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KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE

MICROBIOLOGICAL STUDY OF NASAL CARRIAGE OF PATHOGENIC BACTERIA AMONG THE HOSPITAL PERSONNEL

DLSHAD ABDALLA DEWANA

MASTER’S THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

KAHRAMANMARAŞ, TURKEY 2014

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Thesis submitted in candidature for

the degree of Master in

Department of Bioengineering and Sciences

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DECLARATION

I hereby declare that all information in the thesis has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Dlshad ABDALLA DEWANA

Note: The original and other sources used in this thesis, the Declaration, tables, figures and photographs showing the use of resources, subject to the provisions of Law No. 5846 on Intellectual and Artistic Works.

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HASTANE PERSONELİ ARASINDA PATOJEN BAKTERİLERİN NAZAL TAŞIYICILIĞI İLE İLGİLİ MİKROBİYOLOJİK ÇALIŞMA

(YÜKSEK LİSANS TEZİ)

DLSHAD ABDALLA DEWANA

ÖZET

Sağlık personelinde *Staphylococcus aureus* gibi patojenlerin nazal taşıyıcılığının varlığı, bu patojenlerin hastalara bulaştırılmasında önemli bir rezervuar olabilmektedir. Bu tezin amacı, Şubat 2014-Ağustos 2014 tarihleri arasında Kuzey Irak bölgesinin Qaladze kentinde bulunan Qaladze General Hospital'deki 130 sağlık personeline ait nazal örneklerde patojen bakterilerin araştırılmasıdır. Çalışmada toplanan nazal svap örneklerinden standart mikrobiyolojik yöntemler ile Stafilokoklar izole edilerek, bu bakterilerin Kirby-Bauer disk difüzyon yöntemi ile antimikrobiyal duyarlılık testleri yapılmıştır. Sonuç olarak; 130 örnekten, *Staphylococcus aureus* bakterileri %85.38 oranında, Koagülaz negatif stafilokok (KNS) bakterileri ise %14.61 oranında izole edilmiştir. *S.aureus* izolatlarının % 42.10 oranında metisiline dirençli iken, %57.90'u ise metisline duyarli idi. KNS izolatlari ise, %52.25 oraninda metisiline dirençli iken, %47.75 oraninda duyarlı bulundu. En etkili antimikrobiyaller sırası ile vancomycin ve imipenem iken, bunları ciprofloxacin takip etti ve diğer antibiyotiklerde ise direnç oranı daha yüksek bulundu.

Anahtar kelimeler : Nazal taşıyıcılık, Bakteriyal patojen, Hastane personeli.

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MICROBIOLOGICAL STUDY OF NASAL CARRIAGE OF PATHOGENIC BACTERIA AMONG THE HOSPITAL PERSONNEL

(M.Sc. THESIS)

DLSHAD ABDALLA DEWANA

ABSTRACT

Nasal carriage of *Staphylococcus aureus* in health care staffs may be a significant reservoir for transmission of the infective agent to patients. The aim of the study was to screening for coagulase positive methicillin resistant *Staphylococcus aureus* in healthcare workers and prevalence of nasal colonization with *Staphylococcus aureus* in Qaladize general hospital in Qaladize city from February 2014 to August 2014. Total of 130 nasal specimens were isolated from nasal health care worker in the hospital. The nasal carriage Staphylococci were isolated by standard microbiologic methods and antimicrobial susceptibility testing were detected by the Kirby-Bauer Disk diffusion method. The results showed out of total 130 samples, (14.61%) were coagulase positive *Staphylococcus aureus* and (85.38%) were CoNS. Out of total 19 isolates of CoPS, (42.10%) were found to be resistant to methicillin and (57.90%) CoPS isolates were methicillin susceptible *Staphylococcus aureus*. Out of total 111 isolates of coagulase negative Staphylococcus (CoNS), (52.25%) were found to be resistant to methicillin (Methicillin resistant coagulase negative staphylococci), and (47.75%) of them were Methicillin sensitive coagulase negative staphylococci. The effective antimicrobial agents are vancomycin, imipenem and followed by lower degree of ciprofloxacin, these bacteria have more resistant against to other antibiotics.

Key words: Nasal carriage, bacterial pathogens, hospital personnel.

Kahramanmaraş Sütçü İmam University

Institute for Graduate Studies in Science and Technology

Department of Bioengineering and Sicences, December/2014

Supervisor: Assoc. Prof Dr. Ekrem KİREÇCİ

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ABBREVIATIONS

HCS:	Health care staff
CA-MRSA:	Community-associated methicillin-resistant *Staphylococcus aureus*
HA-MRSA:	Hospital or Health care-associated meticillin-resistant *Staphylococcus aureus*
CFU:	Colony forming unit
MRSA:	Methicillin-resistant *Staphylococcus aureus*
MSSA:	Methicillin-sensitive *Staphylococcus aureus*
MRCoNS:	Methicillin-resistant coagulase negative *Staphylococcus*
MSCoNS:	Methicillin-sensitive coagulase negative *Staphylococcus*
MSCRAMMs:	Microbial Surface component recognizing adhesive matrix molecules
MIC:	Minimum inhibitory concentration
MH:	Muller-Hinton
NCCLS:	National committee for clinical laboratory standards
PVL:	Panton valentine leukocidin
PBP:	Penicillin-Binding Protein
PCR:	Polymerase chain reaction
S.aureus	*Staphylococcus aureus*
SANC:	*Staphylococcus aureus* nasal carriage
SCCmec:	Staphylococcal Cassette Chromosome mec
VISA:	Vancomycin Intermediate *Staphylococcus aureus*
VRSA:	Vancomycin-resistant Staphylococcus aureus
BSAC:	British Society of Antimicrobial Chemotherapy
CLSI:	Clinical Laboratory Standards Institute
EONS:	Early-onest neonatal sepsis
LONS:	Late-onset neonatal sepsis
NICHD:	National Institutes of Child Health and Development
CSF:	Cerebrospinal fluid
HH:	Hand hygiene

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1. INTRODUCTION

In humans and mammalian cells are outnumbered by microbes by a factor of ten; on average an individual consists of 10^{13} mammalian cells and 10^{14} cultivable microbial cells. Most body surfaces that are exposed to the external environment are colonized by microbes. Several factors may affect the number and types of microbes at a specific body site, including age, gender, host genotype, hormones, diet, hygiene, clothing, climate, occupation and living conditions (Sangvik, 2012).

Nose is the sense organ for the sense of olfaction or smell. The receptor cells for olfaction are chemoreceptor's, stimulated by chemicals. The receptor cells along with other cells form the olfactory epithelium. The nose acts as air-conditioning device, which performs the functions of heating, humidifying, and removing particulate and gaseous pollutants from inspired air before it reaches the sensitive lungs (Yu, *et al*., 2014).

The nasal mucosa is characterized by a complex micro vascular anatomy. It consists of a dense sub epithelial network of capillaries (Muller, *et al*., 2014).

Staphylococci were first isolated by Koch (1878)s and Pasteur in 1880s, but Ogston and Rosenbach made the initial detailed studies in 1881s Ogston named the clustered micrococci "Staphylococci" from the Greek word (Staphyle) meaning bunch of grapes (Ibrahem, 2010).

Staphylococci are belonging to the family Staphylococcaceae, order Bacillales, class Bacilli, phylum Firmicutes, and the genus *Staphylococcus* (Muhammad and Al-Mathkhury, 2014).

Staphylococci diameters are between (0.5–1.5) µm and characterized by individual cocci, which divide in more than one plane to form grape like cluster, there are 32 species and eight sub-species in the Genus *Staphylococcus*, many of which preferentially colonies the human body (Harris, *et al*., 2007).

Staphylococcus aureus (*S. aureus*) is a microorganism that colonizes the skin and mucosal surfaces of healthy individuals, but it is also one of the most common causes of community-acquired and hospital infection, Nasal carriage of *S. aureus* represents a major risk factor for the development of infection with this bacterium (Dinic, *et al*., 2013).

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Minor skin infection is pimples, impeligo, boils, carbuncles, scalded skin syndrome, folliculitis, cellulitis, and abscesses, also with serious diseases that includes meningitis, osteomylitis, pneuomonia, endocarditis, chest pain, and toxic shock syndrome (Wakabayashi and Ching, 2012).

Optimal combination antibiotic treatment for Methicillin resistant *S.aureus* (MRSA) bacteremia has not been established and the ability of MRSA to produce severe invasive disease, frequently in otherwise healthy individuals, reflects multiple reflects multiple virulence mechanisms for host tissue invasion and immune evasion (Sakoulas, *et al*., 2013).

The Virulence mechanisms for host tissue invasion and immune evasion. *S. aureus* colonies are found in approximately 30% of the general population in the world (Kirecci. *et al*., 2010).

The anterior are the primary reservoir of *S.aureus* in humans and its nasal carriage is recognized as a higher risk factor for the development of both nosocomial infections and community-acquired (Elazhari, et *al*., 2014).

MRSA It is a type of *S. aureus* which gained resistance to common antibiotic used for *Staphylococcus* infections such as methicillin, Bacteria develop resistance when they acquire new genetic material that encodes for resistance and it is transferred to other strains (Lyer, *et al*., 2014).

MRSA is a multidrug resistant organism that threatens the continued effectiveness of antibiotics worldwide and causes a threat almost exclusively in hospitals and long-term care settings. MRSA is a global public health problem, associated with considerable morbidity and mortality (Buzaid, *et al*., 2011; Ray, *et al*., 2011).

MRSA infections are more difficult to treat than ordinary staph infections, because the strains of staph known as MRSA do not respond well to many common antibiotics used to kill bacteria (Batabyal, *et al*., 2012).

The spread and emergency of both community-associated and health-care MRSA challenges both infection control interventions and treatment (Moremi, *et al*., 2012).

MRSA infection can be prevented by screening programmes, handwashing, proper disposal of hospitalgowns and wastes, judicious use of antibiotics, infectivesurfaces sanitizing (Ahmad, and Asrar, 2014).

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MRSA has been associated with major rates of patient morbidity and mortality than methicillin-sensitive strain. MRSA was first reported in the early 1960s, and rapidly increased and spread in the 1980s (Goyal, *et al*., 2013; Cirkovic, *et al*., 2014).

The major problem in the treatment of staphylococcal infections is methicillin resistance. As methicillin-resistant staphylococci are also resistant to beta-lactam antibiotics which include the penicillins (anti-staphylococcus penicillin such as methicillin, dicloxacillin, nafcillin, oxacillin, etc.), therefore is a need for other antibiotics that can be an alternative to glycopeptides in the treatment of staphylococci infections. In the last ten years, new antibiotics such as daptomycin (lipopeptide), linezolid (oxazolidinones), quinupristin/dalfopristin (streptogramin combination), telithromycin (ketolide), and tigecycline (glycylcycline) have been developed (Oksuz and Gurler, 2013).

Methicillin resistance is conferred on the organism by the presence of a unique mobile genetic element called the SCCmec carrying the mecA gene (Shambat, *et al*., 2012). Staphylococci have five major SCCmec different (I to V) have been recognized, that appear to be variously distributed across *Staphylococcus* sp. (Corrente, *et al*., 2009).

The SCCmec element carries two essential parts, the mec gene complex consist of mecA and its regulator genes and the cassette chromosome recombinase (ccr) complex composed of recombinase gene (Han, *et al.*, 2013).

MRSA infections cause a large number of deaths every year in the worldwide (Koyam, *et al*., 2013).Human MRSA infections have been categorized into three groups based on their putative sources, health care-associated MRSA, community-associated MRSA, and health care-associated MRSA with community onset (Price, *et al*., 2012).

Community-acquired (CA) MRSA is most often associated with skin and soft tissue infections in young healthy individuals with no recent health care exposure; CA-MRSA was initially reported among injection drug users in the early 1980 and has since become the most frequent cause of skin and soft tissue infections. Most CA-MRSA strains carry SCCmec type IV or V but the larger of SCCme types I, II, III, are present in MRSA traditionally associated with infections in health care facilities (Sfeir, *et al*., 2013; Udo and Sarkhoo, 2010).

Beta-lactam antibiotics are capable of killing different types of Gram positive and Gram negative bacteria by impairing the cell's ability to form peptidoglycan, that necessary component for cell structure and stability. Bacteria that have become resistant by

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produced enzymes known as penicillinases and beta-lactamases, which are capable of destroying the beta-lactam ring structure or functional group of these antibiotics. Methicillin Sensitive *Staphylococcus aureus* (MSSA) causes a type of staph infection that is moderately sensitive to these antibiotics (Sharma, et *al*., 2013).

MSSA, MRSA have been implicated in a variety of endemic and epidemic nosocomial infections worldwide, approximately 20% of healthy adults are persistent nasal carriers of this potential pathogen and 60% harbor the organism intermittently and appear to play a key role in the epidemiology and pathogenesis of infection (Akhtar, 2010).

MRSA and MSSA have few structural differences, but their epidemiologies differ profoundly in term of colonization, infection, and transmission (Lucet, and Rengier, 2010).

In patients who are highly allergic to penicillins and cephalosporins, vancomycin or daptomycin can be used to treat MSSA (Karchmer, 2012).

In the MSSA group treated with antimicrobial therapy without an indication to surgery, all patients survived, and in the combined surgical and antimicrobial group 29% died (Hill, *et al*., 2007).

Nasal MRSA and MSSA colonization is seen in the hospital personnel and patients. With regard to MRSA/MSSA carriers, both the individual carriers themselves and the other people around them are at risk, and this bacterium leads to nasal colonization and can then propagate through contaminated hands and hospital materials (Shibabaw, *et al*., 2013).

Gram-positive coagulase-negative staphylococci (CoNS) are a subgroup of staphylococci, which can be distinguished from *S. aureus* by their lack of ability to produce coagulase enzyme (Hira V, 2013). Thirty-eight different species are found within the CoNS group (Chah, *et al*., 2012).

CoNS represent group of opportunistic microorganism that are common inducing agents of bacteremia and other hospital infection, particularly in the patients with medical implants (central and peripheral venous catheters, valvular prostheses, artificial heart valves, pacemakers and orthopedic prostheses), as well as in immunocompromised subjects (Povazan, *et al*., 2014).

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The treatment of patients with these infections is becoming more complex, greatly increasing the costs of both hospitalization and treatment of these patients in public hospitals (Almeida, *et al*., 2014).

CoNS are one of the most frequent causative agents of neonatal nosocomial infections, especially in premature and low-weight newborns (Oliveira, *et al*., 2012).

As the health care staff such as nurses, doctor and laboratory staffs are on continuous exposure to various clinical samples, the probability of their skin flora especially CoNS to become altered by the acquisition of antibiotic resistance are major (Soumya, *et al*., 2013).

Health care staffs (HCS) are at the interface between hospital on one hand and community on the other. They may serve as reservoirs, vectors or victims of drug resistant bacteria and the major source of staphylococcus for nosocomial infections is nasal carriage among hospital personnel (Baragundi, *et al*., 2012).

CoNS are characterized by their ability to adhere to and grow on solid surfaces and, subsequently, to produce a polysaccharide slime; this extracellular slime may protect the bacteria against immunological host defense mechanisms and antimicrobial therapy (Uyanik, *et al*., 2014).

CoNS usually formed as contaminants inclinical specimens e.g. in Swab from the skin, nose, throat, wounds, burns and bedsores, the most clinically significant species in this group (CoNS) are *S.epidermidis* and *S.saprophyticus*. Most of these species have been isolated from humans usually found inhabitants the skin and mucous membranes (Badwi, *et al*., 2012).

CoNS produces an extracellular matrix called slime layer (Biofilm), biofilm in CoNS have been divided in to three tyes, biofilm positive, biofilm weak, and biofilm negative (Al-kadmy, 2013).

The pathogenesis of CoNS infections depends on their ability to form biofilms on polymer surfaces. This biofilms formed by CoNS clinical isolates possess an extremely high level of tolerance to multiple antibiotics. After contact and attachment to surface, the bacterial cells cause embedded in an extralcelluler polymer substance [EPS], which is consist of water, exopolysaccharide, extralcelluler DNA, lipids and proteins (Qu, *et al*., 2010).

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A biofilm is a group of microorganisms that attach to each other on a surface. The biofilm consist of a single species, but many biofilms include a mixture of various species, including bacteria, fungi, yeast, protozoa. Bacterial biofilms are important because they can be helpful (in water and nutrient cycling), but also harmful persistant infections in the human body and build up on medical equipment/prothestic devices (Bateman, *et al*., 2011).

CoNS colonize different parts of the human skin and mucous membranes. Every species of CoNS that has been characterized as a resident in humans (*S. epidermidis, S. capitis, S. haemolyticus, S.warneri, S. hominis, S. lugdunensis, S. saccharolyticus, S. cohnii, S. saprophyticus*) (Wojtyczka, *et al*., 2014).

Methicillin-resistant coagulase negative staphylococci (MRCoNS) in asymptomatic patients is a high source of contamination, therefor by early identification and distinguish this bacteria we can reduce the risk of colonization of patients and cross-transmission between patients and health care staff, especially in hospital environment (Almeida, *et al*., 2014).

MRCoNS are opportunistic pathogens bacteria among HIV-AIDS patients and serve as a major source of staphylococcal cassette chromosome mec (SCCmec) that carries mecA (Saravanan, *et al*., 2014). Resistant to methicillin is regulated by SCCmec element (Corrente, *et al*., 2009).

MRCoNS are resistant organisms causing infections associated with high morbidity and mortality (Shah, *et al*., 2014).

Many of the studies have concentrated only on MRSA and not on MRCoNS which are equally important; MRCoNS are the important agents causing nosocomial infections (Mir, and Srikanth, 2013).

MRCoNS were also found in animals with clinical infection, The SCCmec elements that have in MRCoNS bacteria of food animals are transmissible to human through the food (Han, *et al*., 2013**)**.

CoNS have been reported to have methicillin resistant mediated by an altered pencillin-binding protien (PBP2a), that encoded by the mecA gene (Soumya, *et al*., 2013).

The mecA gene is inducible and encodes polypeptide with high molecular weight (78-kD PBP2a). It occurs in both MRSA and MRCoNS and is highly conserved. MRCoNS are frequent causes of nosocomial infection, extensive burn injuries (Ekrami, *et al*., 2010).

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Vancomycin is the mainstay of therapy in both (MRSA and MRCoNS) meningitis. MRSA or MRCoNS meningitis treated with linezolid between January 2006 and September 2010 in our hospital (Sipahi, *et al*., 2011).

Infections due to MRSA and MRCoNS are of special concern since they are always associated with prolonged hospital stay and increased cost of treatment. Also infection with MRSA and MRCoNS are associated with higher mortality compared to MSSA and MSCoNS due to multidrug resistance (Wei, *et al*., 2014).

MRCoNS is prevalent worldwide, These are considered as the major cause of hospital-aquired infection (HAI) and community-aquired infection (CAI), resulting in increased morbidity and mortality in the hospital settings, methicillin was first introduced in human medicine in 1960s for the treatment of infections caused by penicillin resistant *S.aureus*, however first methicillin resistant *S.aureus* emerged in 1961s in England (Perveen, *et al*., 2013).

MSSA and methicillin-sensitive coagulase negative Staphylococci (MSCoNS) are microorganisms that can present other resistance phenotypes of clinical interest, such as constitutive or inducible resistance to the macrolide, lincosamide, streptogramin B (MLSB) group (Martins, *et al*., 2012).

The Macrolide-Lincosamide-Streptogramin B (MLSB) family of antibiotics is commonly used in the treatment of Staphylococcal infections, and The MLS family of antibiotics has three different mechanisms of resistance: target site modification, enzyme antibiotic inactivation and macrolide efflux pumps (Sood, 2013).

1.1. The Aim of the study

The identification of niches within healthcare facilities that bear the highest burden of MRSA, MSSA, MRCoNS, MSCoNS as well as MRSA in the community may provide targets for treatment and prevention strategies. The aim of study:

1. Isolation and identification of major bacteria responsible for nosocomial infection with particular emphasis on *S.aureus*.
2. To screening MRSA in healthcare staff and prevalence of nasal colonization with *S. aureus*.

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3. Determine the prevalence and risk factors of nasal carriage of *S. aureus* and MRSA, MSSA, MRCoNS, MSCoNS among healthcare staff who work in environments with varying degrees of exposure to patients.

4. Studying the antimicrobial sensitivity profile of MRSA, MSSA, MRCoNS, MSCoNS.

5.To increase the knowledge of *S. aureus* virulence and pathogenesis as well as to Understand pattern of colonization and transmission.

6.To know the prevalence of nasal carriage, methicillin resistance and antibiotic susceptibility pattern of CoNS among healthcare staff.

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2. LITERATURE REVIEW

2.1. *Staphylococcus aureus*

2.1.1. Background

Staphylococci are very widespread bacteria. Their main representative *Staphylococcus aureus* is one of the most important and successful human pathogens, and it can be acquired in both the community and in hospitals. *S. aureus* is among the most ubiquitous of bacteria. The genus of *Staphylococcus* includes over 30 species and sub subspecies and its main representative, it is highly resistant to adverse environmental conditions and it resists drying as well as high NaCl concentrations. Therefore enables probably temporary and even permanent colonization of skin and nasal mucosa (Matouskova and Janout, 2008).

The anterior are the primary reservoir of *S. aureus* in humans and its nasal carriage is recognized as a major risk factor for the development of both community-acquired and nosocomial infections (Elazhari, *et al.,* 2014). *S. aureus* is a known colonizer in humans, and the anterior nares are the most common colonization sits (Deng, *et al.*, 2014).

Multiple body sites can harbor this bacterium. *S. aureus* is a common inhabitant of the skin, perineum and can also be found in the axillae, vagina, gastrointestinal tract, and the upper respiratory tract, particularly the nasal passages. *S. aureus* is easily identified by its ability to produce coagulase and hence clot human plasma (Sangvik, 2012).

S. aureus is the most common pathogen in nosocomial infections. Nasal carriage of the pathogen among hospital personnel is an important source of nosocomial infection (Ravi, *et al*., 2011). *S. aureus* can also cause invasive infections such as bacteremia, sepsis, endocarditis, pneumonia osteomyelitis (Nair, *et al.,* 2013). *S. aureus* is a human commensal colonizing about 30 percent of the population (Stark, 2013).

2.1.2. Identification

S. aureus is a spherical bacterium, non-motile, non-spore forming, facultative anaerobic bacterium, and they are approximately 1 µm in diameter. It cells form grape-like cluster, since cell division takes place in more than one plane. On a rich medium, *S. aureus* identification was performed by colony on sheep blood agar plates that forms medium size "golden" colonies. Colonies of *S. aureus* often cause β-hemolytic. The golden

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pigmentation of *S. aureus* colonies is caused by the presence of carotenoids and is reported to be a virulence factor protecting the pathogen against oxidants produced by the immune system (Plata, *et al*., 2009).

Carotenoids are the best pigments to recognized the major human pathogen, *S. aureus* that impart the eponymous golden color to it, this organism produces multiple carotenoid pigments via a well described biosynthetic pathway that culminates with golden staphyloxanthin as the major product and yellow, therefore the pigments are important, because colors often provide an easy way of identifying certain microbes, they are often used in names of species For example, Rosenbach in 1884 named the golden-colored pathogen *S. aureus* (Latin, ‘‘golden’’) (Liu and Nizet, 2009).

Golden yellow hemolytic or non hemolytic colonies on blood agar, yellow Mannitol fermenting colonies on Mannitol- salt agar (MSA) and mauve colonies on CHROM agar Staph aureus (CAS) were regarded as presumptive *S. aureus* and were confirmed with Gram stain reactions. Colonies of *S. aureus* may be atypical and difficult to differentiate from coagulase-negative staphylococci (CoNS), a large number of agglutination tests may be required to rule out the presence of *S. aureus* a number of culture media have been developed to increase the specificity of *S. aureus* detection, including Manitol-salt agar and Baird-Parker medium (Bakr and Selim, 2007).

Among the staphylococci, *S. aureus* is catalase positive and easily identified by its ability to produce coagulase enzyme and hence clot human plasma, the bound coagulase is also known as the clumping factor which binds with the alpha and beta chains of the fibrinogen in the plasma, and interacts with prothrombin in the blood causing plasma coagulate by converting fibrinogen into fibrin. Blood coagulation is used to distinguish *S. aureus* from other members of the genus (Sangvik, 2012).

S. aureus is one of the most significant pathogens causing nosocomial and skin infection, and it is an opportunistic bacterium, frequently part of the human micro flora, causing disease when the immune system becomes compromised, and it is belonging to Micrococaceae family, *Staphylococcus* is not halophilc, but rather haloduric, in that it can live in or endure high NaCl concentrations. The high salt content in SM1 10 and MSA inhibits other common skin microorganisms (Singh, *et al*., 2013).

The identification of *S. aureus* is generally done by staining methods (simple and Gram’s staining) and biochemical tests such as catalase test, coagulase test and mannitol

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fermentation test and oxidase-negative. It produces different types of virulence factors such as antigens such as capsules and adhesins; Enzymes such as coagulase, catalase, hyalurodinase and staphylokinase and toxins such as α–Toxin, β–Toxin, δ-Toxin, Panton-Valentine Leukocidin (PVL), Enterotoxin, Exfoliative Toxin, and Toxic Shock Syndrome Toxin (TSST) cause several diseases in human beings. According to coagulase enzyme the *Staphylococcus* species are classified into two types: coagulase positive and negative *Staphylococcus*. Usually such strains can produce two types of coagulase, free and bound. As the name suggests, free is secreted extracellular whereas bound is associated with cell wall associated protein (Babu, *et al*., 2014).

Most species of staphylococci have relative complex nutritional requirement; however, in generally they require an organic source of nitrogen, supplied by 5 – 12 essential amino acids, e.g. arginine, valine and B vitamins, including thiamine and nicotinamide. Staphylococci are tolerant to high concentrations of salt (NACL) and resistant to heat (Harris, *et al*., 2002).

Other tests include MASTASTAPH (Rapid latex agglutination slid test for *S.aureus,* MAST GROUP LTD company, UK) is based on the test principle described by Essers, L., and Radebold, K., 1980) who showed that the presence of bound coagulase (clumping factor) and Protein A could be detected using human plasma coated latex particles. MASTASTAPH latex reagent contains particles coated with fibrinogen and Immunoglobulin G (IgG). If mixed on a slide with *S. aureus* organisms, cell bound coagulase reacts with fibrinogen and/or protein A reacts with IgG causing rapid agglutination of the latex particles. MASTASTAPH identifies both clumping factor and protein A. (Ateba, 2010).

Microgen Staph (MICROGEN BIOPRODUCTS LTD company, UK) is a test for the identification of *S. aureus* which overcomes the problems associated with the traditional tests by detecting both coagulase and protein A offering rapid and accurate identification of *S. aureus* in 2 minutes. Microgen Staph contains all ancillary reagents needed to complete the test and is suitable for clinical, food and environmental laboratories, also available separately is the Staph latex control reagent (product code M43CCE), helping to reinforce the accuracy and reliability of the Microgen Staph rapid test. The isolates suspected to be *Staphylococcus spp*. were further identified with Microgen-MID-69 (Microgen Identification -69) microwell test strips. A single colony

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each, from 18-24 hours culture was emulsified in suspending medium supplied in the kit (Anyanwu, 2013).

Deoxyribonuclease (DNase) and tube coagulase tests were done to increase the efficiency of detection of *S. aureus*. In the tube coagulase tests, rabbit plasma and human plasma were used. However, human plasma was not recommended due to several factors (Amini, *et al*., 2012).

Deoxyribonucleases (DNases) are the enzymes which can break phosphodiester linkages of deoxyribonucleic acid (DNA). This method is used to confirm *S. aureus* which also shows positive result for coagulase test. This method helps to identify only those micro organisms which can degrade Deoxyribonucleic acid (DNA) in the medium thus, showing clear zone around the colonies showing DNase activity (Sheikh and Hosseini, 2013).

2.1.3. Virulence factors

Virulence factors help bacteria to invade the host, cause disease and evade host defenses. Virulence factors are protein molecules that are produced by bacteria to allow them to maintain infection in a host and cause disease. This organism produces virulence factors to help its attachment to the host cells, specifically attack and damage them, and prevent host immune system from attacking them (Al-Bayaty, *et al.*, 2012).

The competition between host factors and commensal organisms that resist colonization and *S.aureus* virulence factors that facilitate colonization and, possibly, subsequent infection. The constitutive properties of skin that help to prevent colonization and infection by *S. aureus* are its low temperature and acidic pH, both host coetaneous defense mechanisms and *S. aureus* virulence factors appear to be the focus of actively ongoing co-evolution, leading to major variations between different host species and bacterial strains (Ryu, *et al.*, 2014).

S. aureus is a highly successful pathogen of humans. Its virulence is mediated by an array of secreted and cell wall associated factors, with secreted proteases being amongst the best-studied, the primary goal of virulent factors may be to convert local host tissues into nutrients required for bacterial growth (Carroll, *et al.*, 2012).

The production of multiple virulence factors, as well as the presence of antibiotic resistance genes, makes *S. aureus* a highly pathogenic microorganism. The objective of

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this work was to study the susceptibility profile and toxin production of *S. aureus* strains isolated from various skin, soft tissue, and bone infections. The virulence factors produced by *S. aureus* that cause this organism are highly pathogenic. The known virulence factors include exotoxins, such as exfoliative toxins (ETs), along with toxic shock syndrome toxin-1 (TSST-1), staphylococcal enterotoxins (SEs), leukocidins (Panton-Valentine leukocidin; PVL, LukE/D), and hemolysins (α, β, γ, δ) . Enterotoxins often cause food poisoning, while ETs (also called epidermolysins) act on the skin. *S. aureus* is equipped with a great variety of virulence factors, which include both structural and secreted products participating in pathogenesis of infection (Sina, *et al*., 2013; Plata, *et al*., 2009).

2.1.3.1. Adherence factors

These are attachment devices such as pili, fimbriae and adhesins which enable pathogenic bacteria to adhere to host cells (Donkor, 2013).The host and bacterial factors that facilitate nasal colonisation remain to be fully characterized. *S. aureus* adheres to the squamous epithelial cells found in the nose. Proteins expressed on the surface of *S. aureus*, including clumping factor B (ClfB), we demonstrate that the squamous epithelial cell envelope protein loricrin represents the major target ligand for ClfB during *S. aureus* nasal colonization. In vitro adherence assays indicated that bacteria expressing ClfB bound loricrin most likely by the ‘‘dock, lock and latch’’ mechanism (Mulcahy, *et al*., 2012).

The attachment of *S. aureus* to the host cell surface initiating the colonization process is mediated by several adhesions to colonization of human nasal mucosa and skin, *S. aureus* expresses various factors that facilitate skin surface binding and survival. To bind to host surface components such as fibrinogen, fibronectin and cytokeratins, which are derived from epidermal keratinocytes or nasal epithelium. *S. aureus* utilizes microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), which include fibronectin-binding protein A (Fnbp A) and (Fnbp B), fibrinogen-binding proteins (ClfA and ClfB), iron-regulated surface determinant A (IsdA) and wall teichoic acid (Ryu, *et al*., 2014).

The ability of *S. aureus* to adhere to plasma and extracellular matrix (ECM) proteins deposited on biomaterials is a significant factor in the pathogenesis of device associated infections (Harris, *et al*., 2002). The staphylococcal cell wall is a dynamic, semi-rigid structure. It is composed of three components: peptidoglycan, teichoic acids, and surface protein, this protein contain asignal sequence directing their secretion and the

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LPXTG motif, the LPXTG motif is cleaved by sortase and than the protein covalently attached to the peptidoglycan, protein A, fibronectin binding protein, collagen binding protein and clamping factor A (Plata, 2009).

Attachment of *S. aureus* to the host cell surface initiating the colonization process is mediated by several adhesins. One major class of *S. aureus* adhesins comprises proteins covalently anchored to cell peptidoglycans (via the threonine residue in the sorting signal motif at their C-terminus), which specifically attach to the plasma or extracellular matrix (ECM) components and collectively are termed the microbial surface component recognizing adhesive matrix molecules (MSCRAMMs). Multiple MSCRAMMs contribute to nasal colonization, including ClfB, which binds cytokeratin-10 in nasal epithelial cells, (Bien, *et al*., 2011).

2.1.3.2. *Staphylococcus aureus* exoprotreins

Nearly, all strains of *S. aureus* secret a group of exoproteins such as exotoxins and enzymes, including nucleases, proteases, lipases, hyaluronidase, and collagenase. The main function of these proteins may be to convert local host tissue into nutrients required for bacterial growth (Bien, *et al.*, 2011).

Invasins include a wide range of extracellular enzymes or proteins are enabling bacterial pathogens to invade host tissues. *S. aureus* produces a wide range of invasins including hyaluronidase which breaks down hyalauronic acid of connective tissues, DNases which break down DNA, haemolysins which split red blood cells, staphylokinase which activates plasminogen to plasmin, an enzyme digesting fibrin clots. Several other invasins such as proteases, lipases, nucleases, collagenase and elastase are produced by *S. aureus* (Donkor, 2013).

Many bacteria produce secreted virulence factors called exotoxins. Exotoxins are often encoded by mobile genetic elements, including bacteriophage (phage). Phage can transfer genetic information to the bacteria they infect. When a phage transfers virulence genes to an avirulent bacterium, the bacterium can acquire the ability to cause disease (Casas, *et al*., 2010).

Toxins produced by microorganisms are important virulence determinants responsible for the pathogenicity of the pathogen and/or invasion of the host immune response. The exotoxin is highly thermolabile and rarely shows lethal effect (Kashid, S. And Ghosh, J., 2010).

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S. aureus produces exotoxins the cytolytic activity of the posses. *S. aureus* secretes a large number of cytolytic toxins that, although structurally diverse and with different target specificity, share a similar function on host cells. These toxins form β-barrel pores in the cytoplasmic membranes of target cells and cause leakage of the cell's content (when at low doses) and cell lysis (at high doses). Cytolytic toxins are such as α, β, and γ-hemolysin, leukocidin, and Panton-Valentine leukocidin (PVL) (Costa, *et al*., 2013).

Alpha-hemolysin monomers are secreted by *S. aureus* and integrate into the membrane of a target cell, and the most characterized virulence factor of *S. aureus*. Upon binding to the cell surface, α-hemolysin monomers assemble into a homoheptamer, forming a prepore. The prepore subsequently transitions to a mature β-barrel transmembrane pore. This pore allows the transport of molecules smaller than 2 kD, such as K+ and Ca2+ ions, leading to necrotic death of the target cell. These pores are oligomerized form which is capable of lysin eukaryotic cells (Vandenesch, *et al*., 2012).

Some strains produce additional exoproteins that may be responsible for a particular clinical manifestation, including staphylococcal enterotoxins, the Toxic-Shock Syndrome Toxin-1, exofoliative toxins, and the Panton-Valentine leukocidin (PVL) (Pajic, *et al*., 2014).

2.1.3.3. Panton-valentine leukocidin

The toxin Panton-Valentine Leukocidin (PVL) is a virulence factor produced by some strains of *S.aureus* that destroys leucocytes. PVL-SA predominantly results in skin and soft tissue infections (SSTIs) but may also cause invasive disease, including necrotising pneumonia, which may affect healthy young individuals and has a high mortality (Bourn, *et al*., 2013).

PVL toxin was first described by van de Velde in 1894 due to its ability to lyse leucocytes. It was named after Panton and Valentine when they associated it with soft tissue infections in 1932 Infections caused by PVL positive strains have been documented since 1930 but are much more prevalent now (Pathirage, 2008).

PVL is a *S.aureus* specific exotoxin, which belongs to the family of bicomponent synergohymenotropic toxins. It is a leukocytolytic toxin, which disrupts the membranes of polymorphonuclear neutrophils that insert it into the host's plasma membrane and heterooligomerize to form a pore. It is encoded by two contiguous and cotranscribed genes carried on bacteriophage, causing leukocyte destruction and tissue necrosi, PVL may be

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found in both methicillin-resistant and sensitive *S. aureus* (MR/SSA) and strains. It is encoded by two contiguous and cotranscribed genes, designated as lukF-PV and lukS-PV (Pajic, *et al*., 2014).

Panton Valentine leukocidin (PVL) is a cytotoxin, one of the β-pore forming toxins, and its presence is associated with increased virulence of certain strains of *S. aureus*. It is present in the majority of community associated methicillin resistant *S. aureus* (CA-MRSA) and is the cause of necrotic flesh eating lesions, an aggressive condition that often kills patients within 72h (Al-Mohana, *et al*., 2012).

The PVL genes are more common in community acquired methicillin-resistant *S. aureus* (CA-MRSA) than in community acquired methicillin-sensitive *S. aureus* (CA-MSSA). Musculoskeletal infections caused by (PVL) secreting S. aureus (CA-MRSA) in children have been previously reported (Bukhari, *et al*., 2012).

2.1.4. Pathogenesis

Bacterial diseases constitute an important cause of morbidity and mortality among humans and also animals. Pathogenic bacteria include a wide range of organisms which employ varied mechanisms in pathogenesis, without a functional immune system, we could not resist infections for a long period of time. Many microbes are pathogenic, and we are constantly in danger of infections and diseases caused by them. The immunity may be innate or acquired (Donkor, 2013).

According to the equipment with virulence factors (or the ability to acquire them by horizontal gene transfer) and the resulting pathogenic capabilities, among the staphylococci, *S. aureus* is the species with the highest pathogenic potential. It is responsible for a variety of nosocomial or community acquired infections ranging from boils, furuncles, styes, impetigo, and other superficial skin infections to more serious infections particularly in the chronically ill or immunocompromised patient. Besides *S. aureus* as the leading staphylococcal pathogen, various other members of the genus, mostly belonging to the CoNS, playroles as infectious agents for human or animal hosts (Rosenstein, and Götz, 2012).

The survival of *S. aureus* in the host is important for pathogenesis. The bacteria may be protected by a polysaccharide capsule that inhibits opsonization by complement and thereby escapes phagocytosis. It may also secrete cytolytic toxins and tissue-cleaving

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enzymes. Moreover, *S. aureus* may express a multitude of adhesion factors that mediate interactions with host cells and extracellular matrix (ECM) (Stark, 2013).

S. aureus is primarily an extracellular pathogen, it may, sometimes, survive inside non-professional phagocytes such as fibroblasts, renal cells, and osteoblasts. Intracellular survival may explain the persistent and recurrent nature of certain staphylococcal infections. Intracellular staphylococci often appear as small colony variants which have mutations affecting electron transport, and show slowly growing, nonpigmented colonies with reduced production of virulence factors (McNamara, and Proctor, 2000).

2.1.5. The carriage of *Staphylococcus aureus*

The primary *S.aureus* reservoir in humans is the anterior nares. Extra-nasal colonization sites include skin, throat, perineum, vagina, and gastrointestinal tract. Nasal carriage of *S.aureus* in health care staffing (HCS) may be a significant reservoir for transmission of the infective agent to patients. Exclusively sampling nasal sites to determine whether a person is colonized at a single point in time will miss 50% of those colonized elsewhere; Nasal carriers may fall into two categories–persistent carriers and non-persistent carriers. Approximately 20% of individuals are persistently colonized with a relatively high bacterial load, and the remainder is either never colonized or only intermittently with low numbers of bacteria (Brown, *et al*., 2014).

Rates of *S. aureus* infection are higher in carriers than in non-carriers in a range of clinical settings. This is consistent with the finding that individuals are usually infected with their own carriage isolate. Temporary eradication of carriage has been reported to result in a reduction in nosocomial infection in several patient groups and has been the focus of much recent interest and research. Eradication of *S. aureus* carriage is usually achieved by the topical application of antibiotic to the anterior nose (Gillespie, and Hawkey, 2005).

S. aureus has remained a major human pathogen that colonizes and infects both hospitalized patients with decreased host defenses and healthy immunologically competent people in the community. Humans (many neonates, most children and adults) become intermittently colonized by *S.aureus* and harbor the organisms either in their nasopharynx or on their skin and clothing (Asime, *et al.*, 2014).

The carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection associated with bacteria. Nasal carriage of *S. aureus* (NCSA)

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has been shown to be an important risk factor for nosocomial and community-acquired infections (Baysoy, *et al*., 2007).

Airway epithelial cells play a major role in initiating inflammation in response to bacterial pathogens. *S. aureus* carries a wealth of pathogenic determinants, which promote tissue colonization, tissue damage, and distant diseases. *S. aureus* is able to survive inside host cells and can invade a variety of nonprofessional phagocytes, including fibroblasts, osteoblasts, endothelial, and epithelial cell. After internalization, *S. aureus* may either persist, escaping host defenses and antibacterial agents, or multiply and further disseminate (Bien, *et al*., 2011).

Hospitalized patients are particularly exposed to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections. *S. aureus* infections, during two-year period have revealed that this pathogen is the leading cause of bloodstream, lower respiratory tract and skin soft tissues infections in all regions surveyed. About 20–30% of individuals are persistent carriers of *S. aureus*, which means they are always colonized by this bacterium, and 30% are intermittent carriers (Plata, *et al.*, 2009).

2.1.6. Clinical manifestations

S. aureus is among the most common human pathogens, capable of causing infections of any body parts in mild to fatal forms both in community and hospital settings. In a surveillance study conducted by the National Nosocomial Infection Surveillance (NNIS) from 1990 to 1999, *S. aureus* was the most common cause of nosocomial infections overall. Other studies have shown that *S. aureus* is the leading cause of nosocomial bloodstream infections (Stevens, 2012).

Nasal colonization with *S. aureus* is found in 20–40% of the general population and in over 50% of selected patient populations. Carriage has been identified as a strong risk factor for subsequent infection. In settings such as chronic haemodialysis or preceding surgical procedures, decolonization reduces subsequent infectious complications, while its effect may be less clear in other situation. Nasal application of an antistaphylococcal drug temporarily decolonizes the nose and other body sites, which prevents infection (Seybold, *et al*., 2011).

Nasal carriage of *S. aureus* in hospital inpatients is associated with an increased risk of nosocomial staphylococcal bloodstream infection, dialysis-associated infections, and surgical site infections. The density of nasal colonization with *S. aureus* could be an

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important risk factor for infection-high concentrations of *S. aureus* in the anterior nares of surgical patients are associated with increased risk of subsequent surgical site infection (Simor, 2011).

S. aureus has a tendency to spread to particular body sites including the bones, joints, kidneys, and lungs. Supportive collections at these sites serve as potential foci for recurrent infections. Moreover, a minority of bacteremia or local infections progress to sepsis. Risk factors for sepsis include advanced age, immunosuppression, chemotherapy, and invasive procedures. *S. aureus* is one of the most common causes of sepsis. Severe cases progress to multiorgan dysfunction disseminated intravascular coagulation, lactic acidosis, and death (Baba-Moussa *et al*, 2008; Big, and Malani, 2010).

2.2. Methicillin (R/S) *S.aureus* and Methicillin (R/S) Coagulase Negative *Staphylococcus*

2.2.1. Definition

Methicillin resistant *S. aureus* (MRSA) is a multidrug resistant organism and any strain of *S. aureus* that has developed, through the process of natural selection, resistance to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.), Strains unable to resist these antibiotics are classified as Methicillin sensitve *S.aureus* (MSSA). All MRSA produced penicillin-binding protein, PBP2a or PBP2, resistance occurs when the organism has a mecA gene producing an altered penicillin binding protein, PBP2a (also known as PBP2') and either an oxacillin MIC of 2mg/l or a methicillin MIC of 4mg/l. Infected and colonised patients are the reservoir of MRSA both in hospitals and the community with transmission generally being via contact with health staff (Batabyal, *et al*., 2012).

MRSA infections are more difficult to treat than ordinary staph infections. This is because the MRSA do not respond well to many common antibiotics used to kill bacteria, The effectiveness of penicillin, introduced in the early 1940s, was annulled within a decade owing to the rapid spread of plasmid-encoded *S. aureus* β-lactamase, Increasing resistance to penicillin has led to the development of semi-synthetic groups of penicillin such as methicillin, that are resistant to many genetic variations of the beta-lactamase enzyme (Buzaid, *et al*., 2011).

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MRSA is of serious concern not due its sole resistance to methicillin, but also because of resistance to many other antimicrobials that are used on a regular basis in hospitals. Current therapeutic options for MRSA are limited few expensive drugs like vancomycin, linezolid, teicoplanin, daptomycin and streptogramins (Mir and Srikanth, 2013).

The first MRSA clones had similar genetic and phenotypic properties MSSA clones that were epidemic in the early 1960s in Europe. The initial appearance of MRSA resulted from the acquisition by successful MSSA clones of mecA. MSSA and MRSA have few structural differences, but their epidemiologies differ profoundly in terms of colonization, infection, and transmission. And MSSA are strains that unable to resistant beta-lactam antibiotics (Rodrıguez-Noriega, *et al*., 2010; Lucet and Regnier, 2010).

The widespread use of vancomycin to treat MSSA bacteremia has led to a general belief that vancomycin is as effective as A-lactam antibiotics against this organism. However, several studies have demonstrated a greater frequency of bacteriologic failure among patients with MSSA bacteremia treated with vancomycin versus those treated with A-lactam antibiotics (Karchmer, 2012).

In the MSSA group treated with antimicrobial therapy without an indication to surgery, all patients survived, and in the combined surgical and antimicrobial group 29% died. The mortality in MRSA patients was lowest if combined surgical and antimicrobial therapy was performed, and in MRSA and MSSA patients with antimicrobial therapy due to a contraindication to surgery, the mortality was extremely high. Both MSSA and MRSA have been implicated in a variety of endemic and epidemic nosocomial infections worldwide. Approximately 20% of healthy adults are persistent nasal carriers of this potential pathogen and 60% harbor the organism intermittently and appear to play a key role in the epidemiology and pathogenesis of infection (Hill, *et al*., 2007; and Akhtar, 2010).

CoNS are common inhabitants of human skin and mucous membranes, and are a heterogeneous group of organisms. CoNS are among the most important microorganisms in nosocomial bloodstream infections. These bacteria can be pathogenic, especially in immunocompromised patients with hematological and other malignancies and in patients with foreign bodies, such as invasive catheters, epidemiological studies of CoNS infection are difficult because this organism is a normal commensal on the skin, and often formed as

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contaminants in clinical specimens e.g. in swab from the nose, throat, wounds, burns, and bedsores (Uyanik, *et al*., 2014).

Staphylococci that do not produce coagulase are coagulase negative staphylococci (CoNS). Strains of CoNS such as *S. carnosus*, *S. epidermidis*, *S. saprophyticus* and *S. xylosus* strains are traditionally used in meat fermentation. The species of *Staphylococcus* condiment, *S. piscifermentans*, *S. equorum* and *S. succinus* are often isolated from fermented food and may therefore have the potential for future application as starter or protective cultures (Zell, *et al*., 2008).

The large groups of methicillin resistant coagulase negative staphylococci (MRCoNS) isolates carrying SCCmec elements are a possible reservoir and donor of resistance genes that can possibly be transferred to other gram-positive organisms such as *S. aureus*. However, the possible mechanisms for the horizontal transfer of SCCmec elements from CoNS to *S. aureus* are not know (Mert, *et al*., 2011).

Although many studies have been done on prevalence and antibiogram of *Staphylococcus*, but many of these studies have concentrated only on MRSA and not on MRCoNS which are equally important (Mir and Srikanth, 2013).

MRCoNS are opportunistic pathogens bacteria among patients that they have HIV, and serve as a large reservoir of SCCmec that carries *mec*A. Very little is known about the prevalent SCC*mec* types among MRCoNS from HIV patients and its molecular epidemiology (Saravanan, *et al*., 2014).

Nasal carriage of MRCoNS is highly prevalent in community subjects. MRCoNS strains were identified and characterized by non beta-lactam susceptibility testing. CoNS species are major components of the human skin and mucosal flora including the Nasal microbiota, the high diversity of methicilin resistant *Staphylococcus epidermidis* (MRSE) clones and their ccr and mec gene complex associations contrasted with the high carriage rates in this isolated community, which might reflect the occurrence of SCCmec rearrangement and the generation of new MRCoNS strains (Lebeaux, *et al*., 2012).

MSCoNS which although a normal skin flora most often, could well be a pathogen if the condition is congenial, cheaper quality or improper use may give rise to abrasion, leading to breakage of the skin which might be a portal of infection. It might be speculated that if the earphones are also used in the hospitals during leisure hours (Mukhopadhyay, *et al*., 2008).

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2.2.2. Mechanism and evolution of resistance

Bacteria have acquired different resistance mechanisms in order to deal with the strong antibiotic pressure. Common resistance strategies include enzymatic modification of drugs, mutating drug targets, enhancing refflux pump expression, and decreasing membrane permeability, for example ß-lactam antibiotics, which inhibit cell wall biosynthesis in bacteria by irreversibly binding to the PBP active site, have been extensively used against bacterial infections since the discovery of penicillin (Sun, 2012).

Antibiotic resistance can be acquired either by mutation or by the horizontal transfer of resistance-conferring genes, often in mobile genetic cassette. The relative contribution of these factors depends on the class of antibiotic and on the genetic plasticity of the bacterial species (for example, methicillin resistance in *S. aureus* caused by a resistance cassette) (Palmer, and Kishony, 2013).

MRSA and MRCoNS are cross resistant to other lactam antibiotics such as cephalosporins, These organisms are often resistant to other antibacterial agents such as macrolides, lincosamides, aminoglycosides, fluroquinolos expect vancomycin (Mehdinejad, *et al*., 2008).

Penicillin and its derivatives are very effective against staphylococci. soon after the introduction of penicillin intro clinical use, penicillin-resistant, penicillinase-containing *S. aureus* strains spread in the world, in the fact that penicillin became ineffective against many infectious *S. aureus* strains, the penicillinase-resistant penicillin derivative methicillin was introduced in 1959 (Otto, 2013).

Methicillin, a modified penicillin expressly designed to resist the destructive action of the staphylococcal penicillinase, all MRSA strains carry an acquired genetic determinant – mecA or mecC which encode for a low affinity penicillin binding protein – PBP2A or PBP2A′ that can continue the catalysis of peptidoglycan transpeptidation in the presence of high concentrations of beta-lactam antibiotics which would inhibit the native PBPs normally involved with the synthesis of staphylococcal cell wall peptidoglycan. Methicillin, like all penicillins, exerts its action by blocking the proteins called “Penicillin Binding Proteins (PBPs), which are responsible for the construction and maintenance of the bacterial cell wall. *S. aureus* resistant strains acquired a new protein, called PBP2a, which was not blocked by methicillin and could replace the other PBPs (Kim, *et al*., 2013).

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2.2.3. Hospital versus community associated MRSA

MRSA, first identified in 1961, was primarily a concern for hospitalized patients until the past two decades when new strains of MRSA emerged in the community. Community-associated MRSA (CA-MRSA) is linked to aggressive virulence factors and displays distinctive antibiotic resistance traits, and has become a significant public health threat causing outbreaks of soft tissue infections in otherwise healthy individual. CA-MRSA have ability to spread among athletes, prisoners, military recruits, daycare attendees, injection drug users and other groups of people who live in crowded settings and/or routinely share contaminated items. Poor hygiene practices, such as lack of hand washing, may spread the bacteria easily (Williamson, *et al.*, 2013).

2.2.3.1. Community-associated MRSA

CA-MRSA is associated with conditions of close personal contact, poor hygiene, and exposure to contaminated objects. MRSA, being reported for the first time in the UK in 1961 and in the US in 1968**,** has long been recognized in patients required hospitalization or long term care facilities. CA-MRSA is infection of the skin and soft tissues. Skin and soft tissue infections (SSTI) account for at least 90% of CA-MRSA infections. CA-MRSA SSTI is usually moderately severe to severe, often very painful, and is treated by simple incision and drainage. However, rare cases of very dramatic skin infections, such as necrotizing fasciitis, have been reported with CA-MRSA in contrast to Hospital-associated methicillin-resistant *S.aureus* (HA-MRSA), and also cause infections of the bones and joints, such as osteomyelitis, and respiratory infections, such as pneumonia, sepsis, and urinary tract infections (Otto, 2013).

Almost all CA-MRSA strains carry the Panton Valentine Leukocidin (PVL) gene that allows the production of a necrotizing cytotoxin which may be responsible for the invasiveness and virulence of the organism. In contrast, only about five percent of methicillin susceptible strains of *S. aureus* and HA-MRSA carry the PVL gene. However, the role of PVL production in conferring virulence characteristics to CA-MRSA remains unclear, CA-MRSA can infrequently cause necrotizing pneumonia, empyema, sepsis syndrome, pyomyositis, osteomyelitis, necrotizing fasciitis and disseminated infections with septic emboli, with skin and soft tissue infections (SSTIs) They first appear as reddened areas on the skin, or can resemble pimples that develop into skin abscesses or boils causing fever, pus, swelling, or pain (Drews, 2006).

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CA-MRSA strains contain a methicillin resistance element, known as SCCmec type IV or V , whereas traditional HA-MRSA strains contain a larger SCCmec element type I–III, CA-MRSA infections can occur in healthy in dividuals, suggesting that these bacterial strains havegreater virulence than traditional HA-MRSA strains (Yamaguchi, *et al*., 2012).

2.2.3.2. Hospital-associated MRSA

HA-MRSA is the major problem in nosocomial infections for instance, patients in hospital with open wounds, invasive devices or under immune compromise conditions are at much higher risk of getting HA-MRSA infection, the typical HA-MRSA patient is elderly, with multiple co morbidities, and has had a prolonged hospital course (Al-Kadmy, 2013).

HA-MRSA is one of the primary causative agents of these infections. A few reports are concerning HA-MRSA virulence because the danger of HA-MRSA infections has been primarily confined to immune-compromised persons in healthcare facilities and HA-MRSA is regarded as being less virulent than CA-MRSA which infects individuals with no predisposing risk factors. HA-MRSA typically occurs in hospitals or other healthcare settings such as long-term care facilities and dialysis clinics; therefore HA-MRSA typically causes serious invasive disease such as bacteremia, pneumonia, catheter-related infections, and wound infections (Fukuda, *et al*., 2013).

HA-MRSA is the most common cause of multi-drug resistant healthcare-associated infection. Infection risk is highest in patients that are immunocompromised or have under. Lying disease or other infection risks such as breaks in the skin barrier, HA-MRSA describes the epidemiological behavior of particular MRSA isolates that are highly successful and may be endemic in particular healthcare settings, and cause epidemic levels of infection (Lindsay, 2013).

Increased rates of MRSA colonization among healthcare staff have at least 3 important implications. First, they may become infected by their own MRSA carriage strains; second, they could serve as a route of cross-transmission to patients; and finally, they may introduce the pathogen into their communities (Elie-Turenne, *et al*., 2010).

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2.2.4. Detection of MR/SSA and MR/SCoNS

2.2.4.1. Detection methods

2.2.4.1.1. Agar dilution

Mueller-Hinton agar is a standardized solid medium recommended for antimicrobial susceptibility testing by the agar diffusion or agar dilution method, with the British Society for Antimicrobial Chemotherapy (BSAC) method either medium may be used, Agar dilution is the most commonly used technique to determine the minimal inhibitory concentration (MIC) of antimicrobial agents, including antibiotics and other substances that kill (bactericidal activity) or inhibit the growth (bacteriostatic activity) of bacteria (Wiegand, *et al*., 2008).

The MIC of oxacillin was determined by the agar dilution (AD) method. Briefly, bacterial suspensions were adjusted to a 0.5 McFarland standard, diluted 1:10 and inoculated (10^4 colony-forming unit) on Mueller-Hinton agar plates with 2% (wt/vol) NaCl and varying oxacillin concentrations (0.5-256 µg/mL). The plates were incubated at 35ºC for 24 hr, and are grown on chromogenic agar that is selective for MRSA; known as CHROMagar (Microbiology, Paris, France). The composition of this medium is proprietary. A 0.5 McFarland bacterial suspension was used to inoculate the chromogenic medium using a cotton swab and the tests were read at two time points: after 24 h and 48 h of incubation at 35ºC, The growth of pink/purple-clear colonies was indicative of the presence of MRSA isolates (Matos, *et al*., 2010; CLSI, 2008).

There is no single method that is suitable for all MRSA strains. Standard methods are published by the; - 1) British Society for Antimicrobial Chemotherapy (BSAC) and in the USA, 2) Clinical Laboratory Standards Institute (CLSI), and 3) National Committee for Clinical Laboratory Standard previously known as CLSI. Minimum Inhibitory Concentration [MIC] by the dilution method has traditionally been the reference method. BSAC recommend the use of Mueller Hinton or Columbia agar (MHA) with 2% NaCl and 10^4 cfu/ ml inoculums incubated at 30°C. CLSI recommend MHAwith 2% NaCl and 104cfu/ml inoculums incubated at 33-35°C. And with Agglutination kits are widely available and can be used to confirm *S. aureus* by detecting protein A and clumping factor, although some strains of MRSA have low levels of these proteins. But now the new kits work by also detecting surface antigen. Other latex kits detect PBP2a which occurs within the cell membrane and requires lysis of the cells for detection (Batabyal, *et al*., 2012).

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All positive agar slats from the above were further screened for their methicillin resistance capability. This was done according to the CLSI guidelines using oxacillin in agar screen test. Isolates from a solution adjusted to 0.5 McFarland standards were spot inoculated unto Mueller Hinton agar supplemented with 6μg/mL oxacillin and 4% sodium chloride. The plates were incubated at 35°C for 24 hours (Udobi, *et al*., 2013).

Hypertonic Mannitol Agar was used for the isolation and presumptive identification of CoNS. The isolated bacteria were identified according to established methodology through the morphotinctorial and biochemical characteristics (Bouzada, *et al*., 2010).

2.2.4.1.2. Broth micro-dilution

The method, which requires the use of MH broth with 2% NaCl, an inoculums of 5 × 10^5 CFU/mL and incubation at 33–35°C for 24 h, is the only defined method in general use, Mueller Hinton II Broth is intended for use in quantitative procedures for susceptibility testing of rapidly-growing aerobic and facultative anaerobic bacteria isolated from clinical specimens (Brown, *et al*., 2005).

2.2.4.1.3. Disk diffusion

Oxacillin disk (1μg) diffusion method was carried out on MHA supplemented with 2% Nacl to detect MRSA according to CLSI guidelines. The plates were incubated at 35°C and results were recorded after 24hours of incubation. Isolates were considered resistant when the diameter of inhibition was <10mm, intermediate resistance when the diameter was 10-12mm and sensitive when the diameter was >13mm, MSSA (ATCC 25923) and MRSA (ATCC 43300) were used as negative and positive controls respectively (Kalyani, *et al*., 2012).

Susceptibility to oxacillin was determined by the Disk Diffusion (DD) method on MHA plates (Hi-Media Laboratories) using a bacterial suspension with the turbidity adjusted to a 0.5 McFarland standard. Plates were incubated at 35 °C for 24 h. Results were interpreted according to CLSI guidelines. The interpretive criteria for oxacillin were: *S. aureus*, sensitive ≥13 μg/ml, resistant ≤12 μg/ml; CoNS, sensitive ≥18 μg/ml, resistant ≤17 μg/ml (Jain, *et al*., 2008).

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2.2.4.1.4. E-test (Epsilometer test) method

Direct colony inoculations were performed on Mueller Hinton media same as disk diffusion method but E- test strips were placed on media instead of antimicrobial disk. E-test is done 0.5 McFarland inoculums according to the manufacturer's instruction. The oxacillin and vancomycin E-test strip was placed on to MHA plate supplemented with 2% NaCl, and the plate was incubated at 35°C for 24 hrs. According to CLSI standards, *S. aureus* isolates with oxacillin MICs of ≤ 2μg/ml and ≥4 μg/ml are defined as MSSA and MRSA. *S. aureus* isolates with vancomycin MICs of ≤ 2 μg/ml were considered susceptible. Vancomycin resistant *S. aureus* (VRSA) by MICs of ≥16μg/ml. MHA plates without antimicrobial were used as controls of bacterial growth. *S. aureus* ATCC 25923 was used as control strain (Sasirekha, *et al*., 2012).

E-test to (CoNS) was performed according to the manufacturer's instructions. A sterile swab was dipped into an inoculum suspension (McFarland standard 0.5) prepared in MHA. Then the swab was spread by rotating on the surface of a Muller Hinton agar plate (2% NaCl supplement) to ensure an even distribution of the inoculum. An E-test strip was placed aseptically onto the agar plate. After incubation at 35°C, MIC values were read at 48 hours for CoNS. The MIC was read at the point of intersection between the zone edge and the E-test strip. Isolates that had MIC value ≥0.5 μg/ml for CoNS were accepted as MR (Dine, *et al*., 2009**)**.

2.2.4.1.5. Agar screening method

The clinical specimens were inoculated on 5% sheep blood agar, incubated at 37°C for 24-48 h, and examined for bacterial growth. *S. aureus* was identified using standard methods based on colony morphology, Gram's stain, catalase test, mannitol fermentation and coagulase test (Pillai, *et al*., 2012).

This method has been recommended for screening colonies isolated on routine media and for confirmation of suspect resistance seen in disk diffusion test. The method recommended by the CLSI requires, MHA plates containing 4% NaCl and 6 μg/ml of oxacillin were prepared. Plates were inoculated with 10μL of 0.5 Mc Farland suspension of the isolate by streaking in one quadrant and incubated at 35^{o}C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h and any growth other than a single colony are indicative of resistance (Sangeetha, *et al*., 2012).

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For each isolate, 100 µl of bacterial suspension in Cation Adjusted Muller Hinton broth equivalent to 0.05 McFarland turbidity standard were inoculated onto MSA plates supplemented with 6µg/ml oxacillin (Sigma) for *S.aureus* and with 1µg/ml and 4µg/ml for CoNS, After incubation for 24 hours at 35°C in air, any growth on the plate was interpreted as a positive result of MR (Dine, *et al*., 2009).

2.2.4.1.6. Latex methods

The MRSA-screen latex agglutination test (MRSA-Screen; Denka Seiken Co, Ltd, Tokyo, Japan) – a qualitative slide latex agglutination test for the detection of penicillin-binding protein 2′ (PBP2; also called PBP2a) present in isolates of *S. aureus* was performed according to the manufacturer’s instructions (Mocan, *et al*., 2014).

The latex agglutination kit for detection of PBP2a encoded *mec*A gene is an alternative that could be used in most laboratories and this rapid latex method would be practically useful for urgent confirmation of resistance, a visible agglutination reveals appositive and the presence of PBP2a encoded *mec*A gene product, therefore it is designated as MRSA strain. Negative result indicates that absence of PBP2a the *mec*A gene product, which is taken as borderline oxacillin resistant *S. aureus* BORSA strain (Sucilathangam, *et al*., 2013).

2.2.4.1.7. Molecular methods

The molecular methods used for the detection of MRSA are relying on multiplexed PCR primers detecting genes specific for *S. aureus* (nuc,fem) and *mec*A detecting methicillin resistance. Detection of *mecA* gene by PCR has been shown to be highly discriminatory in analyzing hospital outbreaks and tracking genetic changes which occur in a relatively short time. All isolates of *S. aureus* were tested by PCR for the presence of *mec*A gene. Staphylococcal DNA was extracted using boiling method. PCR is an automated technique used to detect a target sequence of DNA that is unique to an organism. To detect MRSA, Moleculer testing focuses on the mecA gene, which confers methicillin resistant and is located in the SCC-mec Assays simultaneously target sequences within the SCCmec and sequence specific to *S.aureus* with in the *orfX* gene (Rallapalli *et al*, 2008; Rostami, *et al*., 2013).

MRSA advanced test was performed according to the manufacturer's instructions (Roche Diagnostics, Switzerland). This assay targets the integration site of the SCCmec

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cassette into the *S. aureus* chromosome. Essentially, the LC (light cycler) PCR assay is performed on the LightCycler 2.0 instrument and has been validated with three transport media (Liquid Stuart, and Amies gel with or without charcoal) (Molan, *et al*., 2013).

PCR-based methods have been used routinely by reference laboratories as their standard method for detecting the *mec*A gene. Occasional susceptible strains carrying a nonfunctional or non-expressed *mec*A will also be detected but the presence of *mec*A is generally considered to indicate a potential for resistance and is used as a marker to identify MRSA (Brown, *et al*., 2005).

2.2.5. Health problems of (MR/SSA, and MR/SCoNS)

Patients with MRSA have a significantly longer in-hospital stay, aworse prognosis and higher mortality. They also instigate higher costs in their diagnosis and therapy, are subject to social stigma and suffer from greater psychological stress (Kupfer, *et al*., 2010).

MRSA is a global human health problem causing infections in both hospitals and the community. These infections are not attributable to hospital stays or medical procedures within the previous year and can occur in otherwise healthy individuals. MRSA infections appear as skin and soft tissue infections and are transmitted through close contact such as occurs with athletes, prisoners, and in daycare centers (Gould, 2006).

Health care staff (HCS) may be carriers of MRSA acquired either from the community or from hospital patients, and may serve as sources of transmission of nosocomial infections, Health care staff (HCS) may be carriers of MRSA acquired either from the community or from hospital patients, and may serve as sources of transmission of nosocomial infections (San juan, *et al*., 2012).

HA-MRSA describes the epidemiological behavior of particular MRSA isolates that are highly successful and may be endemic in particular healthcare settings, and cause epidemic levels of infection, established risk factors for HA-MRSA infection include hospitalization during the previous year, recent surgical procedure, exposure to broad-spectrum antibiotics, residence in a longterm care facility, receipt of hemodialysis, indwelling percutaneous medical devices and catheters, and intravenous drug use (Rehm, and Tice, 2010).

MSSA colonization usually causes them no problems, but can cause an infection when it gets the opportunity to enter the body. MSSA can cause local infections such as

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abscesses or boils and it can infect any wound that has caused a break in the skin e.g. grazes, surgical wounds. MSSA can cause serious infections called septicaemia where it gets into the bloodstream, The patients with CA-MRSA infections are similar to patients with CA-MSSA infections in respect to their epidemiologic characteristics (Al-Kadmy, 2013).

Several reports involving health care associated strains suggest that SCCmec transfer from MRCoNS to MSSA may occur, although its mechanism remains unknown. MRCoNS may thus act as a source of SCCmec for MRSA, The frequency of methicillin resistance in health care–associated CoNS is currently >60% (Barbier, *et al*., 2010).

CoNS have recently emerged as an important cause of nosocomial infections and reservoirs of drug resistance genes for *S.aureus*. Health care staff (HCS) carrying CoNS can be a potential source of infection to the patients, CoNS are the third most common pathogens in the community and nosocomial blood stream infection, Infections with MRSA and MRCoNS are associated with higher mortality compared to MSSA and MSCoNS due to multidrug resistance (Baragundi, *et al*., 2012).

2.2.6. Epidemiology

2.2.6.1. *S. aureus,* MR/SSA and CoNS

The epidemiology of nasal colonization with *S. aureus* is well defined but extra-nasal colonization has been less well studied, Early studies of *S. aureus* colonization patterns in healthy populations revealed variations in carriage patterns at extra-nasal sites; for example, the rate of oropharyngeal colonization among healthy adults varied from 4% to 60% (Mody, *et al*., 2008).

S. aureus is the most commonly isolated human bacterial pathogen and *S. aureus* infections are increasingly reported around the world (David and Daum, 2010). *S. aureus* was observed to cause infections in population with no known risk exposures. The emergence of such community-associated *S. aureus* has further magnified the challenge of *S. aureus* prevention and treatment practices (Nair, *et al*., 2013).

The epidemiology of MRSA is constantly changing, and both circulating clones and their antibiotic resistance profiles vary considerably throughout regions and countries,

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a complicating factor in interpreting epidemiological data comes from the use of different terms to describe MRSA appearing in hospital and community settings (Mejia, *et al*., 2010).

Although epidemiological data from separate studies are often not comparable owing to differences in study design and populations sampled, the highest rates (>50%) are reported in North and South America, Asia and Malta. Intermediate rates (25–50%) are reported in China, Australia, Africa and some European countries [e.g. Portugal (49%), Greece (40%), Italy (37%) and Romania (34%)]. Other European countries have generally low prevalence rates (e.g. The Netherlands and Scandinavia (Stefani, *et al*., 2012).

First emerging as a major pathogen in healthcare institutions in the early 1960s, the epidemiology of MRSA infections began to change in the late 90s, due to the emergence of CA-MRSA. CA-MRSA has distinctly different microbiological, epidemiological and molecular characteristics from those of HA-MRSA. CA-MRSA strains now afflict patients without previous hospital exposures. Most CA-MRSA outbreaks reported before 1990 occurred in specific populations such as Native Americans, intravenous drug users, and prison inmates, but over the past decade, the prevalence of this organism has risen dramatically in the general population, dissemination of CA-MRSA in communities among otherwise healthy children has become a global problem (Millar, 2008 ; Shapiro, *et al*., 2009).

CA-MRSA clones are spreading rapidly among the population in many regions worldwide, based on Swedish data, the greatest risk of travelers importing CA-MRSA was, in descending order, those from North Africa and the Middle East, sub-Saharan Africa, Oceania, East Asia, South America, the north-east Mediterranean and North America. The risk from most trans-European travelers was low (Stefani, *et al*., 2012).

Molecular typing studies have shown that CA-MRSA from HA-MRSA, in general. CA-MRSA is more virulent compeered to HA-MRSA due to the presence of various virulence factors (Partida, *et al*., 2014).

MSSA & MRSA blood stream infections (BSI) are associated with morbidity, mortality and associated costs. MSSA is an important cause of severe and potentially fatal infection in Neonatal Intensive Care Unit NICU babies. Control of MSSA in the NICU presents unique challenges because colonization is expected, infections are tolerated, and surveillance focuses on antibiotic resistant pathogens such as MRSA. Understanding the

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epidemiology of MSSA may help reduce or prevent serious disease in this vulnerable population (Daniel, *et al*., 2011).

According to the Centers for Disease Control and Prevention National Healthcare Safety Network, CoNS contribute to over 30% of hospital-wide central-line associated bloodstream infections, the most common type of nosocomial infection attributed to CoNS. The most commonly isolated organisms in the hospital setting are CoNS, inclusive of pediatric and neonatal patients. In the mid 1980s, the National Healthcare Safety Network reported that CoNS contributed to 10% and 31% of all general pediatric and NICU nosocomial infections. In more recent epidemiology, CoNS account for more than half of bloodstream isolates obtained from neonates with late-onset sepsis (Nash, *et al*., 2013).

Neonatal sepsis is defined as infection in the first 28 days of life, or up to 4 weeks after the expected due date for preterm infants. Epidemiologists defined two types of infections in neonates: early-onset neonatal sepsis (EONS), which manifests in the first 72 hours of life (up to 7 days) and late-onset neonatal sepsis (LONS), CoNS are the major pathogen involved in LONS, particularly in infants born at a lower gestational age. According to more recent data from the National Institutes of Child Health and Development (NICHD), infection-related mortality in very low-birth-weight (VLBW) infants (birth weight < 1500 grams) averages 10% (Marchant, *et al*., 2013).

Data from the NNIS system collected between 1992 and 1997 revealed that coagulase-negative staphylococci accounted for 36 percent of all bloodstream isolates in intensive care units, making these organisms the most common cause of nosocomial bloodstream infections. A survey from the Surveillance and Control or Pathogens of Epidemiologic Importance (SCOPE) database of nosocomial bloodstream infections in United States hospitals between 1995 and 2002 also identified CoNS as the most common cause of hospital-acquired bloodstream infections, accounting for 31 percent of cases (Tufariello, and Lowy, 2014).

2.3. Vancomycin Resistant *S. aureus* (VRSA)

Before the discovery of antibiotics, mortality rate of *S. aureus* strains was more than 75%, when *S. aureus* acquired resistance against to penicillin and methicillin, in the later decades, resistance rate among *S. aureus* strains increased dramatically and MRSA became a worldwide hazard. In the past ten years, the prevalence of MRSA in many hospitals is reaching 50%, high prevalence of MRSA forced clinicians to use vancomycin,

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which is a glycopeptide antibiotic discovered before methicilin, as the first-line of treatment against MRSA despite its side effects (Askari, *et al*., 2012).

Vancomycin was the first glycopeptides antibiotic discovered in 1956 in United States, and it is the only glycopeptide antibiotic use clinically in the world, Vancomycin is important in treating infections caused by penicillin-resistant Gram positive organisms such as MRSA. many vancomycin molecules were trapped within the cell wall (Thati, *et al*., 2011).

Although vancomycin is often prescribed for the treatment of MRSA, vancomycin-resistant *S aureus* (VRSA) infections remain rare, with only few cases confirmed worldwide—mostly in the USA. The isolation and preliminary characterisation of the first VRSA strain in Europe isolated from a patient in Portugal (Melo-Cristino, *et al*., 2013).

Three classes of VRSA have emerged that differ in vancomycin susceptibilities: vancomycin-intermediate *S.aureus* (VISA), heterogenous vancomycin-intermediate *S. aureus* (hVISA), and high-level VRSA. These strains may also be resistant to meropenem and imipenem. The vancomycin minimum inhibitory concentration (MIC) result reported for this isolate was in the intermediate range (8µg/ml) using interpretive criteria defined by (CLSI) (Ilang, *et al*., 2013).

The mechanism by which vancomycin exerts its action is by preventing the synthesis of peptidoglycan precursors of the bacterial cell wall by blocking the transglycosylation step and subsequently affecting the transpeptidation step also. Both the transglycosylation and transpeptidation steps are essential for bacterial cell wall cross-linking, the concentration of vancomycin required to inhibit most strains of *S. aureus* ranges from 0.5 to 2µg/ml. According to the current guidelines given by the (CLSI). *S. aureus* isolates with vancomycin MICs between 4 and 8µg/ml are classified as vancomycin intermediate and those with MIC $\geq$16µg/ml as vancomycin resistant. These MIC cut-offs are different from those for (CoNS) and enterococci where isolates with vancomycin MIC $\geq$32µg/ml are classified as vancomycin resistan (Sujatha, and Praharaj, 2012).

2.4. Awareness of the problem of MRSA

Knowledge about health problems is crucial to deal with them properly and it is the first step in modifying behavior in relation to physicians' adherence to clinical practice guidelines. Awareness of the risks of colonization and subsequent infection, effective

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treatment and the appropriate use of antibiotic prescribing remain as high priorities to combat MRSA related issues (Easton, *et al*., 2007; Brady, *et al*., 2009).

In a country like the UK, several initiatives had been launched in an attempt to reduce MRSA rates. These include improving cleanliness in hospitals, and requirements for all healthcare workers to receive formal training to show that they are able to reduce the risk of healthcare-associated infections including MRSA (Gill, *et al*., 2006).

2.5. Treatment of isolates MR/SSA and MR/SCoNS infections and colonization

Infected and colonized patients in hospitals mediate the dissemination of MRSA strains, and hospital staff is the main source of transmission. This leads to serious endemic and epidemic MRSA infections. Colonization with MRSA is a risk factor for subsequent invasive MRSA infection. MRSA strains are difficult to eradicate as they are multidrug-resistant leaving glycopeptides as the drugs of choice, Nasal MRSA colonization is not always present in individuals with active CA-MRSA infection. It is possible for individuals to be colonized with CA-MSSA and develop a CA-MRSA infection (Arora, *et al*., 2010).

Vancomycin is often combined with a second antibiotic like rifampicin, gentamycin or linezolid for the treatment of serious MRSA infections. Antimicrobial agents currently recommended for the treatment of MRSA pneumonia include Vancomycin and linezolid in the USA, and Vancomycin, Linezolid, Teicoplanin and Quinupristin/Dalfopristin in Europe (Kalil, *et al*., 2010).

In children presenting with necrotizing fasciitis, pneumonia, osteomyelitis, and other life-threatening invasive conditions, it may be expedient to administer vancomycin until antibiotic susceptibilities of the isolate are known. Alternative antibiotics are intravenous linezolid, quinupristin–dalfopristin, and daptomycin (Paintsil, 2007).

Vancomycin has long been the drug of choice for treating CA-MRSA in the hospital setting, however, a major limitation to its use in the outpatient setting is its lack of oral bioavailability. Clinicians have begun to use alternative antibiotics, including trimethoprim-sulfamethoxazole (TMP-SMX) and clindamycin, as treatment options in outpatient CA-MRSA skin and soft-tissue infections because of their favorable in vitro activity, high oral bioavailability, and excellent tissue penetration, the CA-MRSA is highly prevalent in the USA, accounting for about 59% infections in emergency department (Frei, *et al*., 2010).

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Recently, the search for alternative strategies to efficiently combat staphylococcal infections is urgently demanded to decrease the enormous burden caused by pathogenic staphylococci. In particular, immunological strategies based on vaccine development or therapeutic antibodies may significantly enhance the efficiency of anti-staphylococcal therapy (Ohlsen and Lorenz, 2010).

According to the Centers for Disease Control and Prevention National Healthcare Safety Network, CoNS contribute to over 30% of hospital-wide central-line associated bloodstream infections, the most of nosocomial infection attributed to CoNS. Antibiotics are the mainstay of therapy for CoNS, the glycopeptide vancomycin is the first-line treatment choice for CoNS. Other agents used to treat serious infections caused by CoNS include refampin, aminoglycosides, linezolid, quinupristin and dalfopristin, and clindamycin (Nash, *et al*., 2013).

2.6. Decolonization

Decolonization refers to the process of eradicating or reducing carriage of a particular organism from the skin, nose or other mucosal surfaces. In the case of MRSA carriage, decolonization has been attempted using topical or systemic therapy. The available systemic options include rifampin plus another antistaphylococcal antibiotic such as trimethoprim-sulphamethoxazol, clindamycin, fusidic acid, doxycycline or minocycline. Eradication from the skin can be attempted using topical agents such as chlorhexidine, whereas nasal decolonization usually requires intranasal mupirocin. And decolonization was successful in eradicating CA-MRSA carriage in a daycare facility (Barton, *et al*., 2006).

Decolonization presents an effective strategy to prevent infection in MRSA carriers as well as its transmission to non-carrier population. Many topical and systemic antimicrobial agents and antiseptic body washes have been employed in decolonization regimens to prevent MRSA outbreaks in health-care settings and community. Mupirocin is the best topical antimicrobial currently available, and is a very effective MSSA and MRSA decolonizing agent. Completion of nasal decolonization with mupirocin treatment (2%) twice daily for (5-10) days can successfully decolonize 81.5 to 100% patients (Ray, *et al*., 2011).

Decolonization regimens with a skin antiseptic solution (e.g.,chlorhexidine) for 5–14 days or dilute bleach baths. [For dilute bleach baths, one teaspoon per gallon (≈4 liters)]

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of water [or ¼ cup per ¼ tub or 13 gallons (≈50 liters) of water] given for 15 min twice weekly for ≈3 months can be considered. An oral agent in combination with rifampin, if the strain is susceptible, may be considered for decolonization if infections recur despite above measures (C-III). The concern for emergence of mupirocin resistance is mentioned and a warning that if "bleach baths" are recommended, patients need to be given clear instructions given the potential for skin irritation if not adequately diluted (Liu, 2011).

2.7. Prevention of infection and colonization

Infection prevention is an important part of quality of care and patient safety in healthcare settings, it is well recognized that nasal carriage of MRSA represents a major risk factor for subsequent infection and transmission of this pathogen, such as healthcare-associated infections (HAIs), Approximately one in ten hospital admissions worldwide results in an HAI, responsibility for patient safety within Swedish healthcare organizations has been strengthened. The law directing patient safety declares that care providers have a distinct responsibility to conduct patient safety work to prevent adverse events in healthcare, such as HAIs caused by multidrug-resistant bacterium (MDRB) (Lindberg, 2012).

The MRSA prevention consisted of three elements: use of systems and behavioral change strategies to promote adherence to infection control protocol, enhanced emphasis on hand hygiene and environmental disinfection and active surveillance testing of anterior nares and open wounds within 48 hours after admission to identify patients asymptomatically colonized with MRSA for prompt initiation of contact precautions, MRSA infections are associated with greater length of stay and cost than (MSSA) infections (Ellingson K *et al*, 2011).

The clinician should assume that the organism is resistant, because of the high prevalence of CA-MRSA strains, and agents effective against MRSA include (vancomycin, linezolid, or daptomycin) should be used, step-down to treatment with other agents, such as tetracycline or trimethoprim-sulfamethoxazole, for MRSA infection may be possible. But the best topical agent is mupirocin, also other agents, such as bacitracin and neomycin, are considerably less effective treatments (Stevens, *et al*., 2005).

CoNS are the major cause of late-onset sepsis (LOS) in neonatal intensive care units (NICUs), LOS is defined by the presence clinical signs of sepsis after 72 hours of age, CoNS are thought to be transmitted to the neonates by NICU personnel, therefore

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important factor in prevention of CoNS associated LOS is the prevention of cross-contamination of CoNS strains (Hira, 2013).

2.8. Hand Hygiene

The importance of hand hygiene in preventing hospital-acquired infections was first documented in 1847 by Dr. Ignaz Semmelweis, who noticed that women whose babies delivered by midwives had a lower rate of mortality compared to women whose infants were delivered by physicians and students, hand hygiene is a catch-all term for hand washing, antiseptic hand wash, antiseptic hand rub, or surgical hand antisepsis, hospital-acquired infections (HAIs) constitute a major source of morbidity, mortality and increased cost, hand hygiene (HH) has been singled out as the most important procedure in preventing HAI, HH either by washing hands with water and soap or by using alcohol-based hand rubs, is one of the simplest, but most important, methods to prevent cross-infection and to decrease the rate of HAIs (McLaws, *et al*., 2014).

Observation of health care staff in patient care were performed during morning and afternoon, and compliance were defined as hand hygiene practice before and after any contact with a patient or with the inanimate material inside the patient's room, hand washing with water and detergent and/or the use of alcohol-based hand sanitizers, is the single most important method of preventing nosocomial infections (Borges, *et al*., 2012).

Good hand hygiene has for many years been considered the single most important measure that can be applied to prevent the spread of healthcare-associated infection (HAI), MRSA is one such Healthcare-associated infections(HCAIs) where the UK incidence exceeds many parts of Europe. MRSA is therefore a global healthcare issue with medical and socio-economic consequences for patients, healthcare professionals and hospital trust, Poor hand hygiene is the main source of MRSA transmission within hospital. After applying alcohol gel, 99% of transient organisms, including MRSA, are eradicated, potential sources of MRSA within hospital, includes stethoscopes, marker pens, casenotes, bed control handsets, nebulisers, ventilation grilles, and environmental surfaces (Davis, *et al*., 2010).

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3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Antibiotics

Table 3.1. Antibiotics used in this study.

No.	Antibiotics	Code	Disc potency	Company/Origin
1	Imipenem	IPM	10 mcg	Bioanalyse/Turkey
2	Trimethoprim	TMP	1.25 mcg	Bioanalyse/Turkey
3	Vancomycin	VA	30 mcg	Bioanalyse/Turkey
4	Ampicillin	AM	10 mcg	Bioanalyse/Turkey
5	Ceftriaxone	CRO	30 mcg	Bioanalyse/Turkey
6	Penicillin	P	10 mcg	Bioanalyse/Turkey
7	Ciprofloxacin	CIP	5 mcg	Bioanalyse/Turkey
8	Azithromycin	AZM	15 mcg	Bioanalyse/Turkey
9	Methicillin	ME	5mcg	Bioanalyse/Turkey

3.1.2. Culture media

Table 3.2. Media used in this study.

No.	Culture Media	Company	Origin
1	Blood agar	LAB M	UK
2	Mannitol salt agar	LAB M	UK
3	Mueller-Hinton agar	LAB M	UK

All these media were prepared by the researcher according to the manufacturer's directions.

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3.1.3. Solutions and reagents

Table 3.3. Solutions and reagents.

NO.	Solutions and Reagents	Company	Country
1	Sodium chloride	Pioneer	Iraq
2	Hydrogen peroxide 3%	BDH	England
3	Rabbit plasma	BIO RAD	United States
4	Gram stain reagents	Syrbio	S.A.R.
5	Normal saline	Pioneer	Iraq

3.1.4. Equipment and apparatus

Table 3.4. Equipments and apparatus.

NO.	Equipment	Company	Origin
1	Autoclave	Wise	Germany
2	Incubator	Selecta	Spain
3	Microscope	Olympus CX21	Philippines
4	Centrifuge	Eppendorf	Germany
5	Sensitive balance	Meller	Switzerland
6	Refrigerator	Profilo	Germany
7	Digital Camera	Sonny	Japan
8	Micropippettes	Eppendorff	Germany
9	Petri dishes	Plasti Lab	Lebanon
10	Conical Flask and Beaker	Bro3.3	Germany
11	Disposable Swab	Citotest	China

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3.1.5. Preparation of culture media

3.1.5.1. Blood agar medium

The medium was prepared according to (Atlas, 2010). Blood agar base was prepared according to the manufacturer company construction (the powder 40 gram of Blood agar base was dissolved into one liter of distilled water and then sterilized by autoclaving at 121^{o}C for 15 minutes) that all bacteria, fungi, viruses, and spores are destroyed, then by adding 5% of EDTA (ethylene diamine tetra acetic acid) human blood to the sterilized blood agar base after cooling to 45^{o}C. This medium was storied at 4^{o}C.

3.1.5.2. Mueller-Hinton agar medium

Mueller-Hinton agar medium was prepared according to the manufacturer company construction. The powder 38 gram of this agar was suspended in 1 liter of distilled water. It was then boiled shortly to dissolve completely and then sterilized by autoclaving at 121^{o}C for 15 minutes. After cooling to 50^{o}C the agar was poured into sterile Petri dishes and left to set. Mueller-Hinton agar medium was stored at 4^{o}C.

3.1.5.3. Mannitol salt agar

Mannitol salt agar was prepared according to the manufacturer company construction. The powder 108 gram of this agar was suspended in 1 liter of distilled water. It was then boiled shortly to dissolve completely and then sterilized by autoclaving at 121^{o}C for 15 minutes. After cooling to 47^{o}C the agar was poured in to sterile Petri dishes and left to set.

A single colony was inoculated on Mannitol salt agar (MSA) medium; incubate for 18-24hours, observed for the presence of yellow colored colonies indicating fermentation of mannitol and 7.5% of salt tolerance.

Cream/golden yellow, beta-hemolytic colonies, catalase and coagulase positive with Mannitol fermenting isolates were identified as *S.aureus* but cream/white colonies, catalse positive, coagulase negative, and mannitol non fermenting isolates were identified as (CoNS).

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3.2. Methods

3.2.1 Overview

The study was done in Qaladze district, which is one of the sixteen district of Sulaimaniyah governorate of Iraq. Generally, the study consisted of three parts; the first was a knowledge survey for the assessment of the knowledge of staff working in the hospitals of Qaladze district about nasal carriage Staphylococci. The second part was to detect the prevalence of nasal carriage of *S. aureus* and (MRSA, MSSA, MRCoNS, and MSCoNS) among health care staff working in hospitals and to study the association of some factors related to the job of those staff with the nasal carriage. Finally, the third part of the study was to look for the prevalence of nasal carriage in the community among a special group of population which was working in Hospitals. Qaladze district has a population of about one hundred and twenty five thousand, and it has one general hospital and seven health center. Only two of these were chosen (Qaladze General Hospital and Everia health center) to be the site for data collection.

3.2.2 Prevalence of nasal carriage of MR/SSA and MR/SCoNS among healthcare staff

3.2.2.1. The Setting

As this part of the study was done among health care staff in hospitals, accordingly the (1 hospital and 1 health center) of Qaladze district mentioned in the setting of the knowledge survey were used here too. Qaladze General Hospital is located in Qaladze. This is the main and the only hospital in Qaladze health district providing general medical and surgical health services. It has 100 beds.

3.2.2.2 Data collection

Data collection from each hospital was conducted in one single day, on the day of data collection, all the departments of each hospital were visited and every (doctor and nurse) who were available at the time of visit was asked to participate. Healthcare staff of Qaladze General Hospital consists of (200 nurses and 20 doctors). The samples were collected only from nine doctors and 121 nurses. Because some of them were not ready take nasal swab from him/her. I think they were afraided. The researcher firstly clarified the nature of the survey and an oral consent was then obtained from the participant. The researcher also explained how to fill the questionnaire without explaining the meaning of

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the questions. Then the participant filled the questionnaire in front of the investigator. Demographic data were also covered by the questionnaire including age, sex, credential, specialty, professional qualification and the name of the staff.

3.2.2.3. Time of the survey

Preparation of the questionnaire and printing it started in February 2014. Visiting hospital and interviewing participants started in the same month, and completed in August 2014. Those 130 samples were collected of health care staff. Specimens collected by sampling of the nose by using sterile disposable cotton swabs.

3.2.2.4. The Sample

3.2.2.4.1. Participants

All the health care staff of the involved hospitals had not the chance to participate in this study. By using the term “health care staff”, the administrative and service staffs of the hospitals were excluded from the study. In order to have a sample of hospital staff participated from each hospital, the two hospitals were visited and in each hospital all the departments were visited. Many (hospital staff) who were available at the time of visit were asked to participate and they considered the sample of the survey without using a specific sampling method. When the participant attended firstly the nature of the study was explained to him/her and an informed oral consent was obtained. Then, some personal demographic information was asked. After that a nasal swab was taken.

3.2.2.4.2. Demographic data

These data were filled in a specially designed questionnaire. These included: age, sex, name of the hospital they worked in, type of their work and the department they worked in, as well as the duration of work in that hospital (in years) and the average hours or work per day. Regarding the type of the work and for the sake of grouping and analysis, the participants were divided into two groups as follows:

1. Physicians

2. Other health care staff: This group included the clinical scientists as biologists, bacteriologists, biochemists or chemists. Also included in the group all the medical assistants (those who had the title of assistant in any medical department) including

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anesthesia, laboratory, pharmacy and dentistry. Physiotherapists were also included in this group**.**

The departments as place of work were internal medicine, surgery, gynecology and obstetrics, pediatrics, emergency room, burns, laboratory, radiology, pharmacy, intensive care unit (including cardiac care unit), physiotherapy, and renal dialysis.

3.2.2.4.3 Taking the nasal swab

Nasal swabs were taken by the researcher himself. Firstly, the procedure and its purpose were explained to the participant. Then, after wearing gloves, a sterile swab was opened, few drops of distilled water were added to the tube of the swab, and the cotton tip immersed in the water drops and squeezed on the wall of the tube. The participant sitting with his/her head slightly tilted backward, the tip of the swab inserted to one nostril 1-2 cm and rolled five times against the inside walls of the nostril with slight pressure. Using the same swab, the other nostril swabbed in the same way. The swab was directly spread on a plate of Mannitol salt agar and blood agar, or returned to its tube to be transported to the laboratory for culturing if the time to reach the laboratory is less than two hours.

3.2.2.4.4. Time of the study

The practical work for this part of the study started in February, 2014. Arrangement of places for work and obtaining consent as well as preparation of forms, materials and equipments were done. Collection of nasal swabs from health care staff alongside with laboratory work on them started in the same month and continued up to the end of the August, 2014.

3.3. The Laboratory work

Generally, the laboratory work started immediately after obtaining nasal swabs. These swabs were cultured to detect (*S. aureus* and CoNS) bacteria and then they were checked for antibiotic resistance for detection of MR/SSA and MR/SCoNS.

3.3.1. Setting

The laboratory work started immediately after obtaining nasal swabs. All the work was done in the bacteriology unit of the laboratory of Qaladze General Hospital.

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3.3.2. Bacteriological methods

3.3.2.1. Detection of *S. aureus*

The bacteria were identified as *S. aureus* on the basis of their characteristic growth pattern, catalase test, coagulase production, slide test for clumping factor, the culture of bacteria on Mannitol salt agar was confirmed as *S. aureus* by the following ways:

1. Fermentation of the agar: To confirm fermentation of Mannitol, growth of yellow colonies surrounded by yellow zones after 24 hours of incubation at 35°C indicated a positive result.
2. Gram staining: This staining was done next, and it had to be gram positive staphylococci.
3. Positive slide catalase test: This was done by transferring cells from the center of a well isolated colony to a glass slide with loop or applicator stick, and a drop of 3% hydrogen peroxide was added to the bacteria. The test was considered positive if rapid sustained gas bubbles appeared.
4. The final confirmation was by positive tube coagulase test. A sterile tube was filled by 0.5 ml of rabbit plasma. Bacteria were added by a sterile loop to the plasma and incubated at 37°C and the suspension was observed at half hourly interval for 4 hours. A positive result was indicated by the presence of the insoluble clot of coagulated serum. If the test remained negative until four hours at 37°C, the tube was kept at room temperature to complete 24 hours and observed again for the final result (Plata *et al*, 2009).

3.3.2.2. Detection of MRSA and MSSA by agar screening method

The bacteria, after being confirmed as *S. aureus*, were suspended to the density of a 0.5 McFarland standard. A sterile swab was dipped in the McFarland suspension, the excess liquid expressed and inoculated to the MH agar containing 4% NaCl and 6 mg/L oxacillin. The plates were incubated at 35°C or less for 24-48 hours. Any growth other than a single colony was indicative of resistance (MRSA), but if no growth any colony it was indicative sensitive (MSSA). For positive control, again a sterile swab was dipped in the suspension and inoculated to blood agar plates. The plates were incubated in the same manner (Brown *et al*, 2005).

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3.3.2.3 Antimicrobial sensitivity testing

The susceptibility of the entire isolated organisms to selected antibiotics which were normally used to treat nasal carriage bacteria was tested by Kirby-Bauer disk diffusion technique (Bauer *et al*, 1966). All the isolated nasal carriage bacteria were put into appropriate media for antibiotic susceptibility. Disk diffusion test was carried out according to the recommendations of the CLSI. They pass through the numbers of steps as follows (Cavalieri *et al*., 2005):

1. The tops of 4 to 5 well-isolated uniform colonies (colonies must not be older than 18–24 hours) were touched with an inoculating loop and used to inoculate the tube containing 4 to 5ml of sterile normal saline and mixed well. The tube which contained bacterial suspension was compared with the turbidity of 0.5 McFarland standard solutions, and the density of the test suspension was adjusted visually to that of the standard by adding more bacteria or normal saline, and then the turbidity of the suspensions were compared by placing the tubes in front of a file card with black lines.

2. All tests were performed on Mueller-Hinton agar plates, and before it was used it was allowed to warm to room temperature. The plates were inoculated by dipping a sterile cotton swab into bacterial suspension having visually equivalent turbidity to 0.5 McFarland standard solutions; the excess of inoculums was removed by pressing and rotating the swab firmly against the side of the tube above the level of the suspension.

3. The swab then was used to streak over the dry surface of Mueller-Hinton agar plates in three directions and this will ensure that the inoculums is evenly distributed. The inoculums plates was left to dry with the lid closed for at least 15 minutes at room temperature.

4. The antimicrobial disks were stored at -20°C when not in use; the disks were allowed to reach room temperature before being opened. Various antibiotic discs were placed on the surface of the agar medium by gently pressing using a sterile forceps on the top of the discs (for better contact and effective diffusion of the antibiotics into the medium). The plates were incubated in an inverted position at 35°C for 18 to 24 hours.

5. On the next day, plates were read by taking measurement of zone of inhibition produced by antimicrobial inhibition of bacterial growth. Inhibition zones were measured in millimeter (mm) by using a venire caliper.

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3.3.2.4. Detection of MRSA and MSSA by direct disk method

After being confirmed as MRSA by agar screening method, the bacteria were spread on a plate of MH agar and a methicillin susceptibility disk of 5 mcg was placed on the plate. Then it was incubated at 35°C for 24 hours, and an inhibition zone of less than 9 mm was considered positive for MRSA, but more than 9 mm was considered positive for MSSA (Brown, *et al*, 2005)**.**

3.3.2.5. Detection of (MR/SCoNS) by agar screening method

Susceptibility to oxacillin was determined by the Disk Diffusion (DD) method on Mueller–Hinton agar plates (Hi-Media Laboratories) using a bacterial suspension with the turbidity adjusted to a 0.5 McFarland standard. Plates were incubated at 35 °C for 24 h. Results were interpreted according to Clinical Laboratory Standards Institute guidelines. The interpretive criteria for oxacillin were *S. aureus*, sensitive ≥13µg ml^{-1}, resistant ≤12µg ml^{-1}; CoNS, sensitive ≥18µg ml^{-1}, resistant ≤17µg m (Jain, *et al*., 2008; CLSI, 2008).

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4. RESULTS

4.1. MR/SSA and MR/SCoNS among health care staff in hospitals

4.1.1. Demographic characteristics

A total of one hundred and thirty (130) samples were collected from nasal carriage of health care staff and doctors, from Qaladze General Hospital and Health center in Qaladze city.

About 63% of the total participants (130) were from the age group 25-34 years; Health care staff from Qaladze General Hospital in Qaladze consists of 200 nurses and 20 doctors, and 93.07% of the entire sample was nurses. The highest percentage of study participants (20%) were those working in the operation room department. These numbers and some other demographic characteristics are shown in table (4.1).

Table 4.1. Demographic characteristic of health care staff Sample (n:130)

Characteristic of personal		**No.**	**(%)**
Age (year)	25-34	82	(63.08)
	35-44	31	(23.85)
	>44	17	(13.07)
Sex	Male	66	(50.76)
	Female	64	(49.24)
Hospital name	Qaladze General Hospital	120	(92.31)
	Health center	10	(7.69)
Staff in hospital	Doctor	9	(6.92)
	Nurse	121	(93.08)

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Table 4.1. Demographic characteristic of health care staff Sample (n:130)

Characteristic		**No.**	**%**
Department	Laboratory	20	15.39
	Operation room	26	20
	Pharmacy	10	7.69
	Surgery Doctor	3	2.30
	ICU	9	6.93
	X-Ray	8	6.16
	Blood Bank	10	7.69
	Pediatrics	4	3.07
	Emergency	8	6.16
	ENT Doctor	2	1.54
	Other staff	30	23.07
Duration of work in hospital (year)	5 or less	50	38.46
	6 - 10	38	29.24
	11 - 15	26	20
	> 15	16	12.30
Daily time of stay in hospital (hour)	6 or less	96	73.85
	More than 6	34	26.15

4.1.2. Detection of MRSA and MSSA

In the laboratory, once *S. aureus* was identified as golden yellow colonies on Mannitol salt agar, then Gram stain, catalase test and tube coagulase test were done on them and all were positive as shown in figures (4.1, 4.2, 4.3, and 4.4). Then MRSA were identified by resistant to methicillin disk (5mcg) on Muller-Hinton agar, and identified MSSA by susceptibility to methicillin disk (5mcg).

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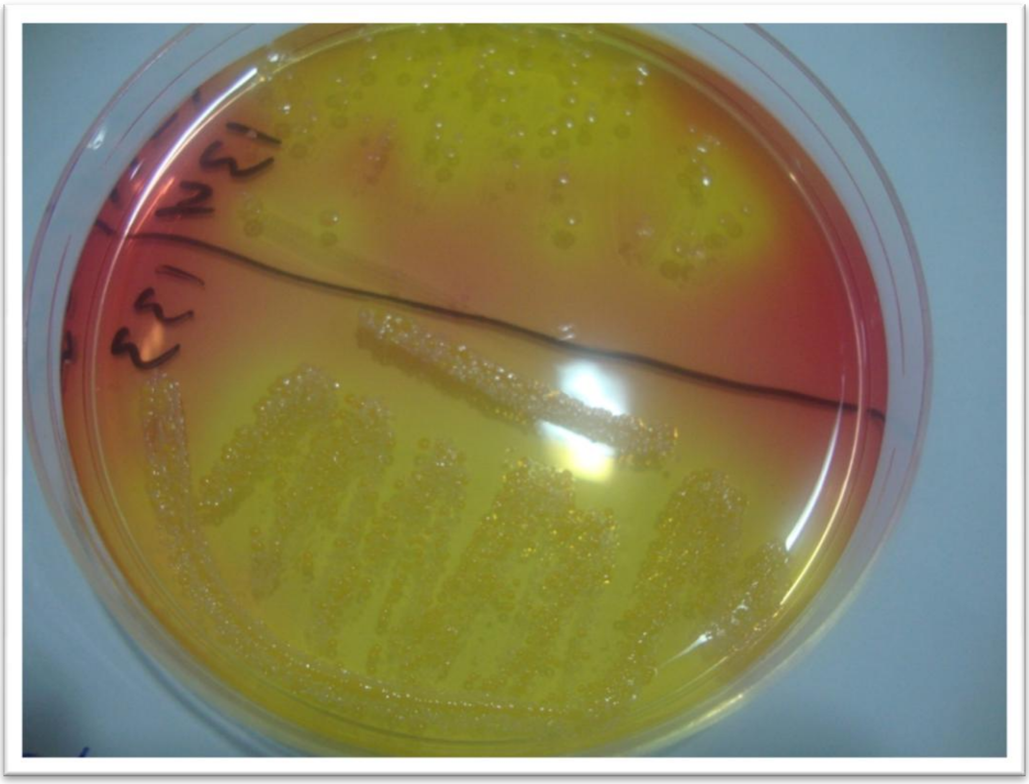

Figure 4.1. Growth of *S.aureus* on Mannitol salt agar.

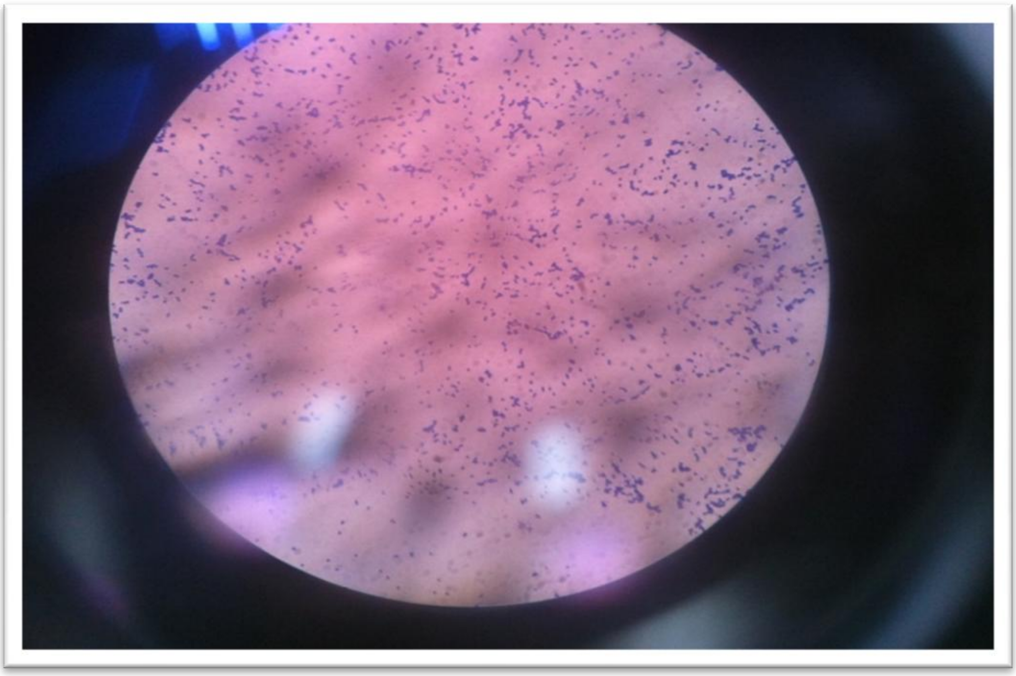

Figure 4.2. *S. aureus* under the microscope

Figure 4.3. Catalase positive test

Figure 4.4. Coagulase positive test

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Figure 4.5. *S. aureus* on blood agar.

4.1.3. Prevalence of nasal carriage of *S. aureus*, MRSA, MSSA.

Table 4.2 and Figure (4.9) shows that the rate of nasal carriage of total 130 samples, *S. aureus* were 14.6%, MRSA was constitute 6.15% of total samples among health care staff, and MSSA was constitute 8.45% of total samples. Moreover, the table reveals that more than half of *S. aureus* isolates were MSSA.

Table 4.2. Prevalence of nasal carriage of *S. aureus*, MRSA, MSSA among health care staff

***S. aureus* carriage**	**MRSA Status**		**Total No.(%)**	**MSSA Status**		**Total No.(%)**
	Yes	**No**		**Yes**	**No**	
Yes	8*(6.15)	11(8.45)	19(14.6)	11*(8.45)	8(6.15)	19 (14.6)
No	Not applicable	111(85.4)	111(85.4)	Not applicable	111(85.4)	111(85.4)
Total - No.(%)	8(6.15)	122(93.85)	130(100)	11(8.45)	119(91.55)	130 (100)

* This constituted (MRSA) 6.15% of *S. aureus* carriers (19), and constituted (MSSA) 8.5% of *S. aureus* (19).

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4.1.4. Association of MRSA nasal carriage with the healthcare staff.

4.1.4.1. MRSA status and association with age

There was a significant association between age and carrying MRSA among health care staff as those aged 25-34 years old had 3 times the odds of carrying MRSA compared to those aged 35-44 years. Of total 19 (14.6%) *S.aureus*, 10 (52.64%) were between (25-34) years old, 7 (36.84%) were between (35-44) years, 2 (10.52%) were above (44) years, However, the association was not significant among those aged 44 years and more. These associations are seen in table (4.3) and figure (4.8)

Table 4.3. Association between age and nasal carriage of (MRSA) among health care staff

Age (years)	**MRSA**		**Total No. (%)**
	Positive No. (%)	**Negative No. (%)**	
25-34	6 (60)	4 (40)	10 (100)
35-44	2 (29)	5 (71)	7 (100)
> 44	0 (0)	2 (100)	2 (100)

4.1.4.2. MRSA status and association with sex

There was 50% of carrying MRSA in the nose of males compared with females 50% are equals, of total 19 (14.6%) *S.aureus*, 8 (42.10%) were males and 11 (57.90%) were females, and this association was significant. As shown in table (4.4) and figure (4.9).

Table 4.4. Association between sex and nasal carriage of (MRSA) among health care staff

Sex	**MRSA**		**Total No. (%)**
	Positive No. (%)	**Negative No. (%)**	
Male	4 (50)	4 (50)	8 (100)
Female	4 (36.36)	7 (63.64)	11 (100)

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4.1.5. Association of MSSA nasal carriage with the health care worker.

4.1.5.1. MSSA status and association with age

There was a significant association between age and carrying MSSA among health care staff as those aged 25-34 years old had less of carrying MSSA compared to those aged 35-44 years. Of total 19 (14.6%) *S. aureus*, 10 (52.64%) were between (25-34) years old, 7 (36.84%) were between (35-44) years, 2 (10.52%) were above (44) years However, the association was not significant among those aged more than 44 years. These associations are seen in table (4.5) and figure (4.8)

Table 4.5. Association between age and nasal carriage of (MSSA) among health care staff

Age (years)	MSSA		Total No.(%)
	Positive No.(%)	Negative No.(%)	
25-34	4 (40)	6 (60)	10 (100)
35-44	5 (71.43)	2 (28.57)	7 (100)
> 44	2 (100)	0 (0)	2 (100)

4.1.5.2. MSSA status and association with sex

There was 36.36% of MSSA nasal carriage were males, that lower chance of carrying MSSA in the nose of males compared to females were 63.64%, of total 19 (14.6%) *S.aureus*, 8 (42.10%) were males and 11 (57.90%) were females and this association was significant, as shown in table (4.6) and figure (4.9).

Table 4.6. Association between sex and nasal carriage of (MSSA) among health care staff

Sex	MSSA		Total No. (%)
	Positive No. (%)	Negative No. (%)	
Male	4 (50)	4 (50)	8 (100)
Female	7 (63.64)	4(36.36)	11 (100)

4.2. Detection of MRCoNS and MSCoNS

In the laboratory, when CoNS grows on MSA, they can’t ferment Mannitol, so the colour of the media around the bacterial colony does not change to yellow, then gram stain, catalse test positive, but tube coagulase test was done on them and all were negative. Then *Staphylococcus* colonies were transferred to on Mueller-Hinton agar, and used methicillin disk, MRCoNS were identified by resistant to methicillin disk (5mcg) on Muller-Hinton agar, and identified MSCoNS by susceptibility to methicillin disk (5mcg). Shown in figure (4.6), that growth Staphylococci on Mannitol salt agar (4.6).

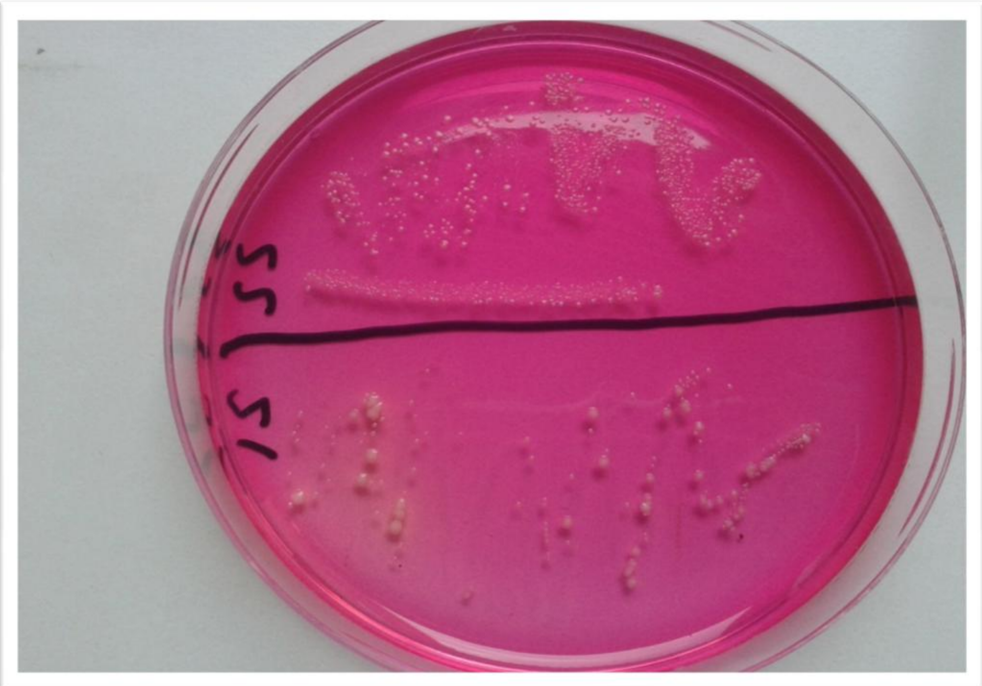

Figure 4.6. Staphylococci on Mannitol salt agar.

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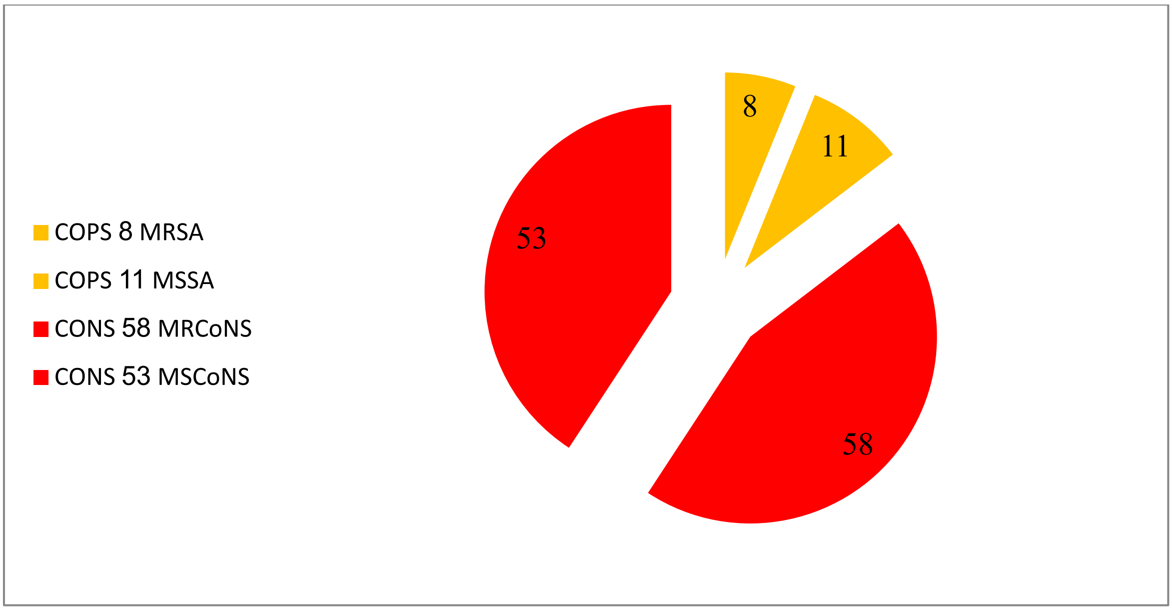

Figure 4.7.distribution of *S. aureus* and CoNS on Mannitol salt agar.

4.2.1. Prevalence of nasal carriage of (MRCoNS and MSCoNS)

Table 4.7 shows that the rate of nasal carriage of CoNS was 111 (85.4%) of total 130 nasal carriage, MRCoNS was constituted 44.63% of total nasal carriage among health care staff, and MSCoNS was constitute 40.77% of total samples. Moreover, the table reveals which more than half of CoNS isolates were MRCoNS.

Table 4.7. Prevalence of nasal carriage of MRCoNS and MSCoNS among health care staff

CoNS Carriage	**MRCoNS No.(%)**		**Total No.(%)**	**MSCoNS No.(%)**		**Total No.(%)**
	Yes	**No**		**Yes**	**No**	
Yes	58(44.62)	53(40.78)	111(85.4)	53*(40.78)	58(44.62)	111(85.4)
No	Not applicable	19(14.6)	19 (14.6)	Not applicable	19(14.6)	19 (14.6)
Total No.(%)	58(44.62)	72(55.38)	130(100)	53(40.78)	77(59.22)	130(100)

*This constituted (MRCoNS) 44.6% of CoNS (111), and constituted (MSCoNS) 40.8% of CoNS (111).

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4.2.2. Association of MRCoNS Nasal Carriage with the Healthcare Staff.

4.2.2.1. MRCoNS association with age

There was a significant association between age and carrying MRCoNS among health care staff as those aged 25-34 years old had more than of carrying MRCoNS compared to those aged 35-44 years. Of total 111 (85.4%) CoNS, 72 (64.86%) were between (25-34) years old, 24 (21.62%) were between (35-44) years, 15 (13.52%) were above (44) years. However, the association was not significant among those aged 44 years and more. These associations as shown in table (4.8) and figure (4.8).

Table 4.8. Association between age and nasal carriage of MRCoNS among health care staff.

Age (year)	**MRCoNS.**		**Total No.(%)**
	Positive No.(%)	**Negative No.(%)**	
25-34	36 (50)	36 (50)	72 (100)
35-44	14 (58.33)	10 (41.67)	24 (100)
> 44	8 (53.33)	7 (46.67)	15 (100)

4.2.2.2. MRCoNS association with sex

There was 46.55% of MRCoNS nasal carriage were males. Which comparable with female, thye are less than MRCoNS nasal carriage of females 53.45%. Of total 111(85.4%) CoNS, 58(52.25%) were males, 53(47.75%) were females. This association was significant, as shown in table (4.9) and figure (4.9).

Table 4.9. Association between sex and nasal carriage of MRCoNS among health care staff

Sex	**MRCoNS**		**Total No. (%)**
	Positive No. (%)	**Negative No. (%)**	
Male	27 (46.55)	31 (53.45)	58 (100)
Female	31 (58.50)	22 (41.50)	53 (100)

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4.2.3. Association of MSCoNS nasal carriage with the healthcare staff.

4.2.3.1. MSCoNS association with age

There was a significant association between age and carrying MSCoNS among health care staff as those aged 25-34 years old had more than of carrying MSCoNS compared to those aged 35-44 years. Of total 111(85.4%) CoNS, 72(64.86%) were between (25-34) years old, 24 (21.62%) were between (35-44) years, 15(13.52%) were above (44) years However, the association was not significant among those aged 44 years and more. These associations as shown in table (4.10) and Figure (4.8).

Table 4.10. Association between age and nasal carriage of MSCoNS, among health care staff.

Age (year)	MSCoNS. Positive No. (%)	MSCoNS. Negative No. (%)	Total No.(%)
25-34	36 (50)	46 (50)	72 (100)
35- 44	10 (41.67)	14 (58.33)	24 (100)
>44	7 (46.67)	8 (53.33)	15 (100)

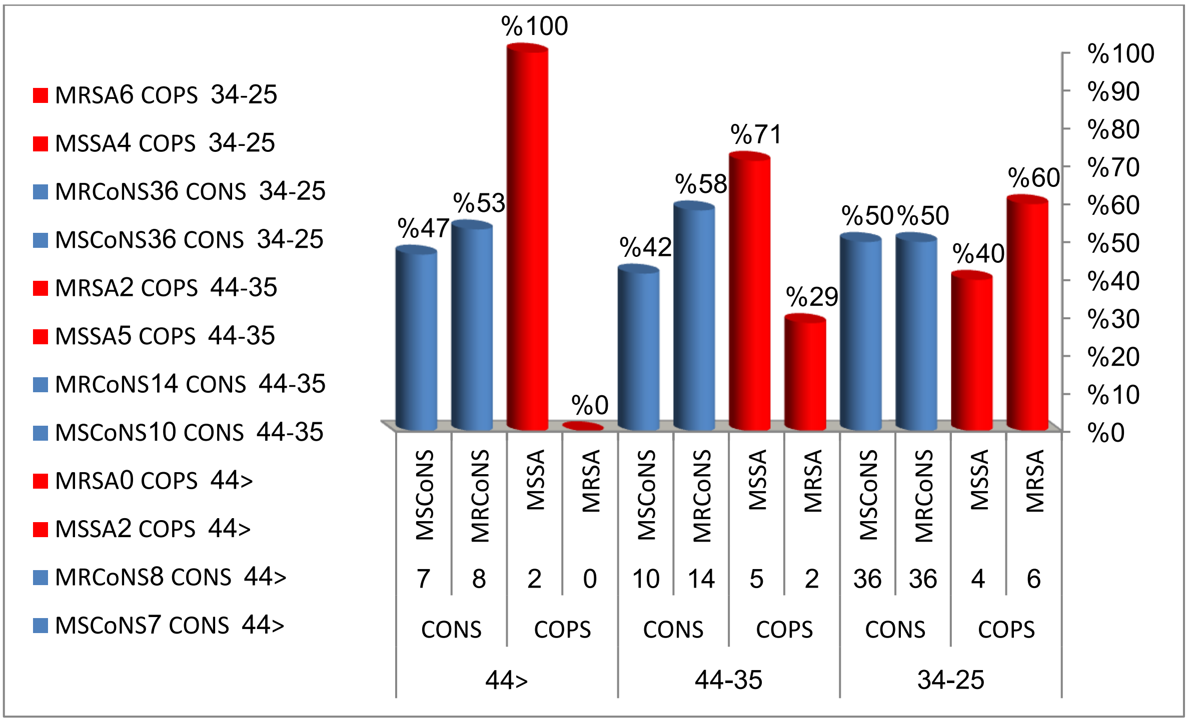

Figure 4.8. CoPS and CoNS distribution among age groups.

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4.2.3.2. MSCoNS association with sex

In the Table (4.11) and Figure (4.9), it appears that the nasal carrying MSCoNS in the nose of males was more than nasal carrying MSCoNS in the nose of females among healthcare staff, and of total 111(85.4%) CoNS, 58(52.25%) were males, 53(47.75%) were females.

Table 4.11. Association between sex and nasal carriage of MSCoNS among health care staff

Sex	**MSCoNS**		**Total No. (%)**
	Positive No. (%)	**Negative No. (%)**	
Male	31 (53.45)	27 (46.55)	58 (100)
Female	22 (41.50)	31 (58.50)	53 (100)

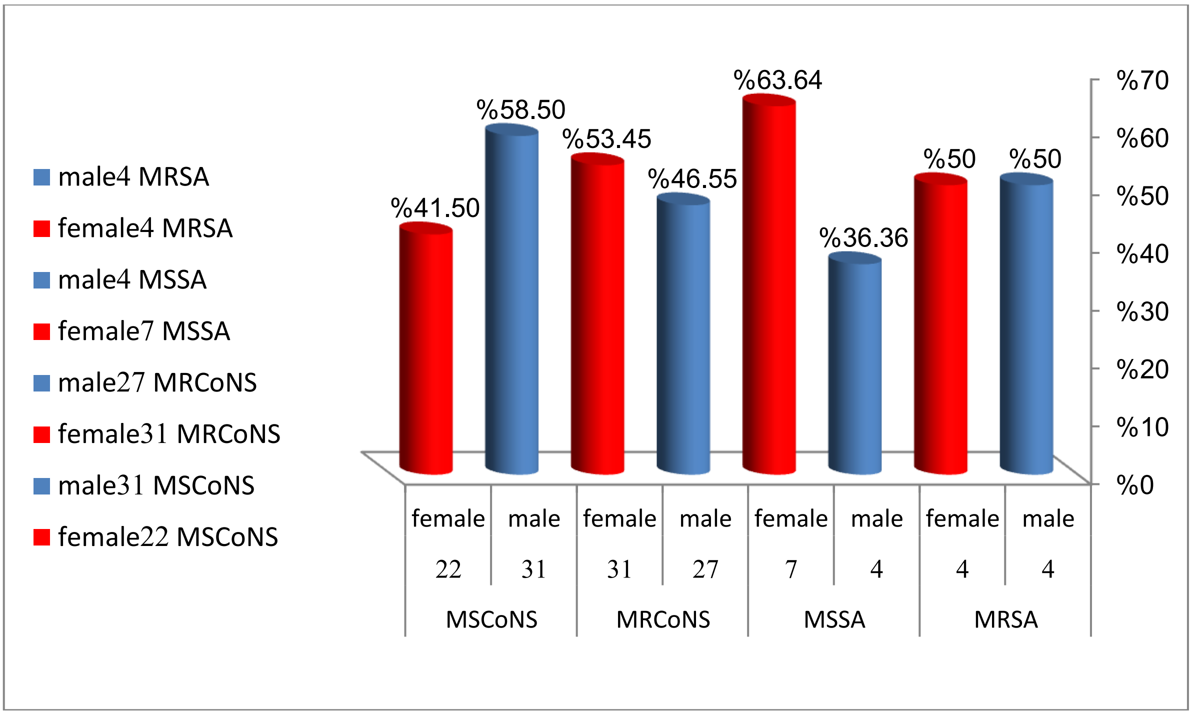

Figure 4.9. Association between sex and isolates among health care staff.

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4.2.4. Isolates and association with the health care staff in the hospital

4.2.4.1. MRSA, MSSA, MRCoNS, MSCoNS and association with the type of work in the hospital

No significant difference were seen between doctors and health care staff with regard to nasal carriage of (MRSA, MRCoNS, MSCoNS), but in nasal carriage of (MSSA), There was a difference between them, because all nasal carriage of (MSSA) was nurses. Of total 19 *S.aureus*, 2(10.53%) were doctors, 17(89.47%) were nurses. But of total 111 CoNS, 7(6.30) were doctors, 104(93.70) were nurses. As seen in table (4.12) and (4.13).

Table 4.12. Association between the type of work and nasal carriage of (MRSA, MSSA) among health care staff.

Work	**MRSA**		**Total No.(%)**	**MSSA**		**Total No.(%)**
	Positive No.(%)	**Negative No.(%)**		**Positive No.(%)**	**Negative No.(%)**	
Doctor	2 (100)	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)
Nurse	6 (35.30)	11 (64.70)	17 (100)	11 (64.70)	6 (35.30)	17 (100)

Table 4.13. Association between the type of work and nasal carriage of (MRCoNS, MSCoNS) among healthcare staff.

Work	**MRCoNS**		**Total No. (%)**	**MSCoNS**		**Total No.(%)**
	Positive No.(%)	**Negative No.(%)**		**Positive No.(%)**	**Negative No.(%)**	
Doctor	5 (71.42)	2 (28.58)	7 (100)	2 (22.22)	5 (71.42)	7 (100)
Nurse	53 (50.96)	51 (49.04)	104 (100)	51 (49.04)	53 (50.96)	104 (100)

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4.2.4.2. Association between nasal carriage of MR/SSA and MR/SCoNS with the department's staff in the hospital

The rate of MRSA carriage in the nose of laboratory and blood bank departments staff was appeared as the same rate(1.53%) more than other departments, but MSSA carriage rate was more appeared in the operation room department, pharmacy department, and other staff as the same rate (1.53%) more than other departments, from laboratory department and other staff had the highest rates of MRCoNS carriage (7.69% and 10.76%) from the other departments, and MSCoNS nasal carriage rate as the same MRCoNS was from laboratory department staff and other staff had the highest rates (5.38% and 10%) respectively, however, the difference of rates of nasal carriage Isolates among healthcare staff in relation to the department of hospital they worked in was not significant, in table (4.14) more appears.

4.2.4.3. Association of MR/SSA and MR/SCoNS with antibiotics

In this study, demonstrated that all isolates bacteria have a different rate of resistant and sensitive to the antibiotics that we used, the discovery only substantiates of the fact that resistance to methicillin predicts resistance to other beta-lactam drugs, as show in Figure (4.10) and depicted in table (4.15)

Figure 4.10. *Staphylococcus sp.* was shown multidrug resistant

* Table (4.14) Association between Nasal Carriage of MR/SSA and MR/SCoNS With the departments Staff in the Hospital

(N=130)

Department Charactreistic	No.	MRSA N=8		MSSA N=11		MRCoNS. N=58		MSCoNS. N=53	
		Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)
Laboratory	20	2 (1.53)	18 (13.84)	1 (0.76)	19 (14.61)	10 (7.69)	10 (7.69)	7 (5.38)	13 (10)
Operation room	26	1 (0.76)	25 (19.23)	2 (1.53)	24 (18.84)	13 (10)	13(10)	10 (7.69)	16 (12.30)
Pharmacy	10	0 (0)	10 (7.69)	2 (1.53)	8 (6.15)	3 (2.30)	7 (5.38)	5 (3.84)	5 (3.84)
Surgery doctor	3	1 (0.76)	2 (1.53)	0 (0)	3 (2.30)	2 (1.53)	1 (0.76)	0 (0)	3 (2.30)
ICU	9	0 (0)	9 (6.92)	1 (0.76)	8 (6.15)	3 (2.30)	6 (4.61)	5 (3.84)	4 (3.07)
X-Ray	8	0 (0)	8 (6.15)	0 (0)	8 (6.15)	3 (2.30)	5 (3.84)	5 (3.84)	3 (2.30)
Blood bank	10	2 (1.53)	8 (6.15)	3 (2.30)	7 (5.38)	3 (2.30)	7 (5.38)	2 (1.53)	8 (6.15)
Pediatrics doctor	4	0 (0)	4 (3.07)	0 (0)	4 (3.07)	3 (2.30)	1 (0.76)	1 (0.76)	3 (2.30)
Emergency	8	0 (0)	8 (6.15)	0 (0)	8 (6.15)	4 (3.07)	4 (3.07)	4 (3.07)	4 (3.07)
ENT doctor	2	1 (0.76)	1 (0.76)	0 (0)	2 (1.53)	0 (0)	2 (1.53)	1 (0.76)	1 (0.76)
Other staff	30	1 (0.76)	29 (22.30)	2 (1.53)	28 (21.53)	14 (10.76)	16 (12.30)	13 (10)	17 (13.07)

Table (4.15) Association of MRSA, MSSA, MRCoNS, and MSCoNS with Antibiotics

Antibiotics	MRSA		MSSA		MRCoNS		MSCoNS	
	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)
IPM	0	100	0	100	1.73	98.27	0	100
TMP	25	75	63.64	36.36	58.6	41.4	60.37	39.63
VA	0	100	0	100	0	100	0	100
AM	100	0	100	0	96.55	3.45	81.14	18.86
CRO	100	0	100	0	94.83	5.17	75.5	24.5
P	100	0	90.90	9.10	94.83	5.17	84.90	15.10
CIP	0	100	27.27	72.72	19	81	24.5	75.5
AZM	62.5	37.5	63.64	36.36	44.83	55.17	28.30	71.70

IPM: imipenem, TMP: trimethoprim, VA: vancomycin, AM: ampicillin, CRO: ceftriaxone, P: pencillin, CIP: ciprofloxacin, AZM: azithromycin

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5. DISCUSSION

5.1. Prevalence of MR/SSA and MR/SCoNS Among the Health Care Staff

Staphylococci are recognised as important cause of disease around the world. Staphylococcal infections are of particular concern because of the causative agent offering resistance to a wide range of antibiotics. Staphylococcal strains have shown disconcerting propensity to develop resistance to antimicrobial agents and has become a challenge for infection control programmer and the clinicians, Resistance to antimicrobial agents is a major concern worldwide by the global spread of Methicillin- resistan *S. aureus* (MRSA) (Sharvari, 2012).

Staphylococcus aureus has long been recognized as an important pathogen in human diseases. MRSA is the name given to a strain of the *S. aureus* bacteria that is resistant to a number of antibiotics. It is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to most of the current antibiotic classes. Whereas hospital-acquired MRSA has been a concern for in-patients since the 1960s, the threat of CA-MRSA has recently been associated with healthy people without traditional risk factors (Kaibni, *et al*, 2009; Elie-Turenne, *et al*., 2010).

Coagulase negative Staphylococci (CoNS) are among the most important microorganisms in nosocomial bloodstream infections. These bacteria can be pathogenic, especially in immunocompromised patients with hematological and other malignancies and in patients with foreign bodies, such as invasive catheters (Uyanik, *et al.*, 2013).

Analyzing our data base, we could take 130 cases of healthcare staff nasal swabs in which 50.76% were males and 49.24% were females. Most of the healthcare staff was between the age group of 25 - 34 years.

The study revealed that *S. aureus* were 14.61%. Those 42.10% nasal carriage rates of *S. aureus* were MRSA among the participated health care staff, 57.90% were MSSA. Higher rates than ours for MRSA were reported from some other countries in the Middle East. For instance, MRSA was found in 38% (111/292) of *S. aureus* isolated from clinical specimens in 1998 at King Abdulaziz University Hospital, Jeddah, Saudi Arabia (Buzaid, *et al*., 2011).

In this study, of 130 *Staphylococcus* strains, 19 (14.6%) were *S. aureus* and 111 (85.4%) were coagulase negative Staphylococci, which is comparable with the study

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conducted by (Date, *et al*., 2012) reported of 140 *Staphylococcus* strains, 70 (50%) were *S. aureus* and 70 (50%)were CoNS and which is comparable with the study conducted by (Kumar *et al*, 2011), was appear of total 84 samples, 40 (47.6%) *S. aureus* and 44 (52.4%) CoNS.

In the present study, of total 130 samples, methicillin disk sensitivity revealed that 8 (6.15%) isolates wereMRSA, 11(8.45%) were MSSA, 58 (44.63%) were MRCoNS, 53 (40.77%) were MSCoNS. Predominant isolates were MRCoNS (44.63%). Date *et al*, reported 13.57% MRSA, 36.43% MSSA, 13.57% MRCoNS, and 36.43% MSCoNS indicating predominance of MSSA. And Kumar *et al*, reported of 40 (47.6) *S.* aureus, were 18 (45%) MRSA, and 22 (55%) MSSA.

Date, *et al*, (2012), results with my results have a high differentiation between them, therefore I think the nature, population number, and geographical place have a major role to indicate the predominant isolates and researches result. Hill *et al*, (2007), reported of total 72 *S.aureus* were 16 (22%) MRSA, and 56 (78%) MSSA, while Shibabaw *et al*, 2013 reported of total 34 *S. aureus* were 15 (44.11%) MRSA, and 19 (55.88%) were MSSA. Akhtar, 2010 reported of total 85 *S. aureus* were 7 (8.23%) MRSA,and 78 (91.76%) MSSA. Baragundi, *et al*, 2012 reported of total 58 samples, 16 (27.58%) were MSCoNS and 42 (72.42%) were MRCoNS.

Rallapalli *et al*, (2008), reported of total 150 samples, 36.66% were MSSA, 26.66% were MRSA, 29.33% were MSCoNS, and 7.33% were MRCoNS. Kirecci, *et al*, 2010, reported of total 135 midwifery student, 14.1% were nasal MSSA carries, and 2.96% were MRSA. Favorable point is no significant increase in the methicillin resistant isolates in our hospital compared with the study by (Kirecci *et al*, 2010).

In this study all of (6.15%) MRSA, (8.46%) MSSA, (44.63%) MRCoNS and (40.76%) MSCoNS were isolated from nasal carriage sample. Other studies have reported wide range of isolation of different Staphylococci strains from clinical sample (Saravanan *et al*, 2014; Ahmad and Asrar, 2014). Soumya, *et al*, (2013), reported of total 30 samples, (72%) were MRCoNS, and (28%) were MSCoNS.

When my study comparable with (Vishwajith *et al*, 2014) report, in our study of totals 130 samples, 66 (50.76%) were males and 64 (49.24%) were females. But in (Vishwajith *et al*, 2014) reported of total 98 samples, 74 (75.5%) were males, and 24 (24.5%) were females.

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Vishwajith *et al*, (2014), reported Among the *Staphylococcus spp*, 50% were MRSA, 33.9% were MSSA, 9% were MSCoNS and 7.1% were MRCoNS. MRSA and MRCoNS are recognized as a major cause of nosocomial infections which result in significant morbidity and mortality (Wei *et al*, 2014). Wei *et al*, 2014 reported, 56.7% were MRSA, and 73.3% were MRCoNS.

Perveen *et al*, (2013) reported, of 350 total samples, 245 (70%) samples were *Staphylococcus aureus* and 105 (30%) were CoNS. Out of total 245 samples, 148 (60.40%) were MRSA, 97 (39.60%) were MSSA, and of total CoNS, 46 (43.80%) were MRCoNS, 59 (56.20%) were MSCoNS.

In this study of total 130 samples, 82 (63.08%) of isolates were between (25 – 34) years old. Were Followed by 31 (23.85%) were between (35–44) years old. Least 17 (13.07%) were above (44) years old.

In this study after the age of 44 years, the rate of all isolates carriage decreased. This may be explained by the fact that those aged more than 44 tend to have a relatively lesser load of work, shorter contact with patients and other healthcare staff, and less time of stay in hospital.

There was no significant association between health care staff nasal carriage of (MRSA, MSSA, MRCoNS, and MSCoNS) and the hospital they worked in. This could be explained by the fact that no special infection control program was applied in any of the involved hospitals. Also this may give the hint that the workload and crowding is mostly the same in all those hospitals, given that overcrowding can lead to failure of all isolates control programs via decreased healthcare staff hand hygiene compliance and increased movement of patients and staff (Clements *et al,* 2008).

Regarding the type of work in hospital, no significant association was found between (MRSA, MSSA, MRCoNS, and MSCoNS) nasal carriage and being a doctor, and nurse. However, nurses had slightly higher prevalence than doctors. This is compatible with other studies that revealed no significant association between the type of work in the hospital setting and the risk of Staphylococci carriage (Zorgani *et al,* 2009; Elei-Turenne *et al*, 2010)

HCS are the persons having contact with the hospital environment and patients. They are highly prone to colonization with multidrug resistant bacteria and can be a

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potential source of hospital acquired infection to the patients they take care of (Baragundi *et al*, 2012).

The department of work had no significant effect on the rate of nasal carriage of MRSA. Likewise, the duration of work in hospital in years as well as the average daily stay in hospital in hours showed no significant effect on the rate of nasal carriage of MRSA. These findings are generally supported by the body of literature (Albrich and Harbarth, 2008; Askarian *et al*, 2009; Elie-Turenne *et al*, 2010).

Of total 130 healthcare staff, (0%) healthcare staff who participated were nasal carriers of VRSA. However, up to 2010, only 9 cases of VRSA have been reported from the US, with 2 additional cases, one from India and another from Iran (Howden *et al*, 2010).

5.2. Antibiotic Sensitivity Tests

Overall, majority 130 (100%) of *Staphylococcus* isolates were sensitive to vancomycin, followed by 129 (99.23%) to imipenem, least 103 (79.23%) to Ciprofloxacin. All of methicillin resistant *S. aureus* (4 male and 4 female) were resistant to ampicillin, ceftriaxone, and pencillin. 25% of MRSA were resistant to trimethoprim, 62.5% MRSA were resistant to azithromycin. While 75% of MRSA were sensitive to trimethoprim, and 37.5% were sensitive to azithromycin.

Trimethoprim-sulphamethoxazole is favored considering the lower rate of resistance (25%) to it by MRSA in this study, comparable with Wei *et al*., (2014) reported MRSA resistant to trimethoprim-sulphamethoxazole, were 19%. In this study, MSSA resistant to ciprofloxacin and trimethoprim-sulphamethoxazole, were (27.27% and 63.64%) respectively, while comparable with Wei *et al*, MSSA resistant to ciprofloxacin and trimethoprim-sulphamethoxazole and were (5.6% and 1.9%) respectively.

In my study all of MRSA resistant to vancomycin were 0%, while comparable with (Mir B and Srikanth, 2013), they reported, 3.5% of MRSA were resistant to vancomycin, of total MSSA (4 male and 7 female), 100% were resistant to ampicillin, and ceftriaxone, 63.64% of MSSA were resistant to trimethoprim, 90.90% to penicillin, 27.27% to ciprofloxacin, 63.64% to azithromycin. Among of all MSSA, 36.36% were sensitive to trimethoprim, 9.10% to penicillin, 72.72% to ciprofloxacin, 36.36% to azithromycin. As shown in table (3.15)

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About of MRCoNS (27 male and 31 female), 1.73% were resistant to imipenem, 58.6% to trimethoprim, 96.55% to ampicillin, 94.83% to ceftriaxone and penicillin, 19% to ciprofloxacin, 44.83% to azithromycin.

Samant, SA., (2012) reported of total 75 nasal swabs of health staff ,29.2% *S.aureus* and 33.3% CoNS were resistant to penicillin, but in my study 95.45% *S.aureus* and 89.86% CoNS were resistant to penicillin.

Khanna, V. (2012) reported all MRSA and MRCoNS were sensitive to vancomycin, that same with my study were all MRSA and MRCoNS in my study sensitive to vancomycin.

Since the emergence of MRSA, the glycopeptides vancomycin has been the only effective treatment for MRSA infections. In the present study all the MRSA and MRCoNS isolates were susceptible to vancomycin. And all the MRSA strains were resistant to penicillin. These results coincide with the findings of. Whereas MRCoNS have become the predominant pathogen in hospitalized patients with the number of infections caused by these pathogens increased dramatically (Perveen *et al*, 2012).

Among of MSCoNS (31 male and 22 female), 13.84% were resistant to trimethoprim, 81.84% to ampicillin, 75.5% to ceftriaxone, 84.90% to penicillin, 24.5% to ciprofloxacin, 28.30% to azithromycin. Prevalence of MSCoNS of total 130 samples in my hospital, were 53 (40.77%) that resistant to (trimethoprim, ampicillin, ceftriaxone, penicillin, ciprofloxacin, and azithromycin, were 13.84%, 81.14%, 75.5%, 84.90%, 24.5%, and 28.30% respectively.

In my study the large number of isolates (92.65%) were resistant to penicillin. and this result was agreed with many others studies that demonstrated the increasing in the rate of penicillin resistance in all Staphylococci isolates (both *S. aureus* and CoNS) especially in the Methicillin resistance strains. The result was agreed with the local studies by AL-Junadi (2005), Zeidan (2007) and Al-Maliki (2009) who showed that Penicillin resistance rates in the *S. aureus* were 90.5%, 97.5% and 87.8% respectively,

This increased in the penicillin resistance isolates among Staphylococci strains can be explained in most cases to the production of β-lactamase enzyme that destroyed the β-lactam ring and inactivated the penicillin antibiotic and this enzyme was encoded by plasmid that easy to transfer among strains (Brooks *et al*., 2007).

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In this study of total 19 samples of *S. aureus*, 16 (84.21%) were sensitive to ciprofloxacin, and of total 111 samples of CoNS, 87 (78.37%) were sensitive to ciprofloxacin, these results agreed with many other studies in the world for example (Majumder, 2001) reported, 97.3% of CoPS and 83.3% of CoNS were sensitive to ciprofloxacin.

In comparison between MRSA and MRCoNS isolates, found that MRSA were more resistance to antibiotics than MRCoNS isolates, that the rates of resistance to five antibiotics (ampicillin, ceftriaxone, penicillin, azithromycin, and ciprofloxacin) were higher in MRSA than in the MRCoNS isolates. But the rates of resistance to (imipenem and trimethoprim) were higher in MRCoNS than in the MRSA isolates. However there were some differences in the mechanism of resistance between the two groups of isolates.

In comparison between MSSA and MSCoNS isolates, found that MSSA were more resistance to antibiotics than MSCoNS isolates, that the rates of resistance to six antibiotics (trimethprim, ampicillin, ceftriaxone, penicillin, azithromycin, and ciprofloxacin) were higher in MSSA than in the MSCoNS isolates.

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6. CONCLUSIONS AND SUGGESTIONS

6.1. Conclusions

1. A total 130 Staphylococcal isolates comprising of 8 (6.15%) MRSA, 11 (8.45%) MSSA, 58 (44.63%) MRCoNS, 53 (40.77%) MSCoNS were recovered from nasal carriage samples.
2. Prevalence of nasal carriage of all isolates among healthcare staff in Qaladze district is, somewhat, lower than what is reported in another developing countries and higher than that in the developed countries.
3. All isolates nasal carriage rate decreased with increasing age of the health care staff in the hospital of Qaladze district.
4. The hospital as a place of work, type of work, sex, department of work, duration of work and average daily stay in the hospital showed no significant association with the prevalence of isolates nasal carriage.
5. The methicillin resistance Staphylococci (both *S. aureus* and CoNS isolates) showed multi-drug resistance pattern, and MRSA isolates were highly resistant to antimicrobial agents than MRCoNS isolates.
6. Screening should be made an essential protocol to assess the carrier transmitted drug resistant strains of Staphylococci from the healthcare worker to another.
7. The study showed that out of total specimens collected from nurses, doctors, were carrier for MRSA, MSSA, MRCoNS, and MSCoNS.
8. The carriage of both MRSA/MSSA and other pathogens in healthcare staff and personnel must be periodically investigated and identified and treated.
9. Trimethoprim-sulphamethoxazole has an important role in the management of MRSA or MRCoNS infections, because (MRSA and MRCoNS) resistant to this antibiotic, were (25% and 58.6%) respectively.

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6.2. Suggestions

1. Emphasis on the subject of MRSA and other isolates in the continuing medical education program activities within all hospitals of Qaladze district. Doctors from every medical department should be encouraged to participate in such activities.
2. Training and educational sessions about MRSA and other isolates to be held for healthcare staff regularly.
3. Infection control programs, especially about MRSA need to be applied in hospitals. Within these programs annual regular screening of healthcare staff should be included.
4. Investigate the effect of other antimicrobial agents, chemical materials, plant extracts, antiseptics or some products of other species of bacteria on the biofilm formation by Methicillin resistance *Staphylococcus sp*.
5. In hospitals, HCS nasal carriage of MRSA must be regularly screened and give an early warning of the presence of antimicrobial resistant pathogens.
6. Contaminated material must be disinfected or sterilized and the gloves and aprons must be used during the contact with the patient.
7. For developing hospital antibiotic policy and for limiting the use of powerful antibiotics like vancomycin as initial treatment and save it for the treatment of resistant and life-threatening staphylococcal infections.
8. Infection control measures such as hand washing and other aseptic techniques must be followed to avoid therapeutic difficulties associated with these resistant pathogens.

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