may become severe with high rate of morbidity and mortality. In present study, we detected 100% sensitivity to Cefoxitin (i.e., MSSA), Vancomycin, Linezolid and Mupirocin antibiotics among the isolated CoNS of HCWs. 21.42% resistance was detected in Tetracycline, 16.07% resistance in Chloramphenicol, 8.92% in Fusidic acid, 7.14% in Gentamicin and 5.35% in ciprofloxacin. In D-test, 7.14% of isolates detected iMLSB, 12.5% in cMLSB and 60.71% isolates were sensitive to Erythromycin and Clindamycin from CoNS among the HCWs.

Screening of CoNS in community settings is a ground level Survillanence to monitor the AMR (Antimicrobial Resistance). AMR is a risk factor as a opportunistic pathogens in immunocompromised patients when such bacteria enter into hospitals. AMR surveillance outside the hospital sector is

needed to reduce the unnecessary complications. 7.5% isolates showed Methicillin resistance i.e., MR-CoNS among patient visitors. Vancomycin resistance was detected only in 7.5% and no resistance was detected in Linezolid. 3.75 % isolates detected resistance to Mupirocin, 8.75% to Fusidic acid, 6.25% to Gentamicin, 8.25% to Co-trimoxazole, 12.5% to Chloramphenicol, 7.5% in Ciprofloxacin, and 26.25% in tetracycline. 8.74% showed iMLSB and cMLSB resistance in D-test 57.5% isolates showed sensitivity to Erythromycin and Clindamycin among patient visitors of CoNS in present study. Among 7.5% MR-CoNS, 50% were resistant in Mupirocin and Vancomycin among patient visitors of CoNS.

4.7 Conclusions:-

In the present study, we concluded that clinical isolate showed maximum resistance to antibiotics than HCWs and Patient visitors. However, Screening of uncommon CoNS among clinical specimens and nasal cavity of HCWs, Patients visitors and its antibiotic resistance has raised major concerns. In present study, we did not detect Linezolid resistance among Nasal colonization of HCWs and Patient visitors. Vancomycin resistance was detected more in *S.aureus* than CoNS and indicates that CoNS have less exposure to Vancomycin antibiotics. Screening of nasal colonization is an important nosocomial infection control practice and it will help to prevent the spread of unwanted nosocomial infection and its antimicrobial resistance in hospitals settings.

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Prevalence of Methicillin Resistant *Staphylococcus aureus* and its Associated SCCmec Types among Healthcare workers and Patient Visitors from Western Maharashtra, India

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Prevalence of Methicillin Resistant Staphylococcus and its associated Staphylococcus cassette chromosome mec (Sccmec) among Clinical isolates, Nasal carriage of Health care workers and Patient visitors

Chapter-5

Prevalence of *mecA* gene and its associated Staphylococcus Cassette Chromosome mec (SCCmec) among clinical isolates, Nasal carriage of healthcare workers and Patient visitors

5.1 Introduction:-

Methicillin is a semi-synthetic antibiotic, introduced clinical use for the treatment of Penicillin resistant bacterial infection in 1960. Methicillin showed resistance to *Staphylococcus aureus* in 1961 and called as MRSA (Methicillin Resistance *S.aureus*)[1]. Methicillin resistance staphylococcus is major pathogens in hospital as well as community setting. It shows resistance to all β-lactum antibiotics except newer generations of Cephalosporin's (Ceftobiprole and Ceftaroline)[2,3]. Penicillin Binding Protein (PBP), is a membrane associated macromolecular protein which plays an important function during cell wall synthesis process. Antibiotic inactivation is caused by the modification of PBP to PBP2a which leads to low affinity of binding β-lactum antibiotics to the bacterial cell wall[4]. Methicillin resistance is influenced by *mecA* gene (2.1 kb) which is found on the Staphylococcus Cassette Chromosome mec (SCCmec) and incorporated into chromosome of Methicillin resistant Staphylococcus.

SCCmec is a mobile genetic element, situated near the origin of replication (orf) of the Staphylococcus chromosome and it is incorporated at *attB* insertion site. SCCmec carries mec gene complex and the genes which is responsible to control its expression namely[5],

1. mecR1- it encodes the signal transduce protein
2. mecI- encodes the repressor protein and acts an carrier protein to exchange the genetic constituents between Staphylococcus species[5].

SCCmec complex contains three basic elements namely, 1. Ccr complex plays a major role of accurate integration and excision of inserting multidrug resistance and heavy metal resistance genes, 2. mec gene complex and 3. Joining (J) region

Based on the nature of the mec gene and ccr complexes, SCCmec are classified into 13 types (types I through XIII). SCCmec types I to V are widely distributed globally. [6]. Type I, IV, V, VI responsible for β-lactum antibiotic resistance [7]. Type II and III contains multi drug resistance properties and it is carried on integrated plasmids pUB110, pI258, pT181, Transposons 554 (Tn554). Plasmid pUB110 codes for Kanamycin, Tobramycin and

Bleomycin resistance genes. Plasmid pI258 codes for Penicillin and heavy metal resistance, plasmid pT181 codes Tetracycline resistance and Transposon Tn554 encodes (carry *ermA*) for Macrolid, Lincosamide and Streptogramin (MLS) resistance genes[7].

CoNS act as a reservoir of SCCmec based on the evidence that transfers the SCCmec elements by transfer of gene from CoNS to *S.aureus* via conjugation process. However, the origin of SCCmec is still unknown. SCCmec I, II and III are widely distributed and observed among *S.epidermidis* species[8].

Based on the epidemiological risk factors, MRSA is classified into 2 types namely, 1. Hospital acquired MRSA (HA-MRSA) and 2. Community acquired MRSA (CA-MRSA). CA-MRSA are sensitive to non-β-lactam antibiotics, whereas, HA-MRSA are resistant to most of the β-lactum antibiotics. CA-MRSA are associated with skin and soft tissue infections, necrotizing pneumonia and severe sepsis. HA-MRSA is frequently associated with pneumonia, blood stream infections and invasive infections. CA-MRSA varies from HA-MRSA by distinct genotypes, carrying *pvl* gene and other enterotoxin genes. HA-MRSA has large SCCmec I, II, and III genes, which are responsible for resistance to other non beta-lactam antibiotics. CA-MRSA carries SCCmec IV and V which are smaller in size and not having any other resistance genes as previously described [10]. In this chapter, we study the dissemination of *mecA* gene and its associated SCCmec types among clinical isolates and nasal colonization of Staphylococcus species and evaluate the SCCmec typing as a tool to differentiate CA-MRSA and HA-MRSA.

5.2 Methodology:-

5.3 DNA Extraction:-

Boiling lysis Method [9]:-

A pure culture of Staphylococci isolates (4-5 discrete colonies) was transferred into 1.5 mL of Brain heart infusion broth (Hi-media, Mumbai, India) and tubes were incubated at 37 ° c for 24 hours. 500µl of bacterial suspension was transferred after 2houres of incubation to a 2mL micro centrifuge tube containing 500µl of nuclease free water.

The bacterial suspension in the tube was heated at 100 ° c for 10 minutes using a thermal mixer (Applied Biosystem), followed by centrifuged at 6000 rpm for 10 minutes. The obtained

supernatant served as a template DNA. The tube contain DNA was stored in a deep freezer (-20°C) for future use..

5.4 Detection of *mecA*, *femA* and *pvl* gene:

mecA gene was detected by conventional multiplex PCR method (Bio-Rad CFX 100). The following PCR cycling conditions and primers (Primers purchased from Syngene Pvt Ltd) were used in this study

Gene	**Sequence**	**Size (bp)**	**References**
mecA	F: 5'-TGCTATCCACC CTCAAACAGG-3' R: 5'-AACGTTGTAAC CACCCCAAGA-3'	286	11
femA	F: 5' – AAAAAAGCAC ATAACAAGCG – 3' R: 5' – GATAAAGAAGA AACCAGCAG – 3'	132	
pvl	F:5'–ATCATTAGGTAAAAT GTCTGGACATGATCCA–3' R: 5'– GCATCAASTGTATT GGATAGCAAAAGC–3'	441	

Table 5.1 Primers for *mecA*, *femA* and *pvl gene*

Table 5.2 Cycling Condition of *mecA*, *femA* and *pvl gene*

Gene	**MecA /fem A/pvl**
Initial denaturation	94°C 120 S
Denaturation	94°C 45 S
Annealing	55°C 30 S
Extension	72°C 45 S
No. of Cycles	35
Final Extension	72°C 120 S

5.5 Detection of SCC Mec typing:-

SCC mec types (I to V) were detected by multiplex PCR[11]

Table 5.3 Primers for SCCmec types (I to V)

Target	Sequence	Size
SCCmec type I	5'GCTTTAAAGAGTGTCGTTACAGG 3' 3' GTTCTCTCATAGTATGACGTCC 5'	613 bp
SCCmec type II	5' CGTTGAAGATGATGAAGCG 3' 3' CGAAATCAATGGTTAATGGACC 5'	389 bp
SCCmec type III	5' CCATATTGTGTACGATGCG 3' 3' CCTTAGTTGTCGTAACAGATCG 5'	280 bp
SCCmec type IVa	5'GCCTTATTCGAAGAAACCG 3' 3'CTACTCTTCTGAAAAGCGTCG 5'	776 bp
SCCmec type IVb	5' TCTGGAATTACTTCAGCTGC 3' 3' AAACAATATTGCTCTCCCTC 5'	493 bp
SCCmec type IVc	5' ACAATATTTGTATTATCGGAGAGC 3' 3' TTGGTATGAGGTATTGCTGG 5'	200 bp
SCCmec type IVd	5'CTCAAAATACGGACCCCAATACA 3' 3' TGCTCCAGTAATTGCTAAAG 5'	881 bp
SCCmec type V	5' GAACATTGTTACTTAAATGAGCG 3' 3' TGAAAGTTGTACCCTTGACACC 5'	325 bp

Table 5.4 Cycling Condition for SCCmec (types Ito V)

Steps	Temperature and Time	Cycle
Initial Denaturation	94ºC for 45 s	
Denaturation	94ºC for 45 s	10 Cycles
Annealing	65ºC for 45 s	
Extension	72ºC for 90 s	
Denaturation	94ºC for 45 s	25 cycles
Annealing	55ºC for 45 s	
Extension	72ºC for 120 s	
Final Extension	72ºC for 10 mins	
Hold	4 ºC	

5.6 Results:-

Total of 200 nasal swabs from Healthcare workers and 400 nasal swabs from Patient visitors were collected. Of these, 184 *S.aureus* and 134 CoNS were isolated.

200 swabs were collected from in-patient visitors, particularly those who visited IPDs, and 200 swabs were collected from outpatient visitors who came with patients. Distribution of Staphylococci is shown in table 5.5

Table 5.5 Distribution of *S.aureus*

	S.aureus	***CONS***
In-patient visitors (n=200)	51 (27.71%)	39 (29.10%)
Out-patient visitors (n=200)	41 (22.28%)	41(30.59%)
Health care workers (n=200)	92 (50%)	56(41.79%)
Clinical Specimens (n=1800)	350 (43.75%)	450 (56.25%)
Total	534	586

Methicillin resistant *S.aureus* (MRSA):-

211 (39.51%) isolates detected Methicillin Resistant *S.aureus* (MRSA). Among these, Clinical isolates showed maximum MRSA prevalence i.e., 160 isolates (45.71%) followed by others [Table 6]. *femA* gene was detected in all MRSA isolates.

Table 5.6 Distribution of MRSA

	MRSA	**MSSA**
Out -Patient Visitors	12(29.26%)	29 (70.37%)
In- Patient Visitors	14 (27.45%)	37 (72.54%)
Health Care Workers	25 (27.17%)	67 (72.82%)
Clinical specimens	160 (45.71%)	190 (54.28%)
Total	211 (39.51%)	323 (60.48%)

Panton-valentine leukocidin (PVL) gene was tested against the MRSA isolates. Out of 211 (39.51%) MRSA isolates, p*vl* gene detected 98 (46.44%) *S.aureus* isolates. Of these, Clinical isolates showed more prevalence rate (35.07%) followed by Healthcare workers (5.21%), Out-Patient visitors (3.31%) and In-patient visitors (2.84%). [Table 5.7] 53.55% isolated MRSA isolates did not detected *pvl* gene.

Table 5.7 Distribution of *Pvl* gene against MRSA isolates

	***Pvl* gene detected**	***Pvl* gene Not detected**
Out -Patient Visitors	7(3.31%)	5 (2.36%)
In- Patient Visitors	6 (2.84%)	8 (3.79%)
Health Care Workers	11 (5.21%)	14 (6.63%)
Clinical specimens	74 (35.07%)	86 (40.75%)
Total	98 (46.44%)	113 (53.55%)

Diversity of Staphylococcal Cassette Chromosome mec (SCCmec) elements among MRSA:

SCCmec was found in 73 MRSA isolates (SCCmec Types I to V), SCCmec type III was most common (20.76 %) in present study followed by SCCmec types IV, II, I, and V. Three isolates could not be typed.. [Table 5.8]

Table 5.8 Diversity of Staphylococcal Cassette Chromosome mec (SCCmec) elements among MRSA:

	Out-patient Visitors (n=12)		In-Patient Visitors (n=14)		Heath care workers (n=25)		Clinical isolates (n=160)		Total
	***Pvl* negative isolates**	***Pvl* positive isolates**	***Pvl* negative isolates**	***Pvl* positive isolates**	***Pvl* negative isolates**	***Pvl* positive isolates**	***Pvl* negative isolates**	***Pvl* positive isolates**	
SCCmec type I	3 (25%)	-	3(21.42%)	-	5(20%)	-	24(15%)	-	34
SCCmec type II	-	-	2(14.28%)	-	3(12%)	-	19(11.87%)	-	23
SCCmec type III	2(16.66%)	-	1(7.14%)	-	4(16%)	-	28(17.5%)	-	31
SCCmec type IVa	-	2(16.66%)	-	2(14.28%)	-	-	-	12(7.5%)	16
SCCmec type IVb	-	-	-	1(7.14%)	-	3(12%)	1(0.62%)	11(6.87%)	16
SCCmec type IVc	-	1(8.33%)	-	-	-	1(4%)	-	3(1.87%)	5
SCCmec type IVd	-	-	-	-	-	1(4%)	-	6(3.75%)	7
SCCmec type V	-	3(25%)	-	2(14.28%)	-	4(16%)	-	26(16.25%)	35
SCCmec type V+ I	-	1(8.33%)	-	-	1(4%)	-	2(1.25%)	4(2.5%)	8
SCCmec type III+ II	-	-	1(7.14%)	-	-	-	1(0.62%)	3(1.87%)	5
SCCmec type III+ IV	-	-	-	-	**1(4%)**	**1(4%)**	**3(1.87%)**	2(1.25%)	**7**
Non typeable	-	-	-	-		**1(4%)**	**3(1.87%)**	**5(3.12%)**	**9**
Total	5 (41.66%)	7 (58.33%)	6 (57.14%)	6 (42.83%)	14 (56%)	11 (44%)	**86 (53.75%)**	**74 (46.25%)**	211

Total 450 (56.25%) CoNS were isolated from clinical specimens. Total of 600 anterior nasal swabs were collected from Healthcare workers and Patient visitors. Among t[56.25%]%) were CONS from Healthcare Workers, 22.67% of CoNS from In-Patient Visitors and 23.83% of CoNS from Out-Patient Visitors [Chapter 3, Table 3.4].

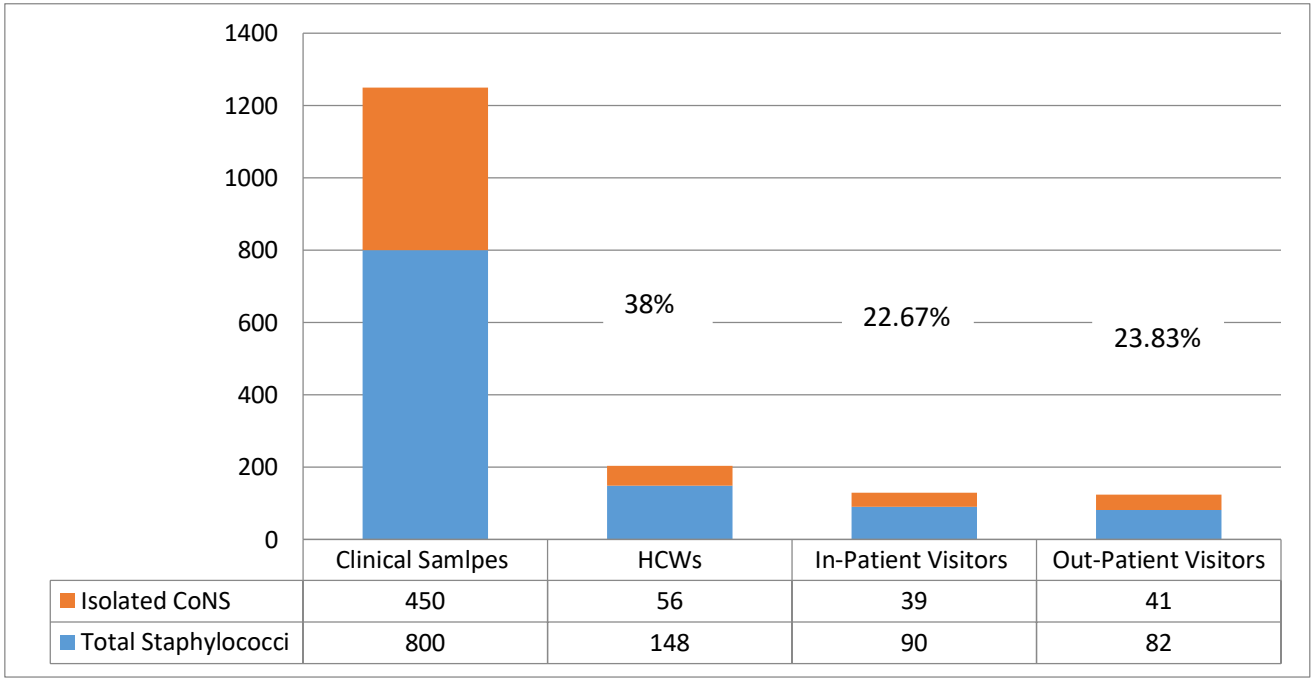


Fig 5.4 Distribution of Coagulase negative Staphylococci (CoNS)

Table 5.9 Distribution of MRCoNS:-

	MRCoNS	MSCoNS
Clinical Specimens (n=450)	88 (19.55%)	362 (80.44%)
HCWs (n=56)	-	56 (100%)
In-Patient Visitors (n=90)	3 (3.33%)	87 (96.66%)
Out-Patient Visitors (n=92)	3 (3.26%)	89 (96.73%)

19.55% MRCoNS was shown among clinical specimens followed by 3.33% MRCoNS was isolated among In-Patient Visitors and 3.26% MRCoNS from Out-Patient Visitors. CoNS isolated from HCWs were shown sensitive to Cefoxitin antibiotic i.e., Methicillin Sensitive CoNS.

Table 5.10 Distribution of *Pvl* gene against isolated MRCoNS

	***pvl* detected MRCoNS**	***pvl* not detected MRCoNS**
Clinical Specimens (n=88)	14(15.90%)	74 (84.10%)
In-Patient Visitors (n=3)	3 (100%)	--
Out-Patient Visitors (n=3)	1 (33.33%)	2 (66.66%)

Table 5.11 Distribution of *pvl* positive MRCoNS species

	S.epidermidis	***S.haemolyticus***	***S.saprophyticus***
Clinical Isolates	6 (42.86%)	5 (35.71%)	3 (21.42%)
HCWs	-	-	-
In-patient Visitors	3 (100%)	-	-
Out-Patient visitors	1 (33.3%)	-	-
Total	10	5	4

Table 5.12 Diversity of Staphylococcal Cassette Chromosome mec (SCCmec) elements among MRCoNS:

	Out-patient Visitors (n=3)		In-Patient Visitors (n=3)		Clinical isolates (n=88)		Total
	***Pvl* negative isolates**	***Pvl* positive isolates**	***Pvl* negative isolates**	***Pvl* positive isolates**	***Pvl* negative isolates**	***Pvl* positive solates**	
SCCmec type I	-	-	-	-	3 3.40%	1 1.13%	4
SCCmec type II	1 33.33%	-	1 33.33%	-	9 10.22%	1 1.13%	12
SCCmec type III	1 33.33%	-	-	-	11 12.5%	2 2.27%	14
SCCmec type IVa	-	-	-	1 1.33%	10 11.36%	0	11
SCCmec type IVb	-	-	-	-	4 4.54%	1 1.13%	5
SCCmec type IVc	-	-	-	-	7 7.95%	-	7
SCCmec type IVd	-	-	-	-	7 7.95%	-	7
SCCmec type V	-	1 33.33%	1 33.33%	-	11 12.5%	2 2.27%	15
SCCmec type III+I	-	-	-	-	2 2.27%	1 1.13%	3
SCCmec type IV + V	-	-	-	-	1 1.13%	-	1
SCCmec type III+ V	-	-	-	-	1 1.13%	1 1.13%	2
Non typeable	-	-	-	-	8 9.09%	5 5.68%	**13**
Total	2	1	2	1	74	14	94

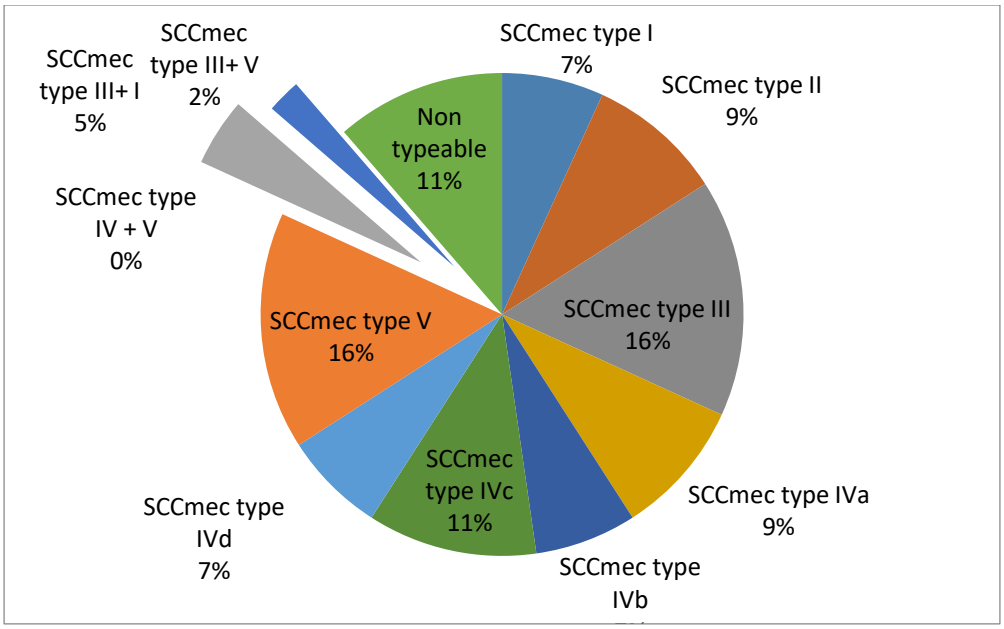


Fig 5.5 Distribution of SCCmec types from *S.epidermidis* among clinical isolates

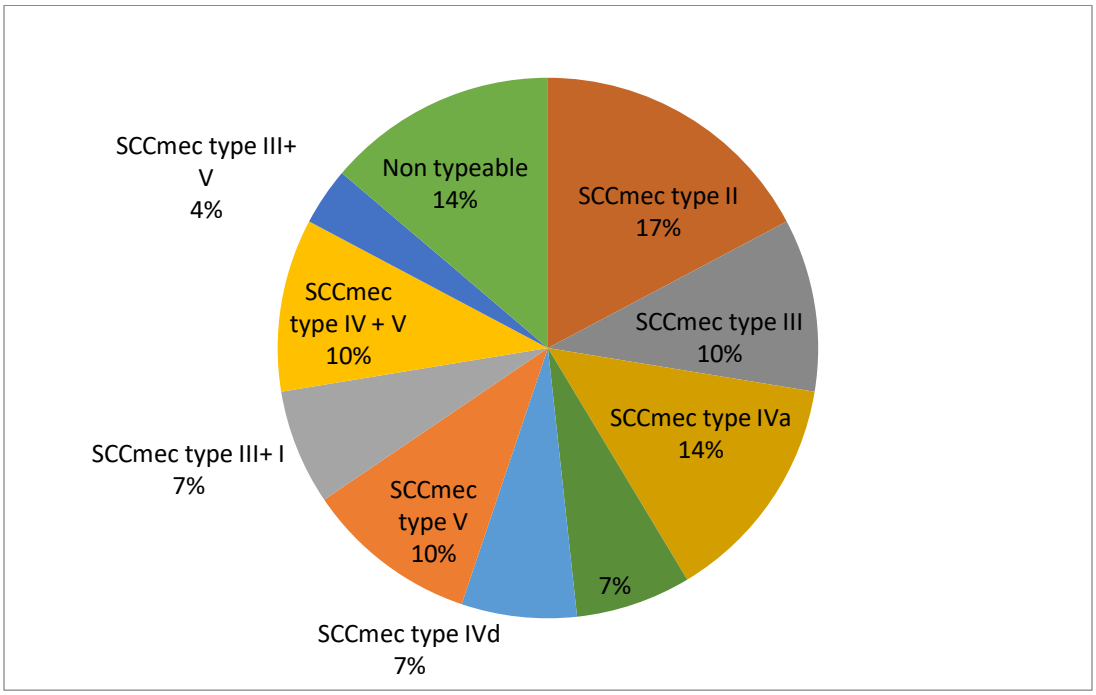


Fig 5.6 Distribution of SCCmec types from *S.saprophyticus* among clinical isolates

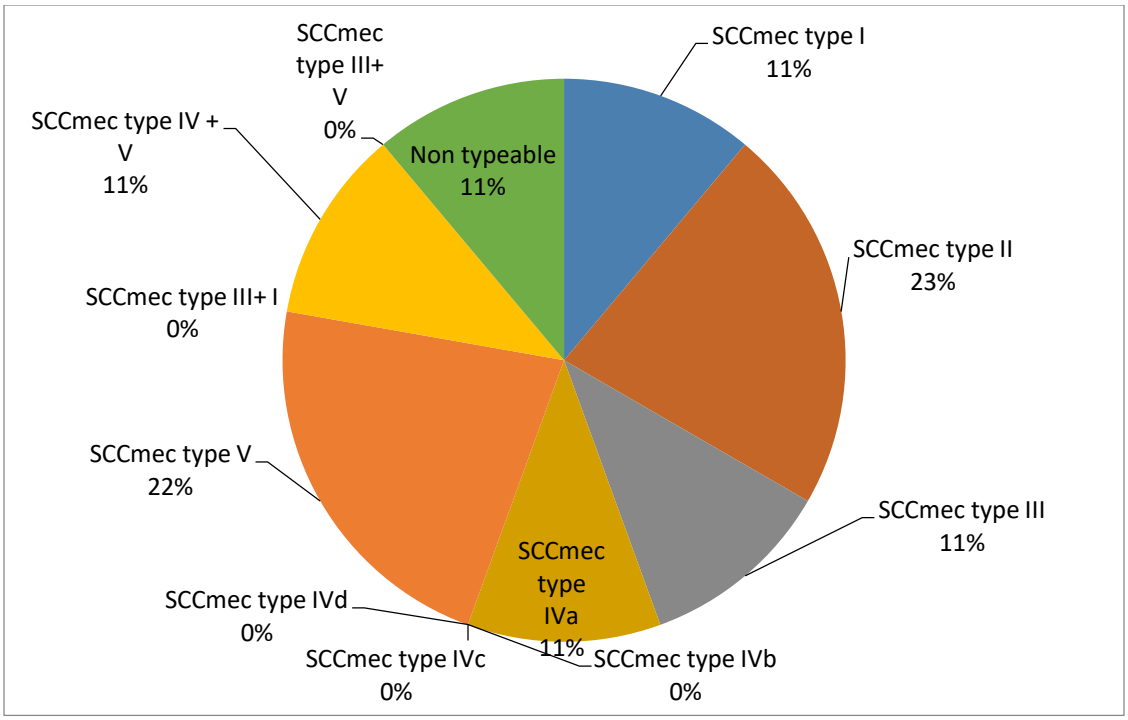


Fig 5.7 Distribution of SCCmec types from *S.haemolyticus* among clinical isolates

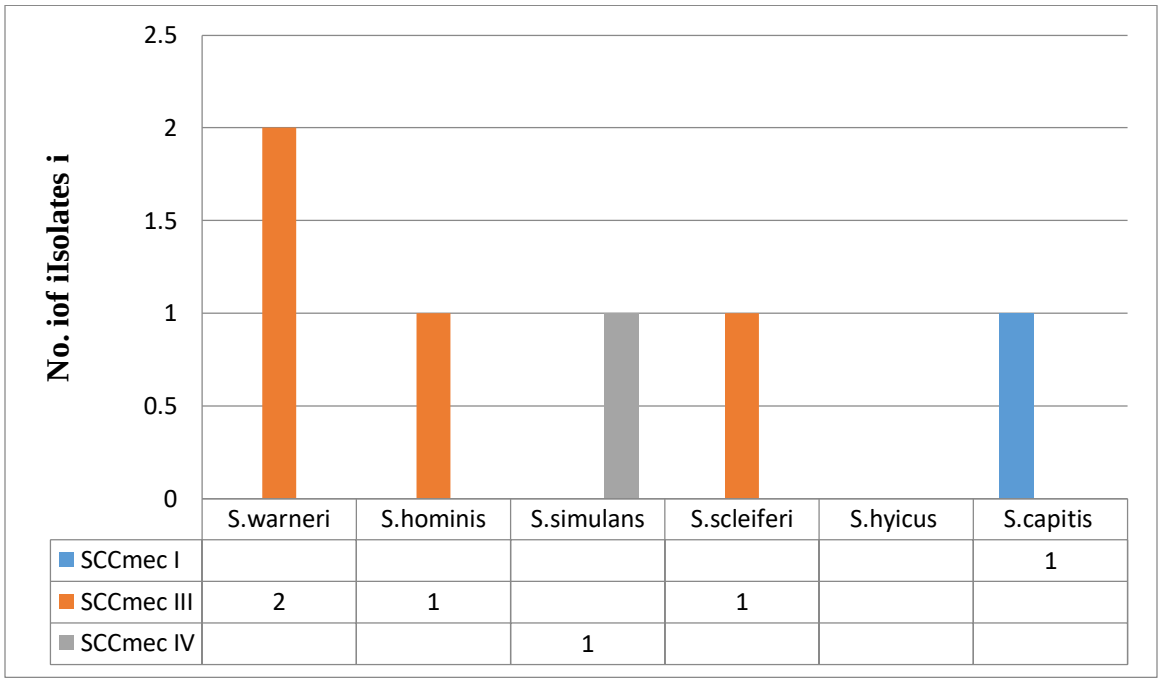


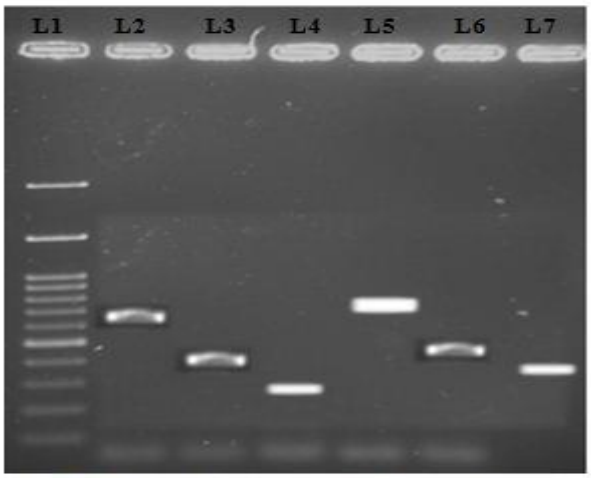
Fig 5.5 Distribution of SCCmec types of other isolated CoNS among clinical isolates

Sccmec type III was more detected than other SCCmec types [Fig 5.5] .

Table 5.13 Distribution of SCCmec types among nasal carriage of Patient visitors

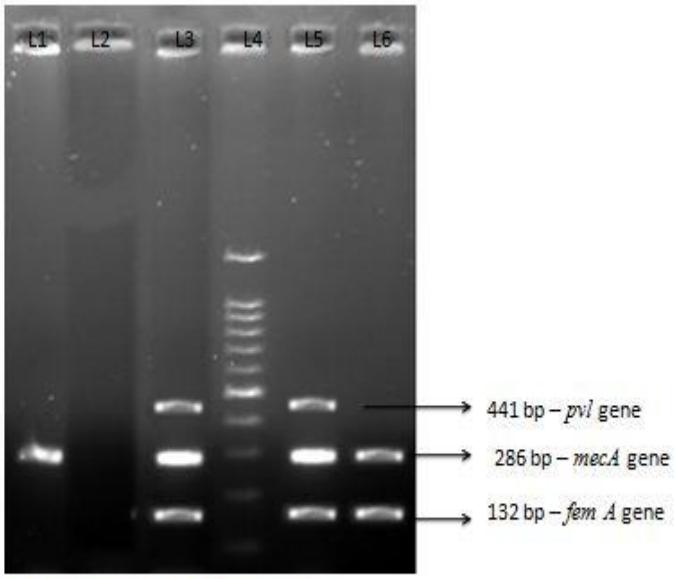
	S.epidermidis	
	Out-Patient Visitors	**In-Patient Visitors**
SCCmec I	-	1
SCCmec II	1	1
SCCmec III	1	-
SCCmec V	1	-

Table 5.13 shows the SCCmec types distribution among patient visitors nasal colonization, 6 isolates were MRCoNS in Nasal carriage of patient visitors [Table 5.9]. Among these, 5 isolates were *S.epidermidis* and one isolate was *S.haemolyticus* which was not typeable in current study which may be other SCCmec types which are common in other Indian studies



L1- Molecular marker(100 bp ladder)
L2- SCCmec type I (613 bp)
L3- SCCmec type II (389 bp)
L4-SCCmec type III (280 bp)
L5- SCCmec type Iva (776 bp)
L6- SCCmec type IV b (493 bp)
L7- SCCmec type V (325 bp)

Fig 5.6 Agarose gel electrophoresis of SCCmec types



L4- Molecular marker (100 bp Ladder)
L3- 441 bp (*pvl*), 286 bp (*mecA*), 132 bp (*femA*)
L6- 286 bp *(pvl)*, 132 bp (*mecA*)

Fig 5.7 Agarose gel electrophoresis of *mecA, femA and pvl* genes

Amplified PCR mixtures were separated on a 1.5% Agarose gel stained with ethidium bromide (0.5 µg/mL) along with a 100 bp DNA ladder (Hi-Media, Mumbai, India) and electrophorized Agarose gel was photographed using a e- gel imager (Applied Biosystem)

5.7 Discussion:-

Methicillin Resistant Staphylococcus (MRS) poses a significant threat to human health. Understanding the disease progression of MRS and its virulence mechanisms are challenging problem in modern world. Understanding the prevalence of virulence factors and genetic markers will be useful for the control of spread of antibiotic resistant determinants among the bacteria[6]. In present study, the *mecA* gene which is responsible for Methicillin resistance, rate of Methicillin resistance among clinical isolates of *S.aureus* was found 45.17% followed by Out-Patient Visitors (29.26%), In-patient Visitors (27.45%) and Health care workers (27.17%). The prevalence of MRSA is varying globally as previously described chapter 4. Identification of *mecA gene* by PCR is a gold standard identification of MRSA compared to other methods. In all *mecA* gene positive isolates in current study *femA* (Factor Essential for Methicillin Resistance) gene was positive. *fem* gene code for protein that influence the level of Methicillin resistance and also *fem* gene present in *Staphylococcus* chromosome are considered as indicator for MRSA[12].

MRSA can be classified into two types based on epidemiological risk factors namely Community Acquired MRSA (CA-MRSA) and Hospital Acquired MRSA (HA-MRSA). CA-MRSA differs from HA-MRSA. CA-MRSA carries *pvl* gene (Panton Valentine Leukocidin), is a cytolysin toxin that causes membrane damage to host immune cells and major virulence factor present in *S.aureus*[13]. *pvl* positive strains are commonly seen in Skin and soft tissue infections, urinary tract infections, necrotizing pneumonia[7]. CA-MRSA also carries SCCmec type 1V and V and usually sensitive to β-lactam antibiotics [7]. In Present study, 35.07% *pvl* gene genes were detected among the clinical isolates of *S.aureus* followed by HCWs (5.21%), Out-patient visitors (3.31%) and In-patient Visitors (2.84%). *pvl* gene detection from other studies shows wide variation, Hu et al 2015[14], showed 10.9% isolates detected *pvl* gene among the skin and soft infections isolates of MRSA. Dekker et al 2016 reported 75% of *pvl* gene detected among invasive disease MRSA isolates [15]. Nikou Bahramin et al 2019 found 17.2% of pvl detected among clinical specimens [16]. The current findings of *pvl* detected among HCWs are agreement with the study conducted by Enas

Mamdouh Hefzy et al 2016 who found prevalence rate of *pvl* detected among HCWs are 2.3%[17]. Detection of pvl gene is not a confirmatory marker to detect CA-MRSA [17].

Conformation of CA-MRSA was done by using SCCmec typing in current study. 46.25% isolates were found *pvl* positive among these, SCCmec type V was found more (16.25%) followed by SCCmec IVa (7.5%), SCCmec IVb (6.87%), SCCmec IVd (3.75%), SCCmec IVc (1.87%). However, other study showed SCCmec IV was predominant , Buntaran et al from Indonesia detected SCCmec V was more prominent among clinical isolates of MRSA[18]. Aqghar et al did not detect any SCCmec IV in his study [19]. This might be due to variation in type of sample used in the study, type of study conducted, geographical distribution of Sccmec types and geographical location. Five isolates (3.12%) were not typeable in current study, it may be having other SCCmec types which are common in Indian isolates. 4 (2.5%) isolates carried SCCmec I+V, 3 isolates (1.87) carried SCCmec III+ IV and 2 isolates (1.25%) carried SCCmec III+1V.

HA-MRSA was confirmed by using SCCmec typing, SCCmec I, SCCmec II and SCCmec III which are common in HA-MRSA. Most of the HA-MRSA did not carriy any *pvl* gene. In present study, 53.75% isolates of MRSA among clinical isolates did not detect any *pvl* gene. Among 53.75% *pvl* not detected isolates, SCCmec III was found more prevalence (17.5%) followed by SCCmec I (15%) and SCCmec II (11.87%). It is comparable to a study conducted by Namvar et al 2014[20] who found SCCmec III was predominant among the MRSA isolates of burn patients. Montazeri and Japoni et al found more SCCmec III in HA-MRSA isolates of various clinical specimens [21]. SCCmec III was predominant type in Asian countries; same was reflected in current study. One isolate (1.25%) carried SCCmec type IVb which is not common in HA-MRSA. It may be transfer from CA-MRSA to HA-MRSA by horizontal gene transfer method; size of SCCmec IV is very small and plays an important role in mobility and easily exposure to MRSA in the community as well hospital. 3 isolates (1.87%) are not typeable, 2 isolates (1.25%) carried SCCmec I+V, 3 isolates carried (1.87%) SCCmec III+ IV, one isolate (0.62%) carried SCCmec II+III.

In current study, 27.17% isolates carried *mecA* gene among the nasal colonization of Healthcare workers. Among 27.17% MRSA isolates of HCWs, 6.63% not detected any *pvl* gene, 5.21% isolates detected *pvl* gene against MRSA isolates of HCWs. Out of 6.63% *pvl* negative isolates, 5 (25%) carried SCCmec I followed by SCCmec III (16%) and SCCmec II (12%). 2 isolates of *pvl* negative MRSA strains among HCWs, detected Co-existence of multiple SCCmec, i.e., 1 isolate (4%) carried SCCmec I+V, 1 isolate carried SCCmec III + IV. Existence of multiple SCCmec types among individual isolates of MRSA and MRCONS have been reported in India but it occurs rare finding in MRSA isolates [22]. Chanchaithong et al, 2014 who found multiple SCCmec types among Coagulase Positive Staphylococcus of animal and animal handlers [23]. Out of 11 (5.21%) *pvl* detected MRSA isolates among HCWs, SCCmec type IV found more (5 isolates) followed by other SCCmec (16%). Among 9 isolates of SCCmec IV 3 isolates carried SCCmec Iva, one isolate carried SCCmec IVc sub types and one isolate carried SCCmec IVd in current study. One isolate are was not typeable and I isolate carried SCC mec III+IV

In current study, 12 isolates (29.26%) from Out-Patient visitors detected *mecA* gene. Among these, 7 (3.31%) isolates detected *pvl* and 5 (2.36%) isolates not detected *pvl* gene. Out of 7 (3.31%) pvl detected MRSA isolates from Out-Patient visitors, 2 isolates(16.66%) detected SCCmec Iva followed by 3 (25%) isolates carried SCCmec V, one isolate (1.8.33%) typed SCCmec V+I. Among 5(2.36%) *pvl* not detected MRSA isolates of Out-Patient visitors, 3 isolates (25%) carried SCCmec I, followed by 2 isolates (16.66%) carried SCCmec III.

We showed, 14 isolates (27.45%) carried *mecA* gene among In-Patient visitors among 51 isolates of isolated *S.aureus* from In-Patient Visitors. Of these 14 MRSA isolates from in-Patient visitors, 6 (2.84%) detected *pvl* gene and 8 (3.79%) isolates not carried *pvl* gene. Among 6 (2.84%) *pvl* positive MRSA strains, 2 isolates (14.28%) carried SCCmec Iva and one isolate typed (7.14%) SCCmec IVb followed by 2 isolates (14.28%) carried SCCmec V and one isolate (7.14%) was not typeable in current study. Among 8 (3.79%) *pvl* negative MRSA isolates of In-Patient visitors, 3 isolates (21.42%) carried SCCmec I followed by 2 (14.28%) isolates detected SCCmec II, one isolate (7.14%0 carried SCCmec III, one isolate

(7.14%) carried co-existence of 2 SCC mec types (SCCmec III+II) and one isolate was not-typeable.

In present study, 19.55% (n=88) isolates detected *mecA* gene which is responsible for Methicillin resistance among 450 isolated coagulase negative Staphylococci. Of these, 14 isolates (15.90%) detected *pvl* gene, which is an cytotoxic gene and important virulence factor in pathogenicity mostly associated with CA-MRSA [24]. There is no adequate data or study is available in prevalence of *pvl* gene and its associated SCCmec diversity among CoNS. Among 14 *pvl* detected MRCoNS, 2.27% isolates carried SCCmec III, SCCmec V and 1.14% isolates carried SCCmec I and II. One isolate detected SCCmec I + III; the Co-existence of 2 SCCmec types among the CoNS are very common. . Co-existence of SCCmec types among single strain of MRCoNS was supported by multiple clonal theory phenomenon [25], which states that the acquisition of SCCmec elements occurs on occasion; this will increase the possibility of integration and addition of other SCCmec elements into the already existing one and give rise to composite SCCmec elements. In present study, 5 isolates are not typeable which might be due to occurrence of the other SCCmec types which are common in other Indian studies.

In the present study, 42.86% isolates of *S.epidermidis* were detected *pvl* gene among 14 *pvl* positive MRCoNS of clinical samples followed by *S.haemolyticus* (35.71%) and *S.saprophyticus* (21.42%). This was supported by other study conducted by Sani NAM et al which showed 8% of the S.epidermidis strains carried *pvl* gene [26], A study from Korea which showed prevalence rate of *pvl* gene among S.epidermidis found 71.4% [27]. Among the CoNS the pvl gene are predominantly seen in *S.epidermidis* followed by others. Out of 88 MR-CoNS in clinical specimens, 50% Methicillin resistance were detected in *S.epidermidis* followed by *S.saprophyticus* (32.95%), *S.haemolyticus* (10.22%) and S.warneri (2.27%). *S.simulans, S.schleiferi, S.capitis, S.hominis* were detected Methicillin resistance rate was 1.13% among the clinical isolates out of 450 isolated CoNS. Among 50% (n=44) Methicillin resistance *S.epidermidis*, SCCmec IV were observed very commonly followed by 15.91% of SCCmec III and SCCmec V, 9.09% of SCCmec II, 6.82% of SCCmec I. 2 isolates carried SCCmec I+ II and one isolates carried SCCmec III + V, 5 isolates of S.epidermidis are not

typeable in current study, as they may belong to other SCCmec types. Among 29 (32.95%) of Methicillin Resistance *S.saprophyticus*, SCCmec IV were detected more in number followed by SCCmec II (17.24%), SCCmec III (13.79%) and SCCmec V (10.39%) .SCCmec I was not detected in Methicillin resistance *S.saprophyticus,* 2 isolates carried SCCmec I+III, 3 isolates of Methicillin resistance *S.saprophyticus* carried SCCmec III +V and one isolate carried SCCmec III+V. 4 isolates of Methicillin resistance *S.saprophyticus* in clinical specimens not typeable. SCCmec II and V were found highest (22.22%) among the 10.22% of Methicillin resistance *S.haemolyticus* (n=9), followed by SCCmec I, Sccmec III and Sccmec IV were detected in 11.11%. One isolate carried SCCmec IV +V and one isolate of *S.haemolyticus* among the clinical samples was not typeable. 2.27% of Methicillin resistance *S.warneri* carried SCCmec III, 1.13% of Methicillin resistance *S.hominis and S. Schleiferi* carried SCCmec III, 1.13% of Methicillin resistance *S.capitis* carried SCCmec I. 1.13% of Methicillin resistance *S.simulans* from clinical isolates carried SCCmec IV

Anterior nasal colonization MRCoNS were not detected among the Healthcare workers in present study. Whereas, In-patient visitors those who came for general visit (Patient relatives, Friends etc in general wards etc) 3.33% (n=3) were detected Methicillin resistant and all isolates (n=3) showed *pvl* cytotoxic gene genes. Among these 3 MR-CoNS from In-patient visitors, 2 isolates of Methicillin resistance *S.epidermidis* were detected and one of each isolate carried SCCmec I and SCC mec II. *S.haemolyticus* was not typeable any SCCmec types among nasal colonization of MR-CONS in current study. 3.32 %(n=3) MRCoNS were detected in Out-Patient visitors (Those who came with out-patients to visit OPD), among these one isolate detected *pvl* gene and carried SCCmec V i.e., *S.epidermidis.* 2 isolates of Methicillin resistance *S.epidermidis* were detected among nasal colonization of Out-patient visitors of *pvl* gene not detected MR-CoNS isolates and carried SCCmec II and III.

5.8 Conclusions:-

Molecular characterization of SCCmec typing among MRSA and MRCoNS is an important epidemiological tool for studying the genetic elements evolution and providing the antibiotic resistance pattern information in Staphylococci spp. In the present study we concluded that there was more prevalence of *pvl* gene among *S.aureus* and CoNS. SCCmec type IV Carried maximum followed by SCCmec V, III, II and I. Multiple clones of SCCmec were observed in same strains of Staphylococcus. Type II and III contains multi drug resistance properties and it is carried on integrated plasmids. Furthermore, CoNS acts as a reservoir and transfer of SCCmec elements to other *Staphylococcus spp* by horizontal gene transfer method. Identification of True CoNS, virulence property and its antibiotic resistance pattern with respective SCCmec types is important to find out the multidrug resistance pattern. The present study showed that Patient visitors carried *mecA* gene and its associated SCCmec elements may transfer these factors to HCWs and Patients. This will leads to poor outcome of the patient with *Staphylococcal* infection especially skin infections. Hospital infection control practices are necessary to prevent this transfer of unwanted antibiotic resistance factors among hospital settings.

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Detection of antibiotic resistant determinant among Clinical isolates, Nasal carriage of Health care workers and Patient visitors

Chapter-6

Detection of Antibiotic resistance determinants of Staphylococcus among clinical isolates, Nasal colonization of Healthcare workers and Patient visitors

6.1 Introduction:-

S.aureus and Coagulase negative *Staphylococci* are well known pathogens to cause hospital and community acquired infections. *Staphylococcus* is a rapidly adapting pathogen in various environments and develops resistance to various classes of routinely used antibiotics. Antibiotic resistance develops through horizontal transfer of antimicrobial resistance determinants or mutation, thus, leading to development of unwanted complications in hospitalized patients [1]. In past 2 decades treatment of Multidrug resistance (MDR) *Staphylococcus* infections is increasing and sometimes leads to treatment failure[2]. Most of the *staphylococcus* is resistant to Macrolid group of antibiotics such as Erythromycin. In Staphylococcus, two mechanisms of Erythromycin resistance have been identified: 1. Ribosomal target site modification, which is mediated by methyltransferases and encoded by the erm (Erythromycin resistant methylase) gene. 2. The msrA (Macrolid specific resistant) gene encodes an efflux pump[3]. Two main mechanisms of Tetracycline resistance against Staphylococcus are identified and very common nowadays namely. 1. Plasmid mediated Active efflux protein is encoded by the genes *tetK* and *tetL* 2. Protection of bacterial ribosome which is encoded by *tetM, tetO* and it is mediated by chromosome or Transposon. Among these, *tetK* showed resistance to Tetracycline only and *tetM* showed resistant to both Tetracycline and Minocycline[4].

Resistance to Aminoglycosides antibiotics is mainly due to the production of Aminoglycoside modifying enzymes (AMEs). AMEs are seen within the mobile genetic elements of Staphylococcus. The most common AMEs in Staphylococcus are *AAC(6')-Ie-aph (2')-Ia, aph(3')-IIIa* and *ant (4')-Ia gene* [5] Mupirocin (Pseudomonic acid A) is a tropical ointment used to treat Staphylococcal skin infections and is effective for Methicillin Resistant Staphylococcus nasal decolonization. [6]. Based on the Minimum Inhibitory Concentration (MIC) Mupirocin Resistance is classified in two phenotypes, low and high level resistance. Low level Mupirocin resistance has a MIC range between 8 - 256 µg/mL and is influenced by point mutations in chromosomosal gene *iles-1* which is encoded by tRNA synthetase. Low level Mupirocin resistance is not-transferable and

stable. High level Mupirocin resistance is mostly by by the acquisition of plasmid borne resistance genes *mupA* and *mupB* by conjugation and a MIC value of ≥512µg/mL. *mupA* gene carries additional antibiotic resistance genes [7]. The most common Fusidic acid extrinsic resistance genes among Staphylococcus spp are *fusB, fusC* and *fusD*. Among Fusidic resistance genes, *fusB, fusC* are widely distributed in *S.aureus* and CoNS. The intrinsic factor *fusD* showed Fusidic acid resistance intrinsically among *S.saprophyticus* [8].

Vancomycin resistance among Staphylococcus spp might be either by mutation or transfer of antibiotic resistant determinants via horizontal gene transfer process. As far now, 11 *van* genes have been described namely *vanA, vanB, vanC, vanD, vanE, vanF, vanG, vanH, vanI, vanM, vanN. VanA* and *vanB* are the most commonly observed Vancomycin resistance genes in hospital settings. *vanA* gene is responsible for Vancomycin and Teicoplanin resistance, while *vanB g*ene seen only in Vancomycin resistance. [9]. The *vanA* gene clusters are carried in transposons Tn1546. They act as mobile genetic element to transfer antibiotic resistance gene from Vancomycin resistance *Enterococci to Staphylococci* [10]. Linezolid is an Oxazolidinone group of antibiotics used against community and, uncomplicated skin and soft tissue infections, bacteremia, hospital acquired pneumonia caused by multidrug resistant Staphylococcal pathogens. Linezolid resistance is mostly by Mutations in ribosomal proteins *L3, L4 and L22* and mutations in *domain V region of 23S rRNA,*. In addition, plasmid mediated horizontal gene transfer, resistance is mediated by *optrA* and *cfrA* (Chloramphenicol-Florfenicol resistance) genes [11]. The rapid and accurate diagnosis of multidrug resistant bacteria in nasal cavity and clinical specimens is very important to inhibit the spread of multidrug resistance pathogens and its resistance determinants. Only few studies are available in India on the distribution of antibiotic resistance determinants among healthy community nasal carriage and clinical isolates. Hence, the present study was designed to find the antibiotic resistant determinants and its SCCmec types among *S.aureus* and CoNS from Kolhapur, Maharashtra, India.

6.2 Methodology:-

Nucleic acid extraction was done by using boiling lysis method as previously described in chapter 5

6.3 Polymerase Chain Reaction (PCR):-

The following primers were used in this study to detect the antibiotic resistant determinants of various classes of antibiotics.

Table 6.1 Primers used in this chapter for identification of antibiotic resistance determinants among Staphylococcus

Sr.No	Gene	Primers	Bp	Ref
1	*Van A*	5'-CATGAATAGAATAAAAGTTGCAATA-3'	1032	12
		5'-CCCCTTTAACGCTAATACGACGATCAA-3'		
2	*Van B*	5'-ACGGAATGGGAAGCCGA-3'	647	
		5'-TGCACCCGATTTCGTTC-3'		
3	*Van C*	5'-ATGGATTGGTACTGGTAT-3'	815	
		5'-TAGCGGGAGTGACCAGTAA-3		
4	*aac (6')-Ie aph (2")-I*	F-CAGGAATTTATCGAAAATGGTAGAAAAG	369	13
		R-CACAATCGACTAAAGAGTACCAATC		
5	*aph(3')-IIIa*	F-GGCTAAAATGAGAATATCACCGG	523	
		R-CTTTAAAAAATCATACAGCTCGCG		
6	*ant(4')-Ia*	F-CAAACTGCTAAATCGGTAGAAGCC	294	
		R-GGAAAGTTGACCAGACATTACGAACT		
7	*tet K*	F- GTAGCGACAATAGGTAATAGT	360	14
		R- GTAGTGACAATAAACCTCCTA		
8	*tet M*	F- AGTGGAGCGATTACAGAA	158	
		R- CATATGTCCTGGCGTGTCTA		
9	*tet L*	F-ATAAATTGTTTCGGGTCGGTAAT	1077	15
		R- AACCAGCCAACTAATGACAATGAT		
10	*tet O*	F-AACTTAGGCATTCTGGCTCAC	514	16
		R-TCCCACTGTTCCATATCGTCA		
11	*ermA*	TATCTTATCGTTGAGAAGGGATT	139	17
		CTACACTTGGCTTAGGATGAAA		
12	*ermB*	CTATCTGATTGTTGAAGAAGGATT	142	
		GTTTACTCTTGGTTTAGGATGAAA		
13	*ermC*	CTTGTTGATCACGATAATTTCC	190	
		ATCTTTTAGCAAACCCGTATTC		
14	*msrA*	TCCAATCATAGCACAAAATC	163	
		AATTCCCTCTATTTGGTGGT		
15	*Domain V of 23SrRNA*	GCGGTCGCCTCCTAAAAG	390	18
		ATCCCGGTCCTCTCGTACTA		
16	*rplC*	AACCTGATTTAGTTCCGTCTA GTTGACGCTTTAATGGGCTTA	822	

17	*rplD*	TCGCTTACCTCCTTAATG	1200	19
		GGTGGAAACACTGTAACTG		
18	*rplV*	CAACACGAAGTCCGATTGGA	350	
		GCAGACGACAAGAAAACAAG		
19	*Cfr*	TGAAGTATAAAGCAGGTTGGGAGTCA	746	
		ACCATATAATTGACCACAAGCAGC		
20	*iles-1*	TATATTATGCGATGGAAGGTTGG, AATAAAATCAGCTGGAAAGTGTTG,	764	20
21	*mupB*	CTAGAAGTCGATTTTGGAGTAG, AGTGTCTAAAATGATAAGACGATC	674	21
22	*FusB*	CCGTCAAAGTTATTCAATCG ACAATGAATGCTATCTCGACA	492	21
23	*FusC*	GGACTTTATTACATCGATTGAC CTGTCATAACAAATGTAATCTCC	411	
24	*Fus D*	AATTCGGTCAACGATCCC GCCATCATTGCCAGTACG	465	
25	*dfrA*	CTCACGATAAACAAAGAGTCA CAATCATTGCTTCGTATAACG	201	22

Table 6.2 Cycling condition of *van A, vanB and vanC*

Initial denaturation	95ºC – 5 minutes
Denaturation	94ºC- 1 minute
Annealing	50ºC-1minute
Extension	72ºC-1 minute
No. of Cycles	35
Final Extension	72ºC – 10 minutes

Table 6.3 ***Cycling condition of aac(6')-Ie aph(2")-I, aph (3')-IIIa,ant(4')-Ia, tetK, tetM, tetL, tetO***

Initial denaturation	94ºC – 5 minutes
Denaturation	94ºC- 40 seconds
Annealing	56ºC-40 seconds
Extension	72ºC-40 seconds
No. of Cycles	35
Final Extension	72ºC – 7 minutes

Table 6.4Cycling condition of ***ermA, ermB,ermC and msrA***

Initial denaturation	94ºC – 5 minutes
Denaturation	95ºC- 1 minutes
Annealing	55ºC-30 seconds
Extension	72ºC- 2 minutes
No. of Cycles	30
Final Extension	72ºC – 10 minutes

Table 6.5 Cycling condition of domain V of 23sr RNA and ***cfr***

Initial denaturation	94ºC – 5 minutes
Denaturation	94ºC- 30 seconds
Annealing	55ºC-30 seconds
Extension	72ºC- 1 minutes
No. of Cycles	30
Final Extension	72ºC – 7 minutes

Table 6.6 Cycling condition of ***rpl C, rpl D and rpl V***

Initial denaturation	94ºC – 10 minutes
Denaturation	94ºC- 30 seconds
Annealing	55ºC-30 seconds
Extension	72ºC- 1 minutes
No. of Cycles	35
Final Extension	72ºC – 10 minutes

Table 7 Cycling condition of iles-1 and mupB

Initial denaturation	94C – 5 minutes
Denaturation	94- 30 seconds
Annealing	60C-30 seconds
Extension	72C- 30seonds
No. of Cycles	30
Final Extension	72C – 5 minutes

Table 6.8 Cycling condition of fus B and fusC

Initial denaturation	94ºC – 5 minutes
Denaturation	94ºC- 30 seconds
Annealing	50ºC-30 seconds
Extension	72ºC- 30seonds
No. of Cycles	30
Final Extension	72ºC – 10 minutes

Table 6.9 Cycling condition of *fusD*

Initial denaturation	94ºC – 5 minutes
Denaturation	94ºC- 30 seconds
Annealing	57ºC-30 seconds
Extension	72ºC- 30seonds
No. of Cycles	30
Final Extension	72ºC – 10 minutes

Table 6.10 Cycling condition of *dfrA*

Initial denaturation	94ºC – 4 minutes
Denaturation	94ºC- 30 seconds
Annealing	54ºC-30 seconds
Extension	72ºC- 30seonds
No. of Cycles	25
Final Extension	72ºC – 5 minutes

6.4 Results:-

Table 6.11 Prevalence and Distribution of antibiotic resistant determinants among isolated Staphylococcus

Genes	***Clinical Isolates***		***Health care workers***		***In-Patient Visitors***		***Out-Patient Visitors***	
	S.aureus (n=350)	***CoNs (n=450)***	***S.aureus (n=92)***	***CoNs (n=56)***	***S.aureus (n=51)***	***CoNs (n=39)***	***S.aureus (n=41)***	***CoNs (n=41***
msrA	60(17.14%)	51(11.33%)	16(17.39%)	8(14.28%)	8(15.68%)	5(12.82%)	8(19.51%)	7(17.01%)
ermC	45(12.85%)	40(8.88%)	7(7.60%)	4(7.14%)	6(11.76%)	3(7.69%)	5(12.19%)	3(7.31%)
ermB	33(9.42%)	35(7.77%)	6(6.52%)	4(7.14%)	6(11.76%)	2(5.12%)	6(14.63%)	3(7.31%)
ermA	17(4.85%)	21(4.66%)	2(2.17%)	2(3.57%)	2(3.92%)	2(5.12%)	2(4.87%)	2(4.87%)
ermB + msrA	11(3.14%)	-	-	2(3.57%)	1(1.96%)	-	1(2.43%)	-
erm C+ msrA	10(2.85%)	2(0.44%)	1(1.08%)	-	1(1.96%)	-	-	24.87%)
erm A+ erm C	4(1.14%)	12(2.66%)	-	-	-	-	-	-
tet M	110(31.4%)	74(16.44%)	5(5.43%)	5(8.92%)	7(13.72%)	7(17.94%)	6(14.63%)	6(14.63%)
tet K	79(22.57%)	47(10.44%)	4(4.34%)	3(3.57%)	4(7.84%)	3(7.69%)	5(12.19%)	6(14.63%)
tet L	5(1.42%)	14(3.11%)	-	-	-	-	1(2.43%)	-
tet O	-	-	-	-	-	-	-	-
tet M + tet K	6(1.71%)	10(2.22%)	1(1.08%)	1(1.78%)	1(1.96%)	1(2.56%)	1(2.43%)	2(4.87%)
aac(6')-Ie aph (2'')-Ia	31(8.85%)	26(5.77%)	4(4.34%)	3(3.57%)	2(3.92%)	3(7.69%)	4(9.75%)	2(4.87%)
aph(3')-IIIa	25(7.14%)	21(4.66%)	2(2.17%)	-	2(3.92%)	-	2(4.87%)	-
ant (4')–Ia	18(5.14%)	12(2.66%)	-	-	-	1(2.56%)	2(4.87%)	-
van A	27(7.71%)	14(3.11%)	6(6.52%)	-	4(7.84%)	1(2.56%)	3(7.31%)	2(4.87%)

domain V of 23S rRNA	4(1.14%)	-	-	-	-	-	-	-
rpl C	2(0.57%)	3(0.66%)	-	-	-	-	-	-
rplD	-	1(0.22%)	-	-	-	-	-	-
cfr	2(0.57%)	-	-	-	-	-	-	-
mupA	38(10.85%)	18(4.00%)	3(3.26%)	-	2(3.92%)	1(2.56%)	3(7.31%)	-
iles-1	5(1.42%)	6(1.33%)	-	-	-	-	1(2.43%)	-
fus B	20(5.71%)	38(8.44%)	3(3.26%)	3(3.57%)	1(1.96%)	2(5.12%)	3(7.31%)	3(7.31%)
fusC	18(5.14%)	26(5.77%)	2(2.17%)	1(1.78%)	-	1(2.56%)	1(2.43%)	-
dfrA	11(3.14%)	8(1.77%)	8(8.69%)	1(1.78%)	3(5.88%)	-	5(12.19%)	-

Table 6.12 Prevalence and Distribution of antibiotic resistant determinants among isolated MRS

	Clinical Isolates		***Health care workers***		***In-Patient Visitors***		***Out-Patient Visitors***	
	S.aureus (n=123)	***CoNs (n=72)***	***S.aureus (n=24)***	***CoNs (n=0)***	***S.aureus (n=14)***	***CoNs (n=9)***	***S.aureus (n=10)***	***CoNs (n=9)***
msrA	11(8.94%)	04(5.55%)	2(8.33%)	-	02(14.28%)	1(11.11%)	2(20.00%)	3(33.33%)
ermC	07(5.69%)	05(6.94%)	1(4.16%)	-	-	1(11.11%)	-	-
ermB	04(3.25%)	01(1.38%)	-	-	01(7.14%)	1(11.11%)	-	-
ermA	3(2.43%)	-	-	-	-	-	-	-
ermB + msrA	1(0.81%)	-	-	-	-	-	-	-
tet M	9(7.31%)	4(5.55%)	2(8.33%)	-	2(14.28%)	2(22.22%)	1(10.00%)	1(11.11%)
tet K	6(4.87%)	2(2.77%)	2(8.33%)	-	-	1(11.11%)	1(10.00%)	2(22.22%)
tet M + tet K	5(4.06%)	-	1(4.16%)	-	-	-	1(10.00%)	-

aac(6')-Ie aph (2")-Ia	8(6.50%)	3(4.16%)	2(8.33%)	-	1(7.14%)	-	-	-
aph (3')-IIIa	5(4.06%)	2(2.77%)	1(4.16%)	-	-	-	-	--
ant (4') –Ia	3(2.43%)	1(1.38%)	-	-	-	-	-	-
van A	27(21.95%)	14(19.44%)	6(25.00%)	-	4(28.57%)	1(11.11%)	3(30.00%)	2(22.22%)
domain V of 23S rRNA	4(3.25%)	-	-	-	-	-	-	-
rpl C	2(1.62%)	3(4.16%)	-	-	-	-	-	-
rplD	-	1(1.38%)	-	-	-	-	-	-
cfr	2(1.62%)	-	-	-	-	-	-	-
mup A	6(4.87%)	17(23.61%)	3(12.5%)	-	2(14.28%)	1(11.11%)	2(20.00%)	-
iles-1	1(0.81%)	3(4.16%)	-	-	-	-	1(10.00%)	-
fus B	6(4.87%)	4(5.55%)	2(8.33%)	-	1(7.14%)	1(11.11%)	1(10.00%)	1(11.11%)
fusC	2(1.62%)	-	-	-	-	-	-	-
dfrA	11(8.94%)	8(11.11%)	2(8.33%)	-	1(7.14%)	-	-	-

Table 6. 11 Distribution of SCCmec types among clinical isolates of *S.aureus* (n=123)

SCCmec	I	II	III	IVa	IVb	IVc	IV d	V	I + V	II +III	III+IV	Total
msrA	4 3.25%	3 2.43%	-	1 0.81%	1 0.81%	-	-	2 1.62%	-	-	-	11 8.94%
ermC	2 1.62%	-	2 1.62%	-	-	1 0.81%	-	2 1.62%	-	-	-	7 5.69%
ermB	-	2 1.62%	-	1 0.81%	-	-	-	-	1 0.81%	-	-	4 3.25%
ermA	-	-	3 2.43%	-	-	-	-	-	-	-	-	3 2.43%
ermB + msrA	-	-	-	-	-	-	-	-	-	1 0.81%	-	1 0.81%
tet M	3 2.43%	2 1.62%	-	2 1.62%	-	1 0.81%	-	1 0.81%	-	-	-	9 7.31%
tet K	2 1.62%	4 3.25%	-	-	-	-	-	-	-	-	-	6 4.87%
tet M + tet K	-	-	-	1 0.81%	2 1.62%	-	-	1 0.81%	-	-	1 0.81%	5 4.06%
aac(6')-Ie aph (2")-Ia	3 2.43%	-	-	1 0.81%	2 1.62%	-	2 1.62%	-	-	-	-	8 6.50%
aph (3')-IIIa	-	3 2.43%	-	-	-	-	-	2 1.62%	-	-	-	5 4.06%
ant (4') – Ia	-	2 1.62%	-	1 0.81%	-	-	-	-	-	-	-	3 2.43%
van A	2 1.62%	2 1.62%	13 10.56 %	-	2 1.62%	-	-	1 0.81%	2 1.62%	2 1.62%	3 2.43%	27 21.95%
domain V of 23S rRNA	2 1.62%	-	-	2 1.62%	-	-	-	-	-	-	-	4 3.25%
rpl C	-	-	-	-	-	-	-	2 1.62%	-	-	-	2 1.62%
cfr	--	-	-	-	-	-	2	-	-	-	-	2

							1.62%					1.62%
mup A	2 1.62%	-	-	-	-	-	-	4 3.25%	-	-	-	6 4.87%
iles-1	1 0.81%	-	-	-	-	-	-	-	-	-	-	1 0.81%
fus B	-	-	-	1 0.81%	-	-	-	5		-	-	6 4.87%
fusC	1 0.81%	-	-	0	1 0.81%	-	-	-	-	-	-	2 2.43%
dfrA	1 0.81%	-	5 4.06%	-	1 0.81%	-	-	2 1.62%	1 0.81%	1 0.81%	-	11 8.94%
Total	**23 18.69 %**	**18 14.63 %**	**23 18.69 %**	**10 8.13%**	**9 7.31 %**	**2 1.62 %**	**4 3.25 %**	**22 17.88 %**	**4 3.25 %**	**4 3.25 %**	**4 3.25 %**	**123**

Table 6.12 Distribution of SCCmec types among Clinical isolates of CoNS (n=72)

SCCmec	**I**	**II**	**III**	**IVa**	**IVb**	**IVc**	**IV d**	**V**	**I + V**	**II +III**	**III+IV**	**Total**
msrA	1 1.38%	2 2.77%	1 1.38%	-	-	-	-	-	-	-	-	4 5.55%
ermC	-	2 2.77%	-	-	-	-	-	3 4.16 %	-	-	-	5 6.94%
ermB	-	1 1.38%	-	-	-	-	-	-	-	-	-	1 1.38%
tet M	2 2.77%	0	2 2.77%	-	-	-	-	-	-	-	-	4 5.55%
tet K	-	2 2.77%	-	-	-	-	-	-	-	-	-	2 2.77%
aac(6')-Ie aph (2")-Ia	-	-	1 1.38%	-	1 1.38%	-	1 1.38%	-	-	-	-	3 4.16%
aph (3')-IIIa	-	1 1.38%	-	1 1.38%	-	-	-	-	-	-	-	2 2.77%

ant (4') – Ia	-	1 1.38%	-	-	-	-	-	-	-	-	-	1 1.38%
van A	-	-	3 4.16%	3 4.16%	2 2.77%	-	3 4.16%	2 2.77 %	-	-	1 1.38%	14
rpl C	1 1.38%	-	-	2 2.77%	-	-	-	-	-	-	-	3 4.16%
rplD	-	-	-	-	-	-	-	-	1 1.38 %	-	-	1 1.38%
mup A	-	1 1.38%	3 4.16%	1 1.38%	2 2.77%	4 5.55%	-	5 6.94 %	1 1.38 %	-	-	17 23.61%
iles-1	-	-	-	-	-	1 1.38%	-	1 1.38 %	-	1 1.38%	-	3 4.16%
fus B	-	-	2 2.77%	-	-	2 2.77%	-	-	-	-	-	4 5.55%
dfrA	-	-	1 1.38%	2 2.77%	-	-	2 2.77%	1 1.38 %	1 1.38 %	-	1 1.38%	8 11.11%
Total	**4 5.55%**	**10 13.88 %**	**13 18.05 %**	**9 12.5%**	**5 6.94%**	**7 9.72%**	**6 8.33%**	**12 16.6 6%**	**3 4.16 %**	**1 1.38 %**	**2 2.77%**	**72**

Table 6.15 Prevalence and Distribution of SCCmec types among *S.aureus* of HCWs (n=24)

SCCmec	I	II	III	IVb	IVc	IV d	V	I + V	II +III	III+IV	Total
msrA	1 4.16%	-	-	1 4.16%	-	-	-	-	-	-	**2 8.33%**
ermC	-	1 4.16%	-	-	-	-	-	-	-	-	**1 4.16%**
tetM	2 8.33%	-	-	-	-	-	-	-	-	-	**2 8.33%**
tetK	-	1 4.16%	1 4.16%	-	-	-	-	-	-	-	**2 8.33%**
tet K+tetM	-	-	-	-	-	-	-	-	-	1 4.16%	**1 4.16%**
aac(6')	1 4.16%	-	1 4.16%	-	-	-	-	-	-	-	**2 8.33%**
aph (3')	-	1 4.16%	-	-	-	-	-	-	-	-	**1 4.16%**
vanA	-	-	2 8.33%	1 4.16%	-	-	2 8.33%	-	1 4.16%	-	**6 25.00%**
mupA	-	-	-	-	1 4.16%	1 4.16%	1 4.16%	-	-	-	**3 12.5%**
fusB	-	-	-	-	-	-	-	1 4.16%	1 4.16%	-	**2 8.33%**
dfrA	-	-	-	1 4.16%	-	-	1 4.16%	-	-	-	**2 8.33%**
Total	**4 16.66%**	**3 12.5%**	**4 16.66%**	**3 12.5%**	**1 4.16%**	**1 4.16%**	**4 16.66%**	**1 4.16%**	**2 8.33%**	**1 4.16%**	**24**

Table 6.16 Prevalence and Distribution of SCCmec types among *S.aureus* of Out- Patient visitors (n=10)

SCCmec	I	II	III	IVa	IVb	V	II +III	Total
msrA	2 14.28%	0	0	0	0	0	0	2 14.28%
ermB	0	1 7.14%	0	0	0	0	0	1 7.14%
tet M	1 7.14%	0	1 7.14%	0	0	0	0	2 14.28%
aac (6')-Ie-aph -Ia	1 7.14%	0	0	0	0	0	0	1 7.14%
vanA	0	1 7.14%	0	2 14.28%	0	1 7.14%	0	4 28.57%
mupA	0	0	0	0	0	2 14.28%	0	2 14.28%
fus B	0	0	0	0	0	0	1 7.14%	1 7.14%
dfrA	0	0	0	1 7.14%	1 7.14%	0	0	2 (14.28%)
Total	4 28.57%	2 14.28%	1 7.14%	2 14.28%	1 7.14%	3 (21.42%)	1 7.14%	14

Table 6.17 Prevalence and Distribution of SCCmec types among *S.aureus* of In- Patient visitors (n=14)

SCCmec	I	III	IVa	IVc	V	I + V	Total
msrA	1 10.00%	1 10.00%	0	0	0	0	**2** **20.00%**
tet M	0	1 10.00%	0	0	0	0	**1** **10.00%**
tet K	1 10.00%	0	0	0	0	0	**1** **10.00%**
tet K + L	0	0	1 10.00%	0	0	0	**1** **10.00%**
vanA	1 10.00%	0	0	0	0	0	**1** **10.00%**
mupA	0	0	0	1 10.00%	0	1 10.00%	**2** **20.00%**
iles-1	0	0	0	0	1 10.00%	0	**1** **10.00%**
fus B	0	0	0	0	1 10.00%	0	**1** **10.00%**
Total	**3** **30.00%**	**2** **20.00%**	**1** **10.00%**	**1** **10.00%**	**2** **20.00%**	**1** **10.00%**	**10**

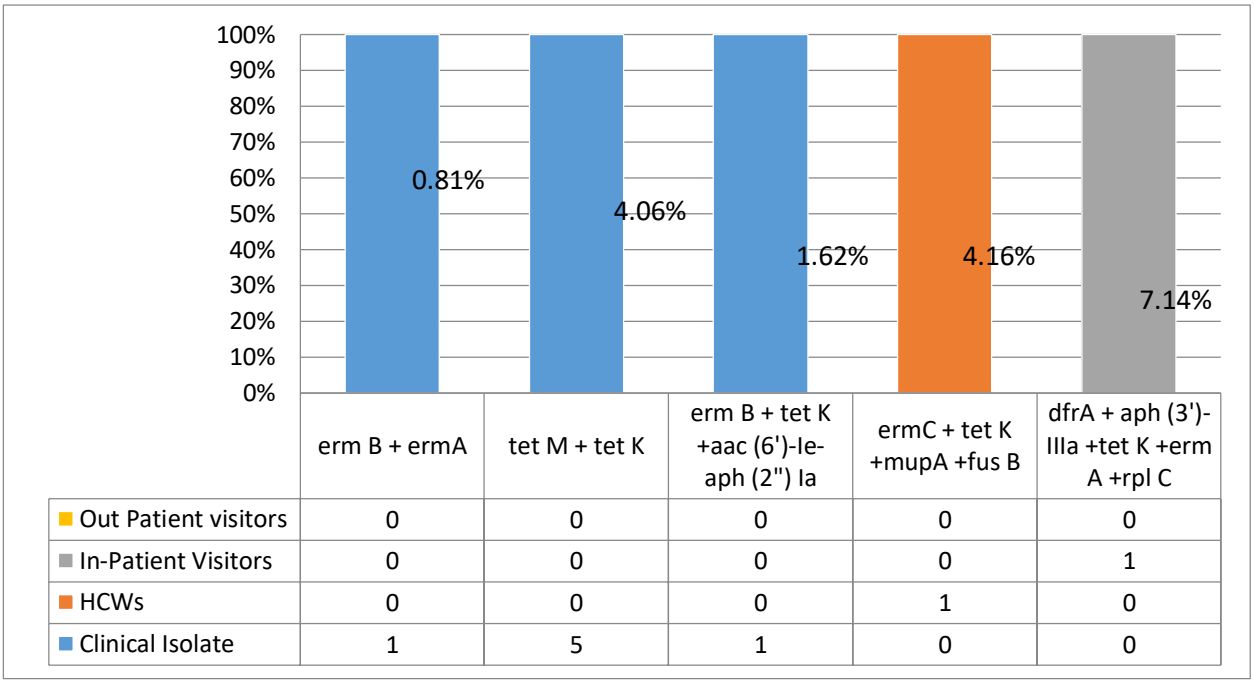
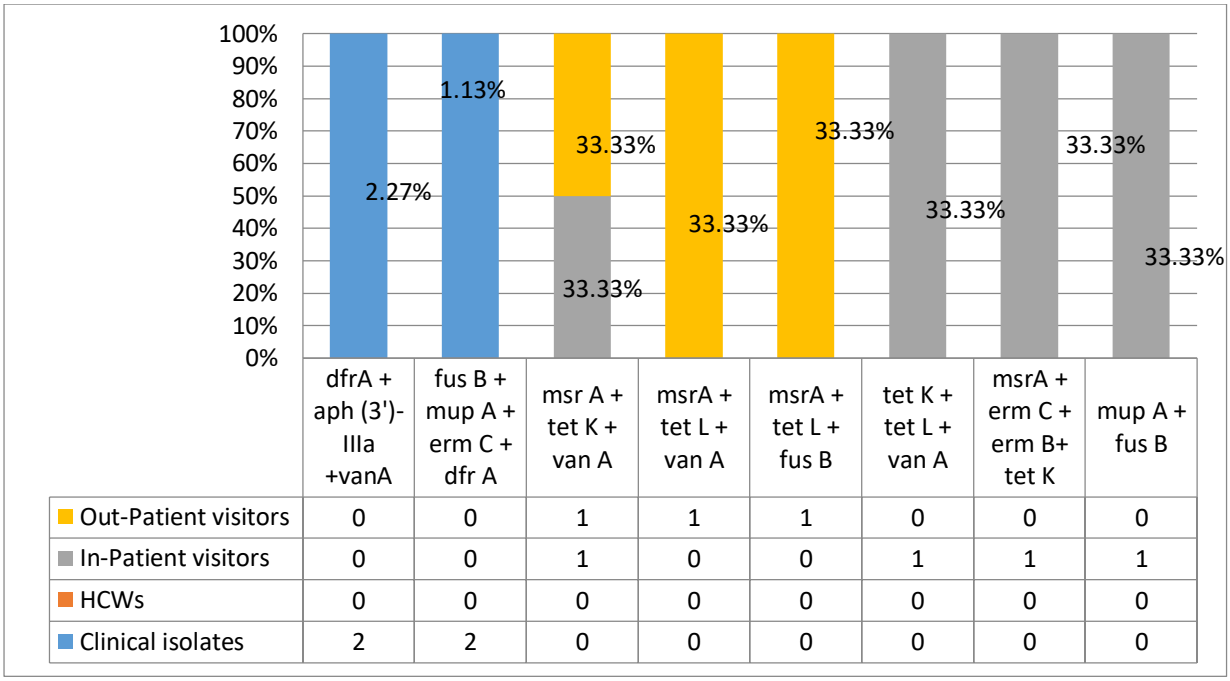


Figure 6.8 Distribution of Co-existence of antibiotic resistant determinants among *S.aureus*

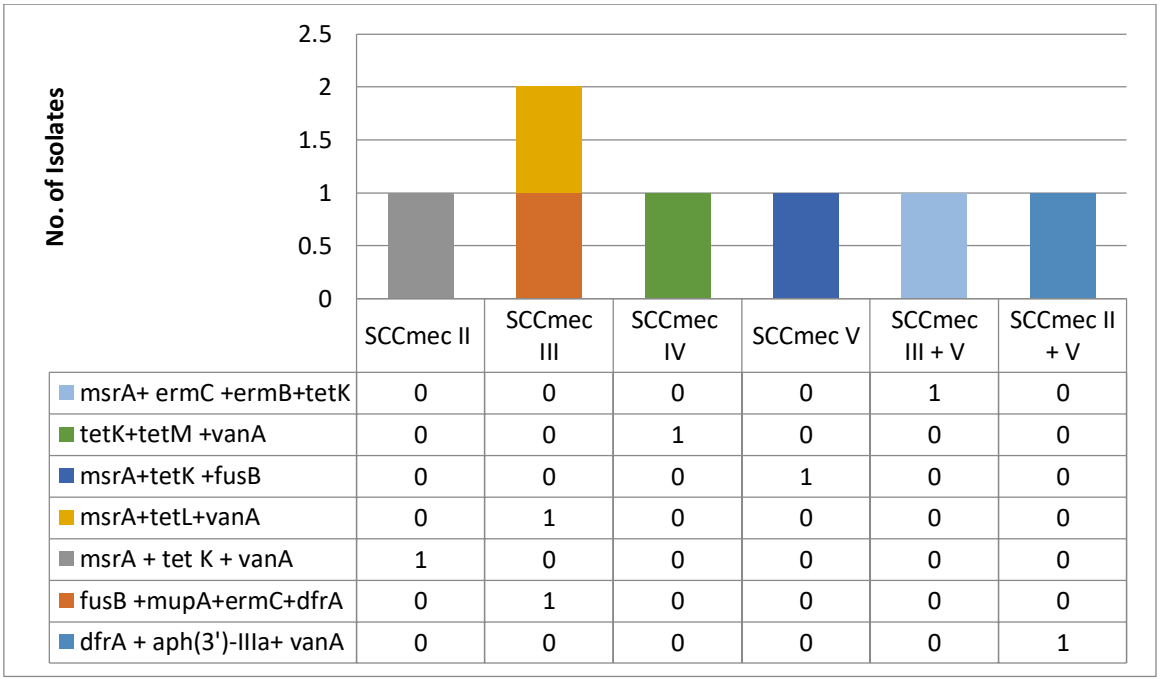
Table 6.13 Distribution of SCCmec types among Co-existence of multiple antibiotic resistant determinants of Methicillin resistant *CoNS*

Resistant determinants	SCCmec types	No.of Isolates
erm B + ermA	SCCmec I	1
tet M + tet K	SCCmec III	1
erm B + tet K +aac (6')-Ie-aph (2") Ia	SCCmec II + III	1
ermC + tet K +mupA +fus B	SCCmec I	1
dfrA + aph (3')-IIIa +tet K +erm A +rpl C	SCCmec 1V	1



	dfrA + aph (3')-IIIa +vanA	fus B + mup A + erm C + dfr A	msr A + tet K + van A	msrA + tet L + van A	msrA + tet L + fus B	tet K + tet L + van A	msrA + erm C + erm B+ tet K	mup A + fus B
Out-Patient visitors	0	0	1	1	1	0	0	0
In-Patient visitors	0	0	1	0	0	1	1	1
HCWs	0	0	0	0	0	0	0	0
Clinical isolates	2	2	0	0	0	0	0	0

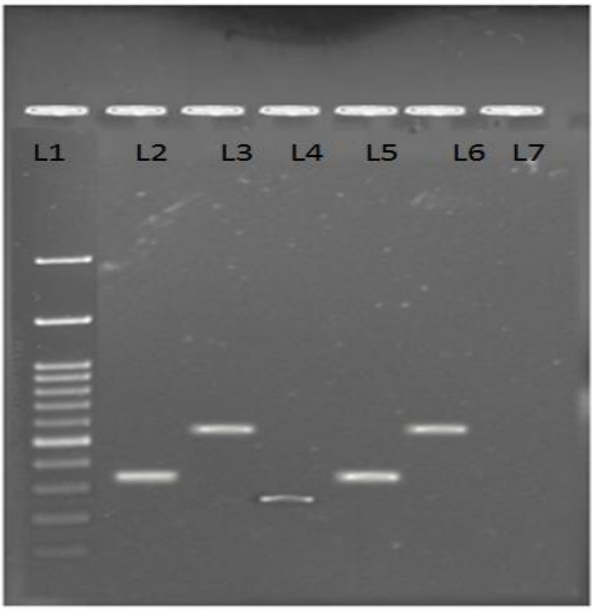
Figure 6.9 Distribution of Co-existence of multiple antibiotic resistant determinant of Methicillin resistant *CoNS*



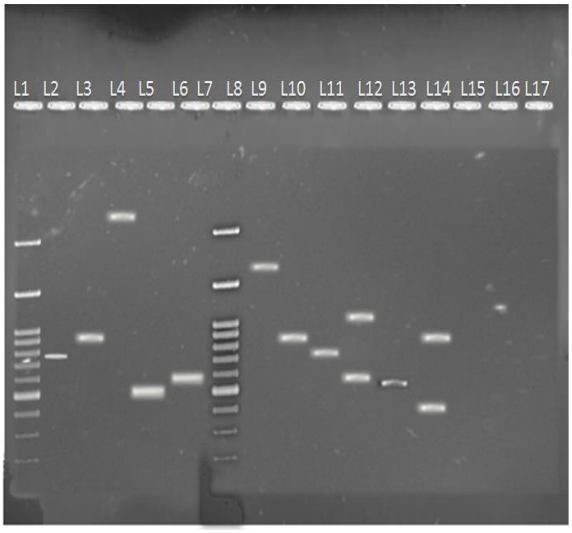
	SCCmec II	SCCmec III	SCCmec IV	SCCmec V	SCCmec III + V	SCCmec II + V
msrA+ ermC +ermB+tetK	0	0	0	0	1	0
tetK+tetM +vanA	0	0	1	0	0	0
msrA+tetK +fusB	0	0	0	1	0	0
msrA+tetL+vanA	0	1	0	0	0	0
msrA + tet K + vanA	1	0	0	0	0	0
fusB +mupA+ermC+dfrA	0	1	0	0	0	0
dfrA + aph(3')-IIIa+ vanA	0	0	0	0	0	1

Figure 6.10 Distribution of SCCmec Types among co-existence of multiple Antibiotic Resistant Determinants of isolated CoNS

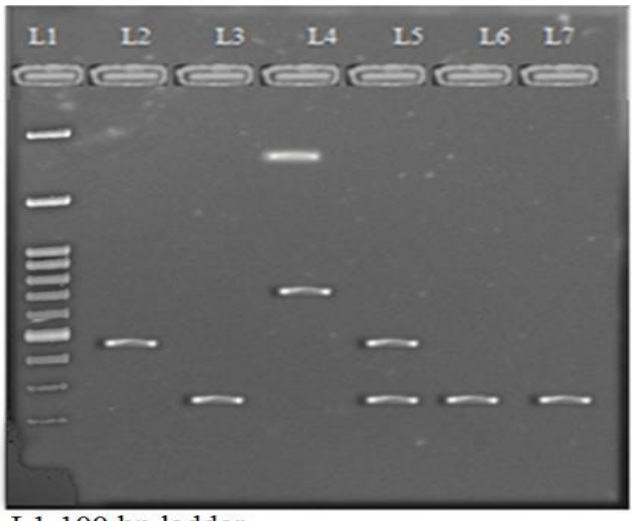
L3 – 100 bp Ladder ,
L1- *erm(C)- 190 bp , L2- erm(C) 190 bp + erm(A)139 bp*
L4-erm(C) 190 bp +erm (B) 142 bp, L6- msr(A) 163 bp



L1 -100 bp (Ladder)
L2- 369 bp aac (6') Ie-aph(2")-Ia
L3- 523 bp aph (3')-IIIa
L4- 294 bp ant (4')-Ia



L1 and L8 -100bp Ladder
L2- 746 bp (cfr), L3- 822 bp (rplC), L4-1200 bp (rplD), L5- 390 bp (domain V of 23SrRNA
L9-1032 bp (vanA),
L10-764 bp (iles-1), L11 – 674 bp (mupB)
L11-492 bp (fusB), L12 – 411 bp (fusC), L14 – 201 bp (dfrA)



L1-100 bp ladder
L2-*tet (K)*- 360 bp
L3-*tet (M)*-158 bp
L4- tet (L)- 1077 bp
L5- *tet(K)* +*tet (M)*- 360 bp + 158 bp

6.5 Discussion:-

Staphylococci are important pathogens in humans all over the world, Antimicrobial resistant (AMR) in bacteria is an important public health problem in modern medical world. CoNS are potential reservoirs of antibiotic resistant determinants which may transfer then to *S.aureus* both in vivo and in vitro [23]. Developing countries, like India generally pay less attention in commensal, low pathogenic and environmental antimicrobial resistance bacteria. These bacteria act as a reservoir of AMR genes that might increase the number of antibiotic resistance determinants and make patients who are hospitalised more harmful. [24]. Erythromycin has a good oral absorption and high tissue penetration and. It remains an important antibiotic in Macrolid groups and is used to treat nosocomial and community associated infections caused by gram positive and gram negative bacteria [25].

In the present study, frequency of Erythromycin resistant determinants was 41.33% among clinical isolates of *S.aureus*. Among these, *msr A* (31.72%) gene found more followed by *erm C* (22.58%), *erm B* (16.66%) and *erm A* (15.59%). *msrA* gene encodes for the active efflux pump protein and it is ATP dependent. This is similar to the findings of Misic et al 2017, Hungary [26] who found *msrA* gene in half of the Macrolid resistant *Staphylococci*. Among *erm* genes *ermC* was more common than other *erm genes*; most of the studies found *ermC* was more prevalent followed by others. This could be due to the presence of *ermC* genes in small plasmids and easier transmission and dissemination from resistant strains to susceptible strains [27]. It was supported by a study conducted in Iran 2019 [28], who found more prevalence in *ermC* gene than others. Some studies did not find *ermB* gene and showed no role in prevalence of Macrolid resistance. But our studies showed *ermB* was 16.66%. This indicates that *ermB, ermA* and *ermC* play important role in Macrolid resistance among *S.aureus*.

We found Co-existence of *ermB + msrA was 6.45%, ermC + msrA 4.30% and ermA+ ermC 2.68%*. It was previously reported and supported by other studies, Faccone D et al 2014 [29], Sazczuka E et al 2016 [30] and Steward CD et al 2005 [31]. Co-existence of Macrolid genes in single isolate indicates that the pathogen plasmids may carry multidrug resistant genes and posses more virulence than the other pathogens. Among MRSA clinical isolates, 8.94% isolates detected *msrA, 5.69% ermC, 3.25% ermB, 2.43% ermA* and 1 isolate (0.81%) detected *erm C +msrA*. Among 11 (8.94%) *msrA* MRSA clinical positive isolates, 4 (3.25%) isolates carried SCCmec I, 3(2.43%) carried SCCmec II,

1.62% SCCmec IV, and 2 (1.62%) detected SCCmec V. Among 7 *ermC* positive clinical MRSA isolates, 2 isolates (1.62%) isolates carried SCCmec I , SCCmec III, SCCmec V and one isolate carried SCCmec IV. *ermB* + *msrA* isolate of Methicillin resistance *S.aureus* from clinical isolates carried SCCmec II + III. One isolate (0.81%) of *ermA* carried SCCmec I + V.

Aminoglycosides are important class of antibiotics which are used for complicated Staphylococcal infections. The common Aminoglycoside resistance mechanisms are Aminoglycoside Modifying Enzymes (AMEs). Among AMEs, the most common AMEs seen in Staphylococcus are *aac(6')-Ie-aph(2")* which confers resistance to Gentamicin, Tobramycin and Kanamycin, *aph(3')-IIIa* which confers resistance to Neomycin and Kanamycin, *ant-(4')-Ia* which confers resistance to Kanamycin, Tobramycin, Amikacin and Neomycin [32-34]. In present study, 15.77% Aminoglycosides resistance were detected genotypically among clinical isolates of *S.aureus*. Of these, *aac(6')-Ie-aph (2")-Ia* was detected more common (43.66%) followed by *aph(3')-IIIa* (35.21%) and *ant (4')-Ia* (25.35%). Among 160 MRSA clinical isolates, 10% of the isolates detected resistance to Aminoglycosides, 6.50% of isolates detected *aac(6')-Ie-aph (2")* followed by *aph(3')-IIIa* (4.06%) and *ant (4')-Ia* (2.43%). Most of the aminoglycoside resistance MRSA carried SCCmec IV followed by SCCmec II, I and V.

In current study, Nasal colonization of Aminoglycoside resistance among *S.aureus* of Healthcare workers, In –patient and Out-Patient visitors was 6.52%, 7.84% and 19.51%. We found *aac (6')-Ie-aph (2")* was more followed by other AMEs determinants. Out of 24 (26.08%) MRSA isolates among HCWs, 3 (12.5%) isolates detected AMEs determinants. Of these, *aac (6')-Ie-aph (2')* found 8.33% and *aph (3')-III a* found 4.16%. One isolate (7.14%) of MRSA from In- patient visitors detected *aac(6')-Ie-aph (2").* Out –Patient visitors did not detect any AMEs resistance determinants towards MRSA isolates.

In current study, most common Tetracycline resistance gene among clinical isolates of *S.aureus* was mediated *tetK* (33.14%) followed by tet M (22.57%) and *tetO* (1.42%). Among 22 MRSA tetracycline resistant *S.aureus* isolates from among clinical specimens, 7.31% of *tetM* , 4.87% of *tetK* were detected. This is comparable to other studies reported by Khoramrooz et al 2017 [4] who found *tetK* (82.57%) to be the most prominent gene followed by *tetM* (56.9%). Jones CH et al 2006 [35] detected *tetK* gene

higher number in a multicenter study among MRSA isolates. We found Co-existence of *tetk +tet M* (4.06%) in MRSA isolates and this was previously reported in a study by Schmitz et al [36]. In the present study no role of *tetO* gene in Tetracycline resistance among clinical isolates of *S.aureus. tetM* gene was found more in nasal colonization *S.aureus* of Healthcare workers, In-patient Visitors and Out-patient visitors i.e, 5.43%, 15.68%, 17.07%. Our finding is comparable to a study conducted by Hui-Ling Ong M et al 2017 [37], who found more prevalence of *tetM* gene. *tetM* gene is more stable and persists for a longer period of time in a bacterial population followed by other *tet* genes in Tetracycline resistance. This is due to the Transposon Tn916, which is a highly stable transposon even without any high antibiotic selective pressure. *tetK* gene seen in a 4.4kb transmissible plasmid (pT181) which infects only a limited host range [38]. SCCmec II was seen more in Tetracycline resistant genes followed by other SCCmec types. However, a study from Belgium [39] reported SCCmec I instead of SCCmec II. This difference between the prevalence of Tetracycline resistance determinants in the SCCmec type may be due to the geographical location and sample types.

Vancomycin is a drug of choice for the treatment of Methicillin Resistant Staphylococcal infection, Vancomycin resistance occurs by two mechanisms 1. Mutation or by horizontal transfer of Vancomycin resistant determinants among bacteria and 2. Increased cell wall thickness leading to decreased intake of antibiotic inside the bacterial cell [12]. The first mechanisms are mediated by *van* gene clusters. Till now 11 *van* gene clusters have been identified. Of these, *vanA and vanB* genes are commonly observed in hospital isolates of *Staphylococcus* [40]. In the Present study, 27 (6%) of isolates carried *vanA* gene and we did not detect *vanB and VanC* gene among the clinical isolates of *S.aureus*. All *vanA* positive isolates showed Methicillin Resistance. This is supported by the study conducted Meera Maharajan et al 2021[41], who found more *van*A gene than *vanB* among the clinical isolates. SCCmec III was the predominant type in *vanA* determinants followed by other SCCmec types; this may be due to its hospital association as previously described in Chapter 5. Co-existence of 2 SCCmec types were identified in clinical isolates of *S.aureus* i.e. SCCmec III+IV (2.63%), SCCmec I+V, SCCmec II+III. 1.62%. Nasal colonization of MRSA isolates from HCWs, In-Patient Visitors and Out-Patient Visitors were found in 6.52%, 7.84% and 7.31% of *vanA* gene and most of the isolates carried SCCmec V followed by other SCCmec types which may be the community associated.

Fusidic acid is a narrow spectrum steroid like antibiotics used against the MRSA infection in systemic and topical ointment forms. Fusidic acid has been used widely in gram positive bacteria because of its minimal side effects and there is no known cross resistance among bacteria [42]. However, the long term continuous usage of Fusidic acid in treatment has been developing a resistance against Fusidic acid. Two major Fusidic acid resistance mechanisms have been reported among Staphylococcus spp namely, 1. Changing the drug target site by mutation at chromosomal level which is mediated by *fusA* or *fusE* gene 2. Acquired Fusidic acid resistance is mediated by *fus B, fusC and fus D,* these determinants are located on plasmids or transposons-like elements [43]. In present study, we analyzed the plasmid mediated acquired resistant determinants i.e., *fusB, fusC and fusD* which are more common in both *S.aureus* and CoNS. 8.44% of *S.aureus* among clinical isolates showed Fusidic acid resistance genotypically. Among these, 52.63% (n=20) detected *fus B* , 47.36% (n=38) detected *fus C gene. fus D* determinants were not detected in clinical isolates of *S.aureus.* 11 isolates (8.94%) of *S.aureus* from clinical isolates showed resistance to Fusidic acid genotypically. Out of these, 4.87% detected *fus B* gene and 1.62% detected *fus C* gene. This is comparable to other studies by Aldasouqi et al 2019 from Jordan [44], Yu F et al 2015 from China[45] who found *fusB* gene more followed by *fusC* gene among MRSA isolates in their studies. However it is in contrast to other study conducted by Boloki et al 2021 from Kuwait[46] who found more *fus C* determinants than *fus B.* These differences may be the geographical distribution of Fusidic acid determinants among MRSA isolates. Majority of *fus B* determinants detected higher SCCmec type V (4.06%) than other SCCmec types. 0.81% SCCmec type I and IV carried *fus C* gene among the clinical isolates of *S.aureus.* Genotypic prevalence of Fusidic acid resistant determinants of *S.aureus* among HCWS, In-patient visitors and Out-Patient visitors in our study were 3.26%, 1.96% and 7.31%. Among these, out-patient visitors carried more Fusidic acid determinants i.e., 8.33% among MRSA isolates followed by clinical isolates (8%) and In-patient visitors (7.14). Most of the MRSA Fusidic acid resistant determinants among HCWS, In-Patient and Out-Patient visitors carried Co-existence of 2 SCCmec type's i.e SCCmec I+V, SCCmec II +III and one isolate carried SCCmec V. Most of the Fusidic acid determinants carried SCCmec V and in Combination this indicates that MRSA may be community associated in those who frequently use Fusidic acid as a topical ointment over the counter.

Mupirocin also called as Pseudomonic acid A, is used to eliminate the nasal colonization of MRSA among healthcare workers and treatment of Staphylococcal Skin and soft tissue infection [6]. Mupirocin inhibits the synthesis of protein by interacting with isoleucine –transfer RNA (tRNA) synthetase (IIeS). Two types of Mupirocin resistance among Staphylococcus have been reported based on the MIC (Minimum Inhibitory Concentration). Low level Mupirocin resistance (LLMR) with an MIC range between 8 to 256 µg/mL which is influenced by point mutation in t-RNA synthetase chromosomal gene of bacteria (*iles-1*). High level Mupirocin resistance (HLMR) with an MIC range ≥512 µg/mL and HLMR is an acquired resistance by plasmid or transposons like element; it is encoded by *mupA or mupB* gene. *mupA* determinant encoding plasmids carrying other antibiotic resistance [47]. In Present study, we detected iles-1 (LLMR) and *mupA* gene (HLMR) among clinical isolates of *S.aureus*. 9.55% Mupirocin resistance was detected genotypically in current study from clinical isolates of *S.aureus*. Among these, *mupA* (HLMR) was detected in 88.37% followed by *iles-1* (LLMR) gene 11.62%. Out 9.55% (n=43) Mupirocin resistance genotypically, 7 isolates showed resistance to Methicillin out of these, 6 isolates (4.87%) showed resistance to *mupA* (HLMR) and 1 isolate (0.81%) showed resistant to *iles-1* (HLMR). Our finding is supported by study conducted by Shivanna et al 2018 [48], Batoorn et al 2012 [49] who found more high level Mupirocin resistance than LLMR. In contrast, Rudresh et al [50] found higher prevalence in LLMR than HLMR. The difference between the studies may be different origin of isolates, geographical areas and patient characteristics etc. Most of the Mupirocin resistant MRSA strains in clinical specimens carried SCCmec V (4.06%) followed by SCCmec I (1.62%) and SCCmec II (0.81%). Nasal colonization of *S.aureus* isolates among HCWs, In-Patient Visitors and Out-Patient visitors showed resistance to Mupirocin as follows ; 3.26%, 3.92% and 7.31%. All these isolates showed resistance to Methicillin and *mup A* (HLMR) gene was observed more than the *iles-1* (LLMR). Most of the Mupirocin resistance strains carried SCCmec IV followed by other SCCmec types. This indicates that the most of the resistance is community origin; due to the increased usage of Mupirocin ointment over the counter. This has developed unwanted resistance against Mupirocin among *S. aureus*.

Linezolid is a bacteriostatic agent that inhibits the synthesis of bacterial protein by binding to the Variable domain of the 23Sr RNA in the bacterial ribosome of 50S subunit [51]. Two main mechanisms have been mediating the Linezolid resistance among

Staphylococcus. 1. Mutations in Ribosomal proteins L3, L4 and L22 encoded by *rplC, rplD and rplV* genes 2. Plasmid mediated resistance by *cfr* (Chloramphenicol-florfenicol resistance) [52]. In Present study, 1.77% of *S.aureus* clinical isolates showed resistance to Linezolid genotypically. Among these, mutation in domain V of 23srRNA was detected in 50% followed by ribosomal mutation protein L3 *(rplC)* 25%, and plasmid mediated resistance *(cfr*) was 25%. Mutation in domain V of 23s rRNA carried SCCmec II, mutation in L3 protein (*rplC*) carried SCCmec V and plasmid mediated *cfr* gene carried SCCmec IV. These results are comparable to study conducted by Yoo IY et al 2020 [11]. All *cfr* positive isolates showed resistance to Chloramphenicol also. *Domain V 23sRNA* mutation are not transmissible and it is associated with overuse of antibiotics in clinical settings or misuse of antibiotics. *Cfr* gene is unstable plasmid, mediated and responsible for multidrug resistance. We did not detect any Linezolid resistance among HCWS, In-patient visiors and Out-patient visitors *S.aureus*. Two resistance mechanisms of Trimethoprim have been discovered till date. 1. Mutation of the chromosomal dihydrofolate reductase (DHFR) and 2. Resistant genes that encode the variant of DHFRs [53]. In present study we detected *dfrA* gene in Staphylococcus, *dfr* mediate variant shows high level resistance to Trimethoprim and key determinant in Trimethoprim resistance in *S.aureus* among humans [54]. We detected 8.94% of *dfrA* gene among clinical isolates of *S.aureus* and all are resistant to Methicillin. Most of the *dfrA* positive isolates carried SCCmec III in present study followed by other SCCmec types. This indicates that all are associated with Hospital acquired HA-MRSA. Nasal colonization of S.aureus among HCWs, In-Patient Visitors and Out-Patient Visitors showed resistance to Trimethoprim *(dfrA)* was 8.69%, 5.88% and 12.19%. *dfrA* gene with MRSA isolates showed 8% in HCWs, 7.14 in In-patient visitors. We did not find any *dfrA+ mecA* gene in Out-patient visitors. All these *mecA+dfrA* isolate carried SCCmec IV.

Antimicrobial Resistance (AMR) is a major challenge in treatment of bacterial infection. To asses risk factor and AMR in Commensal and environmental bacteria is an important factor to control the spread of unwanted superbugs among the living communities [55]. CoNS are typical commensals and share the same ecological niches in the anterior nares of human begin with *S.aureus* and other commensal bacteria [55]. CoNS are opportunistic pathogens. They transfer the AMR determinants through the horizontal gene transfer method and exchange the genetic materials. CoNS acts as a reservoir and source of resistant traits and transfer it across the Staphylococcaceae family. For example

origin of *mecA* gene which is responsible for Multidrug Methicillin resistance whose genetic origin is *Staphylococcus sciuri* [21]. Macrolides are frequently prescribed antibiotics in out-patient basis and Tetracycline is most commonly used antibiotics in animal and agricultural sectors [2]. In current study, the prevalence of erythromycin resistant genes among CoNS of clinical isolates was 35.77%. Of these, 11.55% isolates carried *msrA* gene, 11.42% isolates detected *ermC* followed by *erm B* (7.77%), *erm A* (4.66%). 12 (2.66%) clinical isolates of CoNS carried *erm + erm C* gene and 0.44% isolates detected *ermC +msrA* gene. Among MRCoNS, in Present study, 11.36% isolates detected erythromycin resistant determinants. Among these, 5.68% *erm C*, 4.54 % *msrA* and 1.13% *ermB gene*, most of the MRCoNS positive erythromycin resistant gene carried SCCmec II followed by SCCmec V, II and I. Screening of CoNS in nasal colonization of HCWs and Patient visitors is an important infection control practice to prevent the spread of unwanted antibiotic resistant pathogens in hospital environment. In present study, 35.71% isolates detected erythromycin resistant determinants among isolated CoNS of HCWs, 30.76% of In-patient visitors and 41.46% of Out-Patient visitors. Among these, *msrA* gene is isolated more in number followed by other Erythromycin resistant determinants. HCWs did not detect any MR-CONS but, In-patient visitors and Out-patient visitors carried *mecA* gene which is responsible for Methicillin resistance. Among patient visitors of MRCoNS isolate, out-patient visitors carried *msrA* gene, In-patient visitors carried *msrA, ermB and ermC* and carried SCCmec II, followed by SCCmec III and V.

Tetracycline is a broad spectrum antibiotic, used more frequently in humans as well as in Veterinary medicine. Increased irrational usage of antibiotics will develop resistance towards antibiotics. In present study, 32.22% of Tetracycline resistance determinants were detected among CoNS of Clinical isolates. Among these, 16.44% were *tetM*, 10.44% of *tetK* 3.11% of *tet L* and not detected *tet O* determinant. 2.22% of isolates carried co-existence of 2 genes *(tet K + tet L)*. Among MRCoNS of clinical isolates, 6.81% isolates detected Tetracycline resistant determinants i.e. ., *tetM* (4.54%) and *tetK* (2.27%). Sccmec II was detected in *tetK* isolate and SCCmec I and III were detected in *tetM* isolates of CoNS. 16.07% of Tetracyclines resistant determinants were detected in HCWs nasal cavity , 28.20% was detected in In-Patient visitors and 34.14% in Out-Patient visitors.

13.11% of AMEs genes were detected among Clinical isolates of CoNS. Of these, 5.77% of *aac (6')-Ie-aph (2'') Ia* followed by 4.56% of *aph (3'') IIIa* and 2.66% of *ant (4')-Ia*. 6.81% of MRCONS isolates of clinical specimens showed resistance to Aminoglycosides and carried 3.40% of *aac (6')-Ie- aph (4'') Ia*, 2.27% of *aph (3')-IIIa* and 1.13% of *ant (4')-Ia*. Most of the detected AMEs among MRCoNS was carried SCCmec IV followed by SCCmec II and SCCmec III. Nasal screening of antimicrobial resistance among CoNS are important to monitor and prevent the development of unwanted resistance pathogens in community as well as in hospital. In present study, 10.25% of AMEs determinant were detected in In-Patient Visitors followed by 5.35% in HCWs and 4.87% in Out-Patient visitors. We did not detect any AMEs genes among MRCoNS isolates of HCWs, IN and Out-Patient Visitors.

Fusidic acid is used for the treatment of skin and soft tissue infections as both systemic and topical ointment. Development of antimicrobial resistance among commensal bacteria is a major challenge in modern world. It will transfer the plasmid mediated resistance determinants through horizontal gene transfer method to pathogenic bacteria thus leading to origin of MDR and treatment failure among hospitalized patients. In present study, plasmid mediated *fusB* and *fusC* resistance determinant were detected 8.44% and 5.77% among clinical isolates of CoNS. Only 4.54% of *fusB* gene was detected among MRCoNS isolates. All *fusB* detected isolates in clinical specimens carried SCCmec III and SCCmec IV. Among nasal colonization, 7.31% of *fus B* were detected in Out-Patient visitors followed by 5.35% in HCWs and 5.12% in In-patient visitors. 2.56% of *fusC* genes were detected In-Patient Visitors followed by 1.78% in HCWs and we did not detect *fusC* gene in Out-Patient visitors. Among MRCoNS of nasal colonization, fus *B* was detected in 33.33% of Patient visitors and not HCWs in current study.

Mupirocin is used as nasal decolonization of Methicillin Resistance Staphylococcus and also used as topical ointment for the treatment of skin and wound infections. In present study, 5.33% of Mupirocin resistance was detected genotypically among these, Low level Mupirocin resistance (LLMR) was detected 4% *(iles-1* gene) followed by High level Mupirocin Resistance (HLMR) was *1.33%* (*mupA* gene) and most of the isolates carried SCCmec IV followed by other types. Mupirocin resistance among HCWs and Out-Patient visitors of CoNS was not detected in current study. 2.56% of isolates detected low level Mupirocin resistant (LLMR) determinants. 9.09% MR-CONS of clinical isolates carried *dfrA* gene which is responsible for Trimethoprim resistance

followed by 33.33% of In and Out-Patient visitors. Majority of the *dfrA* positive MR CoNS detected SCCmec IV than other SCCmec types.

3.11% of Vancomycin resistant determinants were detected among CoNS of clinical isolates in current study and all these isolates carried SCCmec IV followed by SCCmec III and SCCmec V. 2.56% of Vancomycin resistant determinants were detected in In-Patient visitors and 4.87% in Out-Patient visitors and carried SCCmec II, III and V. We did not detect any Linezolid resistant determinants in current study among Clinical isolates, HCWs and Patient visitors of CoNS.

Co-existence of more than two resistant determinants among the multidrug resistant Staphylococcus is common. In Current study, 0.81% MRSA isolate of clinical isolates detected *erm B + msrA* carried SCCmec I , 4.06% isolate detected *tet M + tet K*, 1.62% carried SCCmec III detected *erm B+ tet K+ aac(6')-Ie-aph (2'')-Ia,* carried SCCmec II + III one isolate of MRSA from HCWs detected *erm C +tet K +mupA+ fus B,* carried SCCmec I one isolate of MRSA from in-patient visitors detected *dfrA +aph (3')-IIIa + tet M + erm A + rpl C.*, carried SCCmec IV. In Present study, 2.27% of MRCoNS clinical isolates detected *dfrA +aph (3')-IIIa + van A* and carried SCCmec II +V, 1.13% MRCoNS clinical isolates detected *fus B + mup A + erm C +dfr A* and carried SCCmec III. Out-Patient visitors of MRCoNS detected combination of *msrA + tet K + van A* genes in one MRCoNS isolate of In-Patient visitors and carried SCCmec II, one isolate carried *msrA + tet L + van A,* carried SCCmec III and *msrA+ tet L + fus B,* carried SCCmec V. In current study, 3 isolates from In-Patient visitors MRCoNS carried *mup A + fus B* and carried SCCmec III + V, *tet K + tet M + van A* and detected SCCmec IV and *msr A + erm C +erm B + tet K*, detected SCCmec II.

6.6 Conclusions:-

The present study showed the prevalence of antibiotic resistance determinants among the Clinical specimens, Nasal colonization of HCWs and Patient visitors of CoNS and *S.aureus* and its associated SCCmec types. The present chapter concludes that, CoNS are emerging antibiotic resistant pathogens and increased level of antibiotic resistance is detected in *S.aureus.* 35.14% of Methicillin Resistant *S.aureus* and 16.00% of MRCoNS isolates detected antibiotic resistant determinants among clinical specimen. In present study most of the antibiotic resistant determinants carried SCCmec IV followed by

SCCmec type III and V. 9 isolates of *S.aureus* and 5 isolates of CoNS among Clinical isolates, HCWs and Patient visitors detected multiple resistant determinants in single pathogens. Screening of antibiotic resistant determinants and its associated SCCmec types is an major epidemiological tool in hospital infection control practices to prevent the spread of multidrug resistance pathogens.

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80 Recommendation

Chapter-7

80-Recommendations

7.1 Recommdations:-

The study has contributed to understanding the antibiotic resistance profile among Staphylococcus species from Clinical and Nasal colonization. The recommendation as follows

a. In this study, Staphylococcus was identified by conventional Microbiology method. It is recommended to conduct a study on detection of Staphylococcus species by molecular based analysis like species specific RT-PCR, 16s rRNA and whole sequencing. It will yield more accurate data.

b. SCCmec types were done by Multiplex PCR and SCCmec I to Sccmec type V were typed. It is recommended that SCCmec type can be done by sequence analysis of SCCmec, Multi-locus sequence typing, Pulse field electrophoresis and identification of clonal complexes of Staphylococcus will give accurate results of SCCmec types and strain circulation in particular region.

c. Antibiotic resistant determinants were identified by multiplex and simplex conventional PCR. It is recommended that RT-PCR and plasmid analysis can be done which will provide more information about antibiotic resistance mechanisms.

7.2 Summary and Conclusions

Antimicrobial resistance (AMR) is an important public health concerns in modern medicine. Infections caused by multi drug resistance pathogen could lead to prolonged hospitalization, increased mortality, causing heavy financial burden to affected patients, health-care system and hinder the sustainable development goals. Antimicrobial resistance is a natural phenomenon; it develops by the selection pressure of antimicrobials in both animal and human sectors. During the past few decades, novel mechanisms and spread of antibiotic resistant determinants have been identified. For example, Vancomycin Resistance Enterococci (VRE), Multidrug resistance pathogens can be treated with higher end and last resorts of antibiotics. These include ESKAPE group of Microorganisms namely, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella*

pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa and *Enterobacter* species. AMR studies from India are very limited to quantify the nationwide mortality caused by AMR pathogen [1].

In present study, 43.75% of *S.aureus* was isolated from Clinical specimen and 56.25% of CoNS. Among CoNS, *S.epidermidis* was isolated highest in number followed by *S.haemolyticus, S.saprophyticus, S.warneri, S.hominis, S.simulans, S.schleferi, S.hyicus and S.capitis.* Among clinical specimens, more CoNS were isolated from Urine and Wound/Pus swabs. These are the common CoNS isolated from clinical specimens and act as emerging pathogens.

Nasal colonization of *S.aureus* among HCWs, In-Patient Visitors and Out-Patient Visitors were 62%, 29.6% and 23.83%. We isolated CoNS from these groups to be 38%, 22.67 % and 23.83% respectively. We observed more Staphylococcus among HCWs followed by others. This indicates that, both community people and HCWs acts as vectors to transfer the unwanted Staphylococcal pathogen to hospitalized patients and also transfer the antibiotic resistant determinants to those hospitalized patient who are in close contact. Among CoNS we isolated *S.epidermidis, S.haemolyticus, S.saprophyticus, S.warneri, S.hyicus, S.hominis and S.lugdunensis.* All these are isolated CoNS are common skin flora and may acts as opportunistic pathogens. They carry unwanted antibiotic resistance determinants in their plasmids and may transfer it to other pathogens or they may get acquired resistance by high antibiotic pressure.

MRSA is commonly resistant to all beta-lactam antibiotics and considered as multidrug resistant pathogen. In the present study 45.71% of *S.aureus* in clinical specimens detected MRSA; followed by 29.26% in Out-Patient visitors. 27.17% in HCWs, 27.4% in In-Patient visitors by the Cefoxitin disc diffusion method. Bacteriological etiology of HCWs, Patient visitors and Clinical specimens of Staphylococcus and their antimicrobial susceptibility pattern are seen to vary with different populations, types of hospitals in different region and country. The results of this study cannot be exactly compared with other studies as different population; hospital protocols and criteria used for diagnosis are varying from institution to institution. 19.55% MRCoNS were observed in clinical specimens, 7.5% in Patient visitors and MRCoNS were not detected among HCWs. This indicates that CoNS among clinical specimens and Patient visitors are increasing and HCWs may not act as vector to transfer

MRCoNS to other community or hospitalized patient However, HCWs may get MRCoNS in future. Hence, frequent screening is essential to monitor the MRCoNS in HCWs to prevent the spread of resistant factors to others. Hospital health care workers are more prone to acquire these pathogens than the other people and proper infection control practices should be followed to control them.

We observed 11.43% Vancomycin resistant *S.aureus* among In-patient visitors followed by 9.75 % in Out-Patient visitors, 7.71% in clinical specimens and 5.43 % to HCWs. In our hospital antibiotic stewardship policy have maintain strictly and usage of Vancomycin in our hospital is limited hence, the Vancomycin resistance rate is very low than Patient visitors. Vancomycin is used for the treatment of MRSA infection but overuse of Vancomycin in hospital has led to emergence of Vancomycin resistance *Staphylococcus*. In the present study, Vancomycin resistance among CoNS of Clinical isolates and In and Out Patient visitors was 2.66%, 7.69% and 7.31%. We did not detect Vancomycin resistance among CoNS of HCWs. Linezolid is a last resort antibiotics against the *Staphylococcal* infection, we observed Linezolid resistance only in clinical specimens of *S.aureus* (2.57%) and CONS (1.11%). This indicates that Linezolid antibiotic usage is limited in our region. Clinical specimens detected very less Linezolid resistance than other antibiotics.

All MRSA isolates were screened for *pvl* gene. It is a leukocidin toxin which damages the membrane of host immune cells. It is most commonly seen in Skin and soft tissue infections and community associated MRSA infection. In the present study, 39.51% *S.aureus* isolates of clinical specimen, HCWs and Patient visitors detected mecA gene. All MRSA positive isolates were screened for *pvl* gene. Of these, 35.07% of clinical isolates of *mecA* positive *S.aureus* detected *pvl* gene and most of the isolates were from Wound/pus swabs. This clearly states that *pvl* gene is associated with skin and soft tissue infection. 3.31% of Out-Patient visitors, 2.84% of In-Patient visitors and 5.21% of HCWs detected *pvl* gene in our study.

All *mecA* positive *S.aureus* isolates were typed into SCCmec types (I to V) by Multiplex PCR. SCCmec IV was found higher in number followed by SCCmec V, SCCmec III, SCCmec I and SCCmec II. All *pvl* gene positive isolates carried SCCmec IV and V and confirmed that all *pvl* isolates are community associated. 19.55% of CoNS of clinical specimens detected *mecA* gene followed by 3.33% of In-Patient visitors and

3.26% of Out-Patient visitors. All these isolates were screened for *pvl* gene. Among these, 15.90% of Clinical specimens detected *pvl* gene followed by in and Out-Patient visitors. All these isolates were screened for SCCmec types. Among these, SCCmec IV was found maximum in number followed by SCCmec V, SCCmec III, SCCmec II and SCCmec I. 13 isolates of MR CoNS were not typed. It may be due to other SCCmec types which are common in other Indian studies.

In Genotypic detection of Vancomycin resistant determinants, 7.71% *van A* gene was detected in *S.aureus* of clinical specimens followed by In-Patient visitors (7.84%), Out-Patient Visitors (7.31%) and HCWs (6.52%). Among CoNS 4.87% *vanA* was gene detected in Out-Patient visitors followed by Clinical specimens (3.11%) and In-Patient visitors (2.56%). This might be due to the CoNS acting as a reservoir of drug resistance or it can transfer from *Enterococcus* spp to transfer resistance to *S.aureus*. Linezolid resistance was observed only in clinical specimens of *S.aureus* and CoNS. We detected *cfr* gene 0.57% of *S.aureus* and it was not detected in CoNS. All *cfr* positive isolates were resistant to Chloramphenicol. Mutations in ribosomal proteins genes, *rplC* were detected in both *S.aureus* and CoNS. r*plD* was detected only in CoNS, mutation in domain V region of 23S r RNA was detected only in *S.aureus.* Linezolid resistance in our study is lower in number than other Indian studies. We detected higher level Mupirocin Resistance gene (*mupA)* than the low level Mupirocin resistance and considerable amount of Fusidic acid resistance was also detected. A maximum number of resistant were observed in Tetracycline followed by Erythromycin and others [Table 6. 11 in Chapter 6].

Co-existence of more than two resistant determinants among the multidrug resistant *Staphylococcus* is common. In Current study, 0.81% MRSA isolate of clinical isolates detected *erm B + msrA* carried SCCmec I, 4.06% isolate detected *tet M + tet K* and 1.62% *erm B+ tet K+ aac(6')-Ie-aph (2'')-Ia* and carried SCCmec III followed by SCCmec II + III. One isolate of MRSA from HCWs detected *erm C +tet K +mupA+ fus B,* carried SCCmec I. one isolate of MRSA from in-patient visitors detected *dfrA +aph (3')-IIIa + tet M + erm A + rpl C.,* carried SCCmec IV.

In the Present study, 2.27% of MR-CoNS clinical isolates detected *dfrA +aph (3')-IIIa + van A* ,carried SCCmec II +V, 1.13% MRCoNS clinical isolates detected *fus B + mup A + erm C +dfr A* and carried SCCmec III. Out-Patient visitors and In-Patient visitors of MRCoNS detected combination of *msrA + tet K + van A* genes and carried

SCCmec II. Out-Patient visitors of CoNS carried *msrA + tet L + van A*, SCCmec III and *msrA+ tet L + fus B*, carried SCCmec V. In current study, 3 isolates from In-Patient visitors of MRCoNS detected *mup A + fus B* and carried SCCmec III + V, *tet K + tet L + van A*, SCCmec IV and *msr A + erm C = erm B + tet K*, SCCmec II.

Emergence of antibiotic resistance is an emerging and growing problem for public health. Acquired resistance not only found in clinical pathogens, it may get resistance via Horizontal gene transfer method from environmental bacteria, Commensal bacteria etc. Horizontal gene transfer from commensal or environmental bacteria to pathogenic bacteria is significantly important. The emergence of multidrug resistance is significantly increasing and cause serious problem in public health system. The Review on Antimicrobial Resistance, reported by the Government of United Kingdom stated that AMR could kill 10 million people per year by 2050 [2]. If spread of AMR is not checked, it could generate many resistant bacteria and get more lethal in the future than they are today. Major challenge in AMR tackling is to understand the burden of true resistant bacteria pathogens, particularly in locations where surveillance is minimal and data are very limited. There is more literature estimating the effects of AMR like incidence, deaths, hospital length of stay, and healthcare costs but, to our knowledge, no comprehensive estimates covering all locations and a broad range of pathogens and pathogen–drug combinations have ever been published [2]. Our study estimates that the prevalence of antibiotic resistance determinants in tertiary care hospital, would help to understand the prevalence rate and to control the overuse age of antibiotics in hospital and stop the over the counter of antibiotics in community. To control the Antibiotic resistance the following methods should be standardized in hospital practices, Standardized the antibiotic protocols in Skin and Soft tissue infection treatment, minimize the usage of antibiotics in unwanted Uro-pathogens prescribing practices of physicians, antibiotic patterns in emergency treatment, National wide Antibiotic Policy, Active Infection control committee etc.

7.3 Future work:-

- In future, we plan to do multicentric study (Different parts of the Country) and to assess the SCCmec types
- To study the Virulence property of CONS, Plasmid characterization and whole genome sequencing analysis of Multidrug resistant Staphylococcus
- Identification of CRISPR Cas 9 system among Staphylococcaceae family
- To develop a model of CRISPR Cas 9 system to Combat antibiotic resistant among *Staphylococcus aureus.*

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