NASAL CARRIAGE RATE OF *STAPHYLOCOCCUS AUREUS* AND MRSA AMONG HEALTH CARE WORKERS IN TERTIARY CARE CENTER

A Dissertation Submitted to the Department of Microbiology
Pokhara Bigyan Tatha Prabidhi Campus, Pokhara, Nepal
(Affiliated to Tribhuvan University)
In Partial Fulfillment of the Requirements for the Award
of the Degree of Master of Science in Microbiology
(Medical)

By

SUNITA ADHIKARI

Roll No: 25523

T.U. Reg. No: 5-1-48-305-2007

2017

RECOMMENDATION

This is to certify that Mrs. Sunita Adhikari has completed this dissertation work entitled **" Nasal carriage rate of *Staphylococcus aureus* and MRSA among health care workers in tertiary care center"** as a partial fulfillment of M. Sc. Degree in Microbiology (Medical) under our supervision. To our knowledge, this work has not been submitted for any other degree.

……

Mrs. Jyoti Chhetri
Lecturer
Department of Microbiology,
Pokhara Bigyan Tatha Prabidhi Campus
Pokhara, Nepal

……

Dr. Sanjib Mani Regmi, PhD
Assistant Professor
Department of Microbiology
Gandaki Medical College and
Research Centre Pvt. Ltd.
Pokhara, Nepal

Date: ……..

CERTIFICATE OF APPROVAL

On the recommendation of **Mrs. Jyoti Chhetri** and **Dr. Sanjib Mani Regmi, PhD** this dissertation work of **Mrs. Sunita Adhikari** entitled **"Nasal carriage rate of *Staphylococcus aureus* and MRSA among health care workers in tertiary care center"** has been approved for the examination and is submitted to the Tribhuvan University in partial fulfillment of the requirements for M. Sc. Degree in Microbiology (Medical).

……..
Mr. Hari Prasad Khanal
Campus Chief
Pokhara Bigyan Tatha Prabidhi Campus
Tribhuvan University Affiliated
Nayabazar,
Pokhara, Nepal.

Date: ……..

BOARD OF EXAMINERS

Recommended by: ……..

Mrs. Jyoti Chhetri
Supervisor

……

Dr. Sanjib Mani Regmi, PhD

Supervisor

Approved by: ……

Mr. Hari Prasad Khanal
Campus Chief

Examined by: …….

Mr. Krishna Gurung
Lecturer
Pokhara Bigyan Tatha
Prabidhi Campus

Internal Examiner ……
Prof. Dr. Bishnu Raj Tiwari, PhD
Pokhara University
External Examiner

Date: ……..

ACKNOWLEDGEMENTS

First, I express my deepest acknowledgement to my supervisors **Mrs. Jyoti Chhetri**, Lecturer, Department of Microbiology, Pokhara Bigyan Tatha Prabidhi campus and **Dr. Sanjib Mani Regmi**, PhD, Assistant Professor, Department of Microbiology, Gandaki Medical College and Research Centre Private Limited for providing an opportunity to work under their supervision and guidance. Their wisdom, knowledge and commitment to the highest standards inspired and motivated me.

I am much obliged to my Campus Chief Mr**. Hari Prasad Khanal**. It gives me an immense pleasure to thank my respected teachers **Mr. Krishna Gurung Mr. Abhishek Khadka, Mr. Ujwal Koirala** and **Mr. Shiva Lamichhane** for their valuable idea, continuous encouragement and cooperation during the research period.

I am greatly indebted to **Dr. Amar Nagila**, Head of Laboratory Department, the healthcare workers who participated in the study as well as all the laboratory staff of Gandaki Medical College and Research Centre Pvt. Ltd. Pokhara for their kind cooperation and support. My sincere thanks also go to my seniors **Mr. Krishna Banstola** and **Mr. Bharat Baral** my friends **Tulsi Baral, Ratna Kunwar, Shailaza Khanal** and all my classmates.

Finally, I would like to express my gratitude to my husband **Sanjaya Bhandari**, my son **Sushant Bhandari** and sister **Susmita Adhikari** for motivating, inspiring and helping me during data analysis and thesis writing. Last, but not the least, the constant support, blessing and encouragement by my family members during ups and downs are precious in every step of my life.

Sunita Adhikari

Date: …….

ABSTRACT

Staphylococcus aureus is a gram positive bacterium responsible for several bacterial infections. *S. aureus* especially methicillin-resistant *S. aureus* (MRSA), are usually resistant to several antibiotics which is a global public health problem, associated with considerable mortality and morbidity worldwide. Methicillin-resistant *Staphylococcus aureus* continues to be an important nosocomial pathogen and infections are often difficult to manage due to its resistance to multiple antibiotics. Healthcare workers are important source of nosocomial transmission of MRSA.

This study aimed to determine the frequency of staphylococcal nasal carriage of health care workers (HCWs) and antimicrobial susceptibility profile of the isolates in Gandaki Medical College and Research Centre Pvt. Ltd, Pokhara. The study was conducted in altogether 288 samples. For isolation and identification of MRSA culture and different biochemical tests were performed. Out of 58 (20.14%) *S. aureus* isolated 18.97% are MRSA, more MRSA was noticed in female (19.15%) than male (18.18%). However, there is no significant association between gender and MRSA (p=0.723). The prevalence of nasal carrier MRSA is 3.82%. Hence, it was concluded that prevalence of MRSA still emerging. Nasal carriage of *S. aureus* and MRSA among HCWs necessitates the need of control in the frequency of their exposure with the vulnerable patients and need of strict infection control measures to be followed to control the nosocomial infections. The results emphasize the need for high standards of infection control in tertiary care. Vancomycin and Amikacin was found to be most effective (100%) against Methicillin-Resistant *S. aureus*.

Keywords: Health care workers, nasal carriage, *S. aureus*, antimicrobial, MRSA.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

LIST OF APPENDICES

LIST OF ABBREVIATIONS

AIDS	: Acquired Immuno Deficiency Syndrome
ATCC	: American Type Culture Collection
ASM	: American Society of Microbiology
AST	: Antibiotic Susceptibility Test
CA-MRSA	: Community Acquired MRSA
CDC	: Centre for Disease Control
CLSI	: Clinical and Laboratory Standards Institute
CONS	: Coagulase Negative *Staphylococci*
df	: Degree of freedom
DNA	: Deoxyribonucleic Acid
ENT	: Eye Nose Throat
HA-MRSA	: Hospital Acquired MRSA
HCWs	: Health Care Workers
ICU	: Intensive Care Unit
KTM	: Kathmandu
Ltd	: Limited
MA	: MacConkey Agar
MDR	: Multidrug Resistance
MHA	: Muller Hinton Agar
MRSA	: Methicillin Resistant *Staphylococcus aureus*
MSA	: Mannitol Salt Agar

MSSA : Methicillin Sensitive *Staphylococcus aureus*

NA : Nutrient Agar

NCCLS : National Committee for Clinical Laboratory Standards

NICU : Neonatal Intensive Care Unit

OPD : Out Patient Department

ORSA : Oxacillin Resistant *Staphylococcus aureus*

PBPs : Penicillin Binding Proteins

PCR : Polymerase Chain Reaction

PICU : Paediatric Intensive Care Unit

Pvt. : Private

RFLP : Restriction Fragement Length Polymorphysm

SCC : Staphylococcal Cassette Chromosome

SPSS : Statistical Package for the Social Sciences

TUTH : Tribhuvan University Teaching hospital

URTI : Upper Respiratory Tract Infections

USA : United State of America

VISA : Vancomycin Intermediate *Staphylococcus aureus*

VRSA : Vancomycin Resistant *Staphylococcus aureus*

WHO : World Health Organization

CHAPTER-I

1. INTRODUCTION AND OBJECTIVES

1.1 Background

Staphylococci belong to the family Micrococcaceae. They are gram positive spherical cocci. Micrococcaceae cells may occur singly or as irregular clusters (Atlas 1995). These are ubiquitous organisms and the primary natural habitat is mammalian body surfaces. Some are members of man and others are the commonest cause of suppuration (Chakraborty 2007). The genus *Staphylococcus* has at least 40 species. The three most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis,* and *Staphylococcus saprophyticus*. Among them, *Staphylococcus aureus* is a major pathogen for humans. *Staphylococcus aureus* is coagulase positive, which differentiates it from other the other species (Brooks et al 2004).

Staphylococcus aureus is a gram-positive coccus where the round cells, approximately 1 mm in diameter, form grape-like (Greek staphyle) clusters indicative of the ability to divide in more than one plane. They are capable of both aerobic and anaerobic respiration and most strains ferment mannitol aerobically. On nutrient agar they form characteristic golden (Latin aureum) or white colonies. They produce catalase, coagulase and an extracellular cell clumping factor, and some strains produce capsules (Brown et al 2005). The coagulase positive species *Staphylococcus aureus* is well documented as a human opportunistic pathogen. As a nosocomial pathogen, *Staphylococcus aureus* has been a major cause of morbidity and mortality (Murray et al 2003). *Staphylococcus aureus* capable of invading intact normal skin are rare, most able to cause infection, only if they enter through breaks in the skin. *Staphylococcus aureus* causes pyogenic infections like breast abscess, post-operative wound infections, folliculitis, impetigo, furuncles, carbuncles, septic arthritis, lung abscess and etc. Disseminated infections are septicemia often consequent metastatic secondary foci and toxin mediated infections are toxic shock syndrome, staphylococcal scalded skin syndrome, staphylococcal food poisoning (Collee et al 2006). The *S. aureus* transmission occurs by direct

(especially hands) or indirect contact (contaminated surfaces or fomites), especially the colonization in which the individual becomes the carrier of the microorganism, without necessarily showing characteristic signs and symptoms of infection (Carvalho et al 2016).

The staphylococci associated with infections in humans are colonizers of various skin and mucosal surfaces (Tille 2014). There are two types of *Staphylococcus aureus* found in nosocomial environments: permanent and transitory. The former can be found on healthcare-workers and in the hospital environment. The latter can be found in infected patients and in carriers, which are in transitory contact with the hospital (Rashid et al 2012). Presence of *Staphylococcus aureus* nasal colonization can provide an indication of a higher risk for subsequent infection, including with MRSA (Gorwitz et al 2008). Infections caused by *S. aureus* have a poorer prognosis when the infecting strain is MRSA. Treatment of the infections caused by these strains became more difficult since *S. aureus* became resistant not only to usual penicillin related antibiotics but also most other structurally unrelated antibiotics such as rifampicin, chloramphenicol (Cosgrove et al 2005).

Drug resistance is seen mostly in hospital acquired infections than in community acquired infections. This is due to widespread use of antibiotics in the hospital that select for these bacteria. These hospital strains are characterized by developing resistance to multiple antibiotics at the same time. Common examples of such strains of bacteria showing drug resistance include *S. aureus, E. coli* etc. (Parija 2012).

Multi drug resistance (MDR) is a condition enabling a disease causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism. Multi drug resistant isolates are even more likely to be associated with complications in the therapeutic management of patients with infectious diseases. Multi drug resistance is defined as resistance

to two or more antibiotics belonging to different structural classes (CDC 2006). Multi drug resistance among common bacterial pathogen has resulted in treatment failures and economic burden to contain these patients, thus dictating their early and reliable detection (ASM 2009).

Methicillin resistant staphylococci has steadily increased worldwide especially among cases acquired in hospitals (Klevens et al 2007). There are several mechanisms of methicillin resistance in staphylococci, including inactivation by the beta lactamase enzymes, penicillin binding proteins with reduced penicillin binding capacity, and acquisition of the mecA gene which encodes new penicillin binding proteins PBP-2a with low affinity for beta lactams. The later mechanism accounts for the majority of resistance to methicillin and other beta lactams (Brumfitt et al 1996). Strains of *Staphylococcus aureus* that carry the *mecA* gene, which encodes for PBP are referred to as methicillin resistant *Staphylococcus aureus*. The *mecA* gene is carried on a mobile DNA element (SCC *mec*) that mediates wide dissemination of the antibiotic resistance (Tille 2014). Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all currently available β-lactam antimicrobial agents, including β-lactamase–stable penicillin and cephalosporin, and have been recognized as a source of healthcare-associated infections since the 1960s (Gorwitz et al 2008).

In the 1960s and 1970s MRSA was not feared because several other treatment options existed, including use of tetracyclines, macrolides and aminoglycosides (Hugo and Russell, 1993). Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all currently available β-lactam antimicrobial agents, including β-lactamase–stable penicillins and cephalosporins, and have been recognized as a source of healthcare-associated infections since the 1960 (Gorwitz et al 2008). Methicillin-resistant isolates are not effectively treated by most antibacterial agents and are a major challenge for chemotherapy (Niemeyer et al 1996). Since the emergence of Methicillin resistant *S. aureus*, the glycopeptides vancomycin has been the only uniformly effective treatment for staphylococcal infections. In May 1996, the world's first documented clinical infection due to *S. aureus* with intermediate resistance to glycopeptides (glycopeptides-intermediate *S.*

aureus) was diagnosed in a patient in Japan (Hiramatsu et al 1997). The recommended treatment for multi-drug resistant MRSA is glycopeptides, particularly vancomycin. Since the emergence of vancomycin resistance in enterococci in 1988 and its in vitro demonstration that its resistance genes (Van A and Van B) are transmissible to other bacterial species including *S. aureus*, emergence of vancomycin resistance in clinical staphylococci has become a great concern. Clinicians are continually being challenged by infections caused by *S. aureus*. The treatment of suspected *S. aureus* infections is becoming increasingly more complicated and clinical significance of these strains requires further investigation (Yabuta et al 1997 and CDC 2002).

Approximately 10 to 40% of people carry *S. aureus* in their anterior nares. Nasal carriage of MRSA can be found among 1 to 2% of the general population but in as many as 10 to 15% of patients admitted to acute-care hospitals and intensive care units (Quezada Joaquin et al 2013). Nasal carriage of *S. aureus* has been identified as a risk factor for community-acquired and nosocomial infections. Healthy hospital personnel may carry pathogenic hospital strains in their nose and skin and may spread these pathogens to the community leading to more dreadful condition (Sah et al 2013). HCWs who are at interface between the hospital and the community may serve as agents of cross contamination of hospital acquired and community acquired MRSA (El Aila et al 2017).

The prime focus of this study is to determine the nasal carriage rate of *S. aureus* and MRSA among healthcare workers at Gandaki medical college and research centre private limited, Pokhara. The study will also demonstrate the sensitivity pattern of different antibiotics used against it. This study is useful for the healthcare personnel to maintain necessary universal control measures to prevent possible transmission to vulnerable patients.

1.2 OBJECTIVES

1.2.1 General objective

- To determine the nasal carriage rate of *S. aureus* and MRSA among healthcare workers in tertiary care centre.

1.2.2 Specific objectives

- To isolate and identify *S. aureus* from nasal swab of HCWs.
- To determine carriage rate of *S. aureus* among health care workers.
- To identify Methicillin resistance *S. aureus* by using cefoxitin disc following Kirby Bauer disc diffusion method.
- To observe susceptibility pattern of those *S. aureus* isolates.

CHAPTER-II

2. LITERATURE REVIEW

2.1 General characteristics of staphylococci

The staphylococci are gram positive spherical cells, usually arranged in grape like irregular clusters (Brooks et al 2004). The term *Staphylococcus* is derived from the Greek term (staphyle, meaning bunch; kokkus, meaning berry) (Chakraborty 2007). Staphylococci are gram positive cocci and non-motile, non-spore forming, catalase-positive in nature (Cheesebrough 2008). These are ubiquitous organisms and the primary natural habitat is mammalian body surfaces. Some are members of the normal flora of skin, skin glands and mucous membrane of man and birds are the commonest cause of suppuration (Murray et al 2003 and Chakraborty 2007). The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins (Brooks et al 2004).

2.2 Clinical significance of Staphylococci

Staphylococcal infections are among the most common of bacterial infections and range from the trivial to the fatal. Staphylococcal infections are characteristically localized pyogenic lesions. Common staphylococcal infection are folliculitis, furuncle, wound infection, carbuncle, osteomyelitis, arthritis, bursitis, pyomyositis, tonsillitis, pharyngitis, sinusitis, otitis, lung abscess, empyema, meningitis, intracranial thrombophlebitis, bacteremia, septicemia, pyemia and staphylococci are uncommon in routine urinary tract infections, though they do cause infection in association with local instrumentation, implants or diabetes (Anantanarayan and paniker 2009).

The genus *Staphylococcus* has at least 40 species. The three most frequently encountered species of clinical importance are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. *S. aureus* is a major pathogen for human causing pyogenic infections and is coagulase positive in nature, which differentiates it from the other species (Brooks et al

2004; Anantanarayan and paniker 2009). It can cause significant opportunistic infections under appropriate conditions although this organism is a part of the normal human microflora (Sharan 2016).

Coagulase negative staphylococci (CoNS) are skin commensals that can cause opportunistic infections, often associated with implanted devices, such as joint prostheses, shunts, and intravascular catheters, especially in very young, old, and immune- compromised patients. Approximately 75% of this infections caused by coagulase negative staphylococci are due to *S. epidermidis*. Infections due to *S. lugdunensis, S. warneri, S. homonis,* and other species are less common. *S. saprophyticus* is a relatively common cause of urinary tract infections in young women, although it rarely causes infections in hospitalized patients (Brooks et al 2004 and Chakraborty 2007).

2.3 General characteristics of *Staphylococcus aureus*

Staphylococcus aureus is a spherical gram positive cocci arranged in irregular grape like clusters. Cluster formation is due to successive cell division in asymmetric three planes. The organism has a diameter of 1μm in an average and liquid culture shows the arrangement of cocci in single, pairs, tetrads, or short chains of three or four cells. A few strains have capacity to produce capsules in young cultures (Chakraborty 2007). *S. aureus* is catalase and coagulase positive, Novobiocin sensitive and fermentative organism. It grows rapidly under aerobic or anaerobic conditions and is positive towards Voges Proskauer (VP) test (Greenwood et al 2002).

2.3.1 Clinical significance of *Staphylococcus aureus*

As a nosocomial pathogen, *Staphylococcus aureus* has been a major cause of morbidity and mortality. Approximately, 25.0% of all nosocomial infections are caused by *S. aureus*, affecting both surgical and non-surgical patients and leading hospital stay, antibiotics use costs and mortality (Thapa 2011). *S. aureus*

can infect almost all tissue and organs (Deleo et al 2010). *Staphylococcus aureus* infections are often acute and pyogenic and, if untreated, may spread to surrounding tissue via bacteremia to metastatic sites. Some of the infections caused by *Staphylococcus aureus* involve the skin, these include furuncles or boils, impetigo, cellulitis and postoperative wound infections of various sites. Some of the more serious infections produced by *Staphylococcus aureus* are bacteremia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, cerebritis, meningitis, chorioamnionitis, scalded skin syndrome, and abscesses of the muscle, urogenital tract and central nervous system, and various intra-abdominal organs (Murray et al 2003).

2.3.2 Nasal carrier of *Staphylococcus aureus*

Staphylococcus aureus is an endogenous microorganism colonizing the nasal cavities, skin, gastrointestinal, anuses, finger tips and vaginal vaults of healthy women. The anterior nares are a common colonization site because its primary habitat is moist squamous epithelium of the anterior nares and can lead to dissemination of other body sites (Sanford et al 1994). There are three types of nasal carrier states associated with *Staphylococcus aureus*: persistent carriers that harbor a single strain for an extended period of time, intermittent carriers that harbor different strains over time, and then individuals that do not harbor any organisms or non-carriers. Because the carrier state is common among the human population, infections are frequently acquired when the colonizing strains gain entrance to a normally sterile site as a result of trauma or abrasion to the skin or mucosal surface (Tille 2014).

The nose provides a major habitat for *S. epidermidis* and is the dominant ecological niche for *Staphylococcus aureus*. Three *Staphylococcus aureus* carriage patterns have been described in the healthy adult population, with approximately 20% of individuals being persistent *Staphylococcus aureus* carriers, about 60% intermittent carriers, and 20% persistent non carriers. Rates of *Staphylococcus aureus* carriage are higher in those infected with human immunodeficiency virus compared with both health care workers and patients

with chronic diseases. Carriage rates are higher in individuals with insulin dependent diabetes, in patients on continuous ambulatory peritoneal dialysis and hemodialysis, and in intravenous drug abusers when compared with the healthy population (Borriello et al 2009). When it comes to health professionals and students, colonization rates range from 20 to 40%, with high percentages of multi-resistant strains, especially among health professionals who work in hospitals and are a great source of infection, more particularly to patients treated by them every day (Carvalho et al 2016). Colonization with *S. aureus* has been identified as an important risk factor for the development of *S. aureus* infections in both community and hospital settings (Chen et al 2011). Carriage among healthcare workers (HCW) may act as a source of infection in hospitals (Hogan et al 2016).

Carriage of *Staphylococcus aureus* in nose appears to play main role in epidemiology and pathogenesis of disease (Khanal and Jha 2010). Colonization is a strong risk factor for subsequent infections, although most persons colonized with *Staphylococcus aureus* do not develop clinical disease (Gorwitz et al 2008).

2.4 Antibiotic resistance

An antibiotic was originally defined as a substance, produce by one microorganism, which inhibit the growth of other microorganisms. The advent of synthetic method has, however resulted in a modification of this definition and an antibiotic now refers to a substance produced by a microorganisms or to a similar substance (produced wholly or partly by chemical synthesis), which in low concentrations inhibits the growth of other microorganisms (Hugo and Russell 1993). Common source of antibiotic are fungi and actinomycetes. Now many synthetic antibiotics are being manufactured for the desired spectrum (Das 2010). Synthetic antibiotics are produced in industries by using different chemical processes, for example, Chloramphenicol is now usually produced by this process. Semi- synthetic antibiotics are obtained from a part of molecule

that is produced by a fermentation process using the appropriate microorganism and the product is further modified by a chemical process (Pelczar et al 1993).

Bacterial resistance to drugs is a condition in which the bacteria which were earlier susceptible to antibiotics develop resistance against antibiotics and are not susceptible to the action of same antibiotics. Antibiotic resistance among bacteria is a major concern in the treatment of patient. Emergence of antibiotic resistance to the old as well as new antibiotics by bacteria is passing a major challenge in the treatment of infection caused by bacteria (Parija 2012).

Resistance to other antibiotics is achieved by a number of different mechanisms, depending on the class of antibiotic; these include membrane permeability, alteration of the target side and enzymatic degradation of antibiotic (Collee et al 2006). Antibiotic resistance in bacteria can also be achieved when mutations in a ribosome or protein change the site where an antibiotic binds (Criswell 2004). Some bacteria have primary resistance which possess an innate property of resistance to certain drug and acquired resistance results from mutation or gene transfer (Chakraborty 2007). Acquired resistance is temporary or permanent ability of an organism and its progeny to remain viable or multiply under environmental conditions that would otherwise destroy or inhibit other cells (Hugo and Russell 1993).

Mostly the resistance genes are encoded or carried on plasmids but chromosome and transposon have also significant role. Transposon play major role in disseminating resistance genes among bacterial species and also exchange genetic material between plasmids and chromosome. Bacterial resistance to antibiotics can be caused by either the enzymatic modification of an antibiotic which makes it ineffective, the modification of its target site so that it is unable to exert its effect, active physical removal of the antibiotic from the cell, or by reduction of uptake into the bacterial cell due to changes in membrane permeability (Tenovar 2007). The main resistance mechanism is the enzymatic

inactivation of an antibiotic and the most clinically important example of this is beta-lactamase enzymes, the enzymes that hydrolyze beta-lactam antibiotics (Livermore 2012).

2.4.1 Emergence and spread of antimicrobial resistance

Antibiotic resistance arises by chance through mechanisms that represent the legacy of natural competition among mechanisms. The factors playing significant role in the variation of prevalence of resistant strains include

- Host and clone specificity
- Plasmid and clone specificity
- Virulence
- Interactions with other commensal flora
- Duration of the selection pressure and
- Variable gene expression

The two major reasons for the association between the emergence of antimicrobial resistance phenotypes and the clinical (or other) use of antimicrobial agents which the resistance is directed are-

- Not testing for resistance to antibiotics that are not in clinical use
- Nature abhors vacuum, and so when an effective antimicrobial eliminate susceptible members of the flora, resistant varieties soon fill the niche (Sharan 2016).

The staphylococcal chromosome cassette *mec* (SCC*mec*) has been characterized as a novel, mobile resistance element that differs from both transposons and bacteriophages. MRSA typically spreads through clones; however, it is known that the mec gene has been transmitted between *S. aureus* strains and, possibly, between other staphylococcal species (Appelbaum 2007).

2.5 Methicillin resistant *Staphylococcus aureus*

Treatment of *S. aureus* infections before the 1950s involved the administration of benzyl penicillin (penicillin G), a β-lactam antibiotic. The introduction of penicillin in the 1940s, strains of *Staphylococcus aureus* unaffected by

penicillin were reported in 1945. Resistant strains typically produced an enzyme, called a β-lactamase, which inactivated the β-lactam. Efforts were made to synthesize penicillin derivatives that were resistant to β-lactamase hydrolysis. This was achieved in 1959 with the synthesis of methicillin to treat these infections, but in 1961, shortly after the introduction of methicillin, *Staphylococcus aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *Staphylococcus aureus*, MRSA) were reported (Stapleton and Taylor 2002 and Askarian et al 2009). Methicillin is a synthetic antibiotic related to penicillin with modified radicals designed to protect the penicillin ring against the bacterial enzyme penicillinase (Lee et al 2005). Unfortunately, Methicillin-resistant *S. aureus* (MRSA) was reported 2 years after its introduction in the United Kingdom. MRSA is a strain of that is resistant to a large group of antibiotics, called "beta-lactams' (Ike et al 2016). Methicillin resistance was mediated by the production of a beta lactamase enzyme that inactivates drugs such as penicillin, ampicillin and amoxicillin. Consequently, beta-lactamase-stable drugs (e.g. methicillin and cloxacillin) as well as beta-lactamase inhibitors (e.g. clavulanic acid and sulbactam) that could be combined with the antibacterial drugs were developed. Strains of *S. aureus* resistant to these penicillin stable antibacterial drugs have acquired a novel gene (*mecA*) that codes for a novel penicillin-binding protein; these strains are termed methicillin-resistant *Staphylococcus aureus* (MRSA) (WHO 2014).

Since its emergence in 1961, methicillin-resistant *Staphylococcus aureus* (MRSA) has become an important nosocomial pathogen, and morbidity and mortality rates associated with this pathogen have increased markedly in recent years (Ibrahim et al 2011). Treatment of *S. aureus* infections has now become more challenging with the emergence of MRSA, which are often multidrug resistant too (Khanal and Jha 2010).

Methicillin-resistant *Staphylococcus* aureus strains are not only a problem in hospital as distinct strains have emerged in community too, which are referred to as Community acquired MRSA (CA-MRSA). CA-MRSA strains have spread

in community settings and have also entered healthcare facilities. Healthcare workers (HCWs) who are at interface between the hospital and the community may serve as agents of cross contamination of Hospital acquired MRSA (HA-MRSA) and CA-MRSA (Khanal et al 2015).

2.5.1 Mechanism of methicillin resistance

Resistance is primarily mediated by the production of an altered penicillin-binding protein (PBP 2a). The bacterial cell wall contains penicillin-binding proteins (PBPs), which have an enzymatic role in the synthesis of peptidoglycan. Normally, PBPs have a high affinity for beta-lactam antibiotics; in MRSA this affinity reduced resulting in gene. A low-affinity penicillin-binding protein, PBP2a encoded by the chromosomally located gene *mecA*, mediates methicillin resistance among both *Staphylococcus aureus* and coagulase-negative staphylococci. Moreover, *S. aureus* has the ability to acquire new genes, (e.g. antimicrobial resistance genes) mainly through mobile genetic elements, enabling the bacterium to adapt to new environmental conditions or selective pressures (Geha et al 1994; Askarian et al 2009 and Campanile et al 2015).

Penicillin binding proteins are the targets beta-lactam antibiotics. These of antibiotics inhibit cell growth by covalently to the binding active sites of essential PBPs. Although deactivation by beta-lactamase is the most common of resistance mechanism to these drugs mutational alteration resulting in low affinity of PBPs for beta-lactam target been proposed antibiotics has as mechanism of another resistance these drugs in some to bacterial strains of methicillin-resistant species including *Staphylococcus aureus* (Chambers et al 1985). The *mecA* gene encodes this protein and is located on a mobile *SCCmec* cassette chromosome. This genetic element confers resistance to most currently available β-lactam antibiotics (Appelbaum 2007).

2.5.2 Epidemiology of MRSA

Recent studies suggest that the epidemiology of MRSA may be changing, as the isolation of MRSA is no longer limited to hospitalized patients or persons with predisposing risk factors. However, the prevalence of MRSA colonization in healthy persons in the community has been shown to be low, even when MRSA is highly endemic in hospital settings (Salmenlinna et al 2002). Colonized healthcare workers (HCWs) transfer such strains to patients or they transfer the organisms from one patient to another by their hands leading to epidemics in chronic care facilities (Shrestha et al 2009).

Acquisition of MRSA frequently brings about asymptomatic colonization; however it may be associated with infection resulting in significant morbidity and mortality particularly in vulnerable patients. The epidemiology of MRSA is further complicated by the emergence of strains of community-acquired MRSA that transmit efficiently in otherwise healthy people who do not have ongoing interaction with the healthcare system (Malqueen et al 2007). In the current study, Pourakbari et al (2017) reported that in Iran, the prevalence of MRSA nasal carriage was 6.6% (29 out of 438) and 2.8% (29 out of 1046) respectively.

Shakya et al (2010) conducted a research at National medical college teaching hospital, Birgunj, Nepal and found highest MRSA prevalence rate was among health-care personnel (10.0%), followed by visitors/patient attendants (8.2%) and the patients (3.2%). Mondal et al (2016) had reported 18.97% health care workers of Burdwan Medical College and Hospital were MRSA colonized. Al-Talib et al (2013) had been also reported 21.5% health care workers of Hospital in Kelantan, Malaysia were MRSA colonized. In Pakistan, Rashid et al (2012) reported 13.95% hospital personnel were colonized by MRSA. Similarly, Shaibaw et al (2013) found 12.7% of all HCW were identified as MRSA carriers in Ethiopia.

2.5.3 Nasal colonization of *S. aureus* and MRSA among hospital care workers

Humans are natural reservoir for *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) colonize most frequently in the anterior nares of the nose and cause serious infections all over the world (Kilic et al 2008 and Shrestha et al 2009). In context of Nepal, there were not much more studies regarding the nasal carriage rate of MRSA among health personnel. First, the MRSA among patients, staff and hospital environment of a tertiary medical care centre (teaching hospital) in Kathmandu, Nepal has reported to be 29.1% (Rai et al 1990). Out of 204 healthcare workers, 15.7 % were nasal carriers of *S. aureus* and among them 21.9% were carrier of MRSA. Overall nasal carriage rate of MRSA was 3.4 %. Highest MRSA nasal carriage rate of 7.8 % was found among nurses. Healthcare workers of both surgical wards and operating room accounted for 28.6 % of MRSA carriers each (Khanal et al 2015). Out of 129 Hospital care workers, 27.13% were identified as nasal carriers of *S. aureus* and MRSA was isolated from 2.32% (Shrestha et al 2009).

Methicillin resistant *S. aureus* nasal carriage rates of hospital staff and outpatient control group were found to be 6.0% and 2.6% respectively in a hospital in Turkey (Dimitrov et al 2003). Similarly in an Iranian hospital, 19.7% hospital personnel were nasal carriers of *S. aureus*, of whom 8% were MRSA colonized and 96.2% were persistent carriers (Armin et al 2007). Some studies also showed relatively higher nasal carriage rate of *S. aureus* and MRSA among health workers which indicates the dispersion of MRSA among healthcare personnel is global trend. The study carried out in children hospital, Pakistan reported the prevalence of *S. aureus* carriage was 48%, out of these 29% were MRSA (Farzana et al 2008). The prevalence of nasal carriage of methicillin-sensitive *S. aureus* (MSSA) was 25.7% and of MRSA was 5.3%, with the highest nasal carriage of MRSA in surgical wards and the emergency department (Askarian et al 2009).

2.5.4 Hospital acquired MRSA

The intensive use of penicillin resulted in the presence of penicillin-resistant *S.aureus* isolated in the hospitals and community. Due to this penicillin resistance, new antimicrobials are were needed. End of 1950, a semi synthetic version of penicillin was produced, named methicillin. Only two years after the first clinical use, Methicillin resistant Staphylococcus aureus strains emerged in the hospital environment, resulting the name hospital acquired or HA-MRSA (Jevons et al 1963).

In case of HA-MRSA, patients who already have an MRSA infection or who carry the bacteria on their bodies but do not have symptoms are the most common source of transmission. The main mode of transmission to other patients is through human hands, especially healthcare worker's hands (Armin 2007). Healthcare workers, who have direct contact with persistently colonized patients, or contaminated objects in the immediate environment of such patients, can colonize or contaminate their hands and subsequently transmit the organism to other patients complicating the treatment and recovery. Some may harbor the organism for prolonged periods (persistent carriers) and spread the organism to patients by direct contact constantly (Boyce, 1989).

2.5.5 Community acquired MRSA (CA- MRSA)

In the 1990s, the first case of MRSA were seen in people who were not hospitalized in Australia, followed by USA and is now highly prevalent worldwide (Otto et al 2010). Any MRSA infection would be considered CA-MRSA if it is diagnosed among outpatients earlier than 48h after hospitalization. CA-MRSA strains are especially aggressive, causing skin and soft tissue infections, fasciitis, necrotizing pneumonia, and blood stream infections. CA-MRSA also has the ability to survive and spread in the community, leading to an increasing number of colonized persons in the general population (Bektas et al 2016).

Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has become an important threat to public health (Huh and Chung 2016). The growing number of community acquired infections caused by methicillin-resistant *S. aureus* in children and healthy adults is a major problem (Rocha et al 2017). CA-MRSA infections continue to become more widespread, additional investigation into the risk factors for infection will be vital to the development and implementation of effective prevention and control measures (Campbell et al 2004). Outbreaks of community-acquired MRSA infection are extremely rare (Borer et al 2002).

2.6 Multidrug resistance

Definition

- Multidrug resistant bacteria are those, which showed resistance to at least three or more antibiotics of different structural classes (Sahm et al 2000).
- Multidrug resistance is defined as resistance to two or more classes of antimicrobial agents (CDC 2006).
- Multidrug resistance is defined as resistance to at least two antibiotics of different classes including aminoglycosides, chloramphenicol, tetracyclines and/or erythromycin (Pandey et al 2012).

2.7 Treatment of nasal carrier

Moreover, when the nares are treated topically to eliminate nasal carriage, in most cases the organism also disappears from other areas of the body (Kluytmans et al 1997). Elimination of nasal carriage has been reported to cause reduction in the incidence of *S. aureus* infections (Onanuga and Temedie 2011). Carriers of MRSA and treated them with mupirocin ointment for 3 times in a day for 5 days and also excluded them from their work for 48 hours from the start of mupirocin ointment (Bhatiani et al 2017).

CHAPTER-III

3. MATERIALS AND METHODOLOGY

3.1 Materials

A complete list of materials, equipments, media, chemicals, reagents and antibiotics used in this study are listed in Appendices B, C, D and H.

3.2 Methods

3.2.1 Study site

The study was conducted at the Microbiology Laboratory of Gandaki Medical College and Research Centre Pvt. Ltd. Pokhara, Nepal

3.2.2 Study period

The duration of the study from 9th October 2016 to 9th April 2017.

3.2.3 Type of study

This is hospital based descriptive type of study.

3.2.4 Sample size and types of sample

Altogether 288 HCWs were enrolled in this study. Nasal swabs from all hospital care workers were collected.

3.2.5 Inclusion Criteria

All the hospital care workers included doctors, nurses, lab workers and attendants were enrolled in this study.

3.2.6 Exclusion Criteria

Hospital care workers with history of upper respiratory tract infection, fever, recent nasal surgery, diabetes, immunocompromisation, use of nasal medications, or antimicrobial therapy were excluded.

3.2.7 Ethical Consideration

The permission for sample collection was taken from the Head of Hospital Administration of Gandaki Medical College and Research Centre Pvt. Ltd. The

inform consent was given to each HCWs included in the study regarding the details of the research work and privacy of their identity for the further dissemination of results.

3.3 Specimen collection:

Sterile cotton swab dipped in sterile physiological saline was used for the collection of samples from anterior nares. The swab was introduced into first nostril 1-2 cm inside, which was rotated 2-3 times both clockwise and anticlockwise with gentle pressure for 3-5 seconds and the swab was transferred to the second nostril and the process was repeated. After collecting the sample, the swab was kept immediately in the sterile test tube and was plugged with cotton. The samples were labeled with sample code number and other required information (Khanal et al 2015 and Khatri et al 2017).

3.4 Sample transportation:

The collected nasal swabs were put into sterile glass tube, and transported to the laboratory immediately for inoculation into culture media.

3.5 Sample processing

All the samples selected for the study were processed using standard protocols. After receiving and labeling the samples, they were inoculated into Mannitol salt agar, MacConkey agar, and Blood agar.

3.6 Bacteriological identification of *S. aureus*

The inoculated culture plates were incubated at 37˚C for 24 hours. *S. aureus* colonies were identified and confirmed by studying colony morphology, and gram's stain reaction and biochemical tests. Isolates that were gram-positive cocci, yellow colonies on MSA, pink colonies on MA and β-haemolytic colony on BA were considered as *S. aureus* in this study (Chessbrough 2000). Then the culture were sub cultured on nutrient agar (NA) at 37˚C for 24 hours for further processing. Colony having round, convex, opaque, smooth-glistening surface with colony diameter 2-3 mm were indicative of Staphylococci. Most staphylococci produced soft butyrous colony with golden yellow pigment. For

further confirmation of *S. aureus*, various tests like gram staining, catalase test, slide and tube coagulase test were performed from isolated colonies. Standard protocol provided by Chessbrough 2000; Collee et al 2006 and Tille 2014 was followed for confirmatory identification of *S. aureus*.

The procedures for Gram's staining, Catalase test, Coagulase test for the confirmatory identification of *S. aureus* are mentioned in the Appendix E and F.

3.7 Antibiotic susceptibility testing

All *S. aureus* isolated from nasal screening process were subjected to in-vitro antimicrobial, susceptibility test by Kirby-Bauer disc diffusion method as recommended by CLSI (formerly NCCLS). In this study the antibiotics used were Ciprofloxacin, Cloxacillin, Erythromycin, Gentamicin, Cefoxitin, Oxacillin, Penicillin, Amikacin, Vancomycin, Tetracycline, Ceftriaxone and Cefotaxime. The MRSA strains were identified by testing with Oxacillin and resistant strains were also screened against cefoxitin disc, those strains resistant to both discs were considered as MRSA strains in this study (Chessbrough 2000 and Collee et al 2006).

3.8 Quality Control

3.8.1 Quality Control monitoring and regular evaluation of laboratory equipment, reagents and media

Laboratory equipment like incubator, refrigerator, autoclave, and hot air oven were regularly monitored for their efficiency. The temperature of the incubator and refrigerator was mentioned every day.

Reagents and media were regularly monitored as recommended by the manufacturing company. After preparation, they were properly labeled with preparation date, expiry date and were preserved at 2°C- 8°C in the refrigerator. The quality of media prepared was checked by incubating one plate of each lot for sterility and using standard control strains for performance testing.

3.8.2 Quality control during identification

During the identification test, pure culture of the isolated colony of the organism was used. The fresh plasma was used for performing tube coagulase test. The coagulase test was always run along with positive control.

3.8.3 Quality control during antimicrobial susceptibility testing

Muller Hinton Agar and antibiotic discs were checked for their lot number, manufacturing and expiry date, and proper storage. For the standardization of Kirby-Bauer test for performance testing of antibiotics and MHA, control strain of *S. aureus* (ATCC 25923) was tested primarily. Quality of sensitivity tests was maintained by maintaining the thickness of MHA at 4mm and the pH at 7.2-7.4. Strict aseptic condition was maintained while carrying out all the procedures.

3.9 Disposal of materials

Cotton swabs, glass slides and other infected materials were dipped in to the vial containing disinfectant solution (0.5% Hypochlorite solution). All the disposable petri dishes as well as reusable glass petri plates were autoclaved.

3.10 Data analysis

All raw data obtained from laboratory investigation were tabulated and presented in defined tables to explore the major findings in MS Excel program version 2013. The data were statistically analyzed by Chi-square ($\chi 2$) test at 5 % level of significance by entering the data in the computer based SPSS (Statistical Package for the Social Sciences) program version 20.0 and WinPepi 2007 version 3.8.

CHAPTER-IV

4. RESULT

The study was done in Gandaki Medical College and Research Centre Private Limited, Pokhara. Study period was from 9th October 2016 to 9th April 2017.

4.1 Gender wise Distribution of sample

Out of total 288 nasal sample taken from HCWs, 65(22.57%) were male and 223(77.43%) were female.

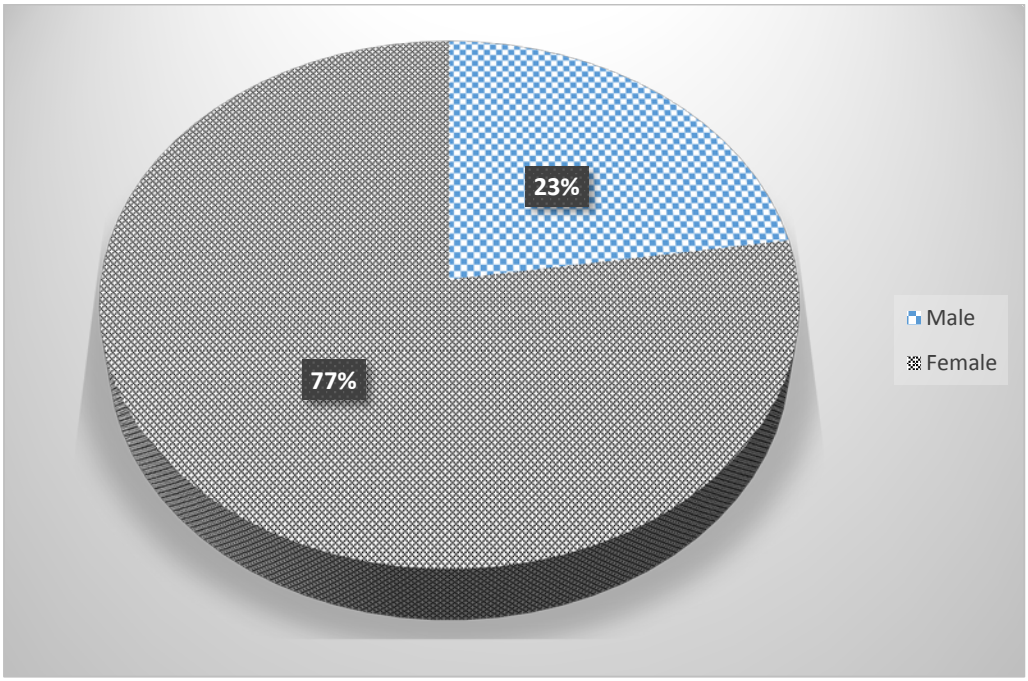


Figure 1: Gender wise distribution of sample

4.2 Distribution of nasal carrier *Staphylococcus aureus*

4.2.1 Gender wise distribution of *S. aureus* carrier

Out of total, only 58 showed *S. aureus* positive, 11(18.97%) *S. aureus* were from male and 47(81.03%) *S. aureus* were from female. Among 288 health care workers, 20.14% were carrier of *S. aureus*. The association between gender and nasal carrier of *S. aureus* is not statistically significant (p=0.463).

Table 1: Gender wise distribution of *S. aureus* carrier

Sex	*S. aureus* carrier		Total	*p*- value	*p*- value
	Positive	Negative			
Male	11(16.92%)	54(83.07%)	65	0.000	
Female	47(21.08%)	176(78.92%)	223	0.000	
Total	58(20.14%)	230(79.86%)	288(100%)		0.463

4.2.2 Comparative study of nasal carrier of *S. aureus* isolated from different age group

Highest nasal carrier of *S. aureus* was found in age group 31-40 year 23.81%. This percent was followed by 21-30 year 20.39% and 41-50 year 14.29%. Nasal carrier of *S. aureus* was not isolated in age group ≥ 20 year and 51-60 year. The association of nasal carrier of *S. aureus* among different age group was not statistically significant ($p = 0.5$).

Table 2: Comparative study of nasal carrier of *S. aureus* isolated from different age group

Age group	*S. aureus* carrier	Non carrier	No of sample	*p*- value	*p*- value
≥20	-	6(100%)	6		`
21-30	41(20.39%)	160(79.61%)	201	0.000	
31-40	15(23.81%)	48(76.19%)	63	0.000	0.5
41-50	2(14.29%)	12(85.71%)	14	0.000	
51-60	-	4(100%)	4		
Total	58(20.13%)	230(79.86%)	288(100%)		

4.2.3 Distribution of *S. aureus* carrier among different department

The nasal swab samples were collected from different departments. Higher prevalence of nasal carrier *S. aureus* was found in department of ENT 40% followed by post-up 36.36%, medicine 28.57%, NICU 26.67%, surgery, laboratory and PICU 20%, ICU 18.9%, emergency 18.75%, paediatric 17.35%, orthopedic 15.63% and gyanecology and obstetrics 11.11%. Lowest percentage of *S. aureus* was found in maternity ward 4.75%. The association of nasal carrier of *S. aureus* among different department was not statistically significant (p = 0.365).

Table: 3 Distribution of *S. aureus* carrier among different department

Department	*S. aureus* carrier		No of sample	*p*-value
	Positive	Negative		
Emergency	3(18.75%)	13(81.25%)	16	0.365
ENT	8(40%)	12(60%)	20	
Gyane & Obst.	3(11.11%)	24(88.89%)	27	
ICU	7(18.9%)	30(81.08%)	37	
Laboratory	6(20%)	24(80%)	30	
Maternity	1(4.76%)	20(94.24%)	21	
Medicine	6(28.57%)	15(71.43%)	21	
NICU	4(26.67%)	11(73.33%)	15	
Orthopedic	5(15.63%)	27(84.37%)	32	
Paediatric	4(17.39%)	19(82.61%)	23	
PICU	3(20%)	12(80%)	15	
Post-up	4(36.36%)	7(63.64%)	11	
Surgery	4(20%)	16(80%)	20	

ENT: Eye nose throat, ICU: Intensive care unit, NICU: Neonatal care unit PICU: Paediatric intensive care unit and Post-up: Post-operative

4.2.4 Comparative study of nasal carrier of *S. aureus* isolated from different professional group

The occupation was recorded in the questionnaire during a short interview while collecting nasal swabs. The involved hospital personnel related to health care facility were further classified in to doctor, nurse, attendant and lab worker. Total nasal carriage rate of *S. aureus* was found highest in nurses 24.83% followed by doctors 16.41%, lab worker 16% and attendant 13.75%. The association of nasal carrier of *S. aureus* among different health occupational group was statistically insignificant (p = 0.220).

Table 4: Comparative Study of Nasal carrier of *S. aureus* among professional group

Profession	*S. aureus* carrier		No of sample	*p*-value
	Positive	Negative		
Doctor	11(16.41%)	56(83.59%)	67	
Nurse	36(24.83%)	109(75.17%)	145	0.220
Lab worker	4(16%)	21(84%)	25	
Attendant	7(13.75%)	44(86.25%)	51	
Total	58(20.14%)	230(79.86%)	288(100%)	

4.3 Drug susceptibility pattern of *S. aureus* and distribution of MRSA

4.3.1 Antibiotic susceptibility pattern of *S. aureus* isolates

All the strains of *S. aureus* isolated from nasal swab of HCWs were tested with specific antibiotics by using Kirby Bauer disc diffusion method. The following table depicts sensitivity and resistance pattern shown by *S. aureus* isolates towards different antibiotics. It was found that 18.97 %(11/58) were methicillin resistant *Staphylococcus aureus*. From the table, it was observed that the most sensitive drug for *S. aureus* strains were vancomycin (100%) and amikacin (100%) which is followed by tetracycline (94.83%), gentamicin (75.86%),

ceftriaxone (75.86%) and cloxacillin (72.41%). The isolated strains showed highest resistant to penicillin (68.97%) and oxacillin (68.97%) which is followed by erythromycin (50%) and ciprofloxacin (46.55%).

Table 5: Antibiotic susceptibility pattern of *S. aureus* isolates

Antibiotics	Sensitive	Resistant	Total *S. aureus*	*p*-value
Amikacin	58(100%)	-	58	
Cefotaxime	25(43.1%)	33(56.9%)	58	0.358
Cefoxitin	47(81.03%)	11(18.97%)	58	0.000
Ceftriaxone	44(75.86%)	14(24.14%)	58	0.000
Ciprofloxacin	31(53.45%)	27(46.55%)	58	0.347
Cloxacillin	42(72.41%)	16(27.59%)	58	0.001
Erythromycin	29(50%)	29(50%)	58	1.104
Gentamicin	44(75.86%)	14(24.14%)	58	0.000
Oxacillin	18(31.03%)	40(68.97%)	58	0.005
Penicillin	18(31.03%)	40(68.97%)	58	0.005
Tetracycline	55(94.83%)	3(35.17%)	58	0.000
Vancomycin	58(100%)	-	58	

4.3.2 Antibiotic resistance pattern of MRSA

The susceptibility testing of MRSA isolates revealed high resistance towards ciprofloxacin (81.8%), erythromycin (72.73%), Cefotaxime (63.64%), ceftriaxone (45.45%). Low resistance towards tetracycline (18.18 %) and gentamicin (37%). All MRSA strain were susceptible towards vancomycin and amikacin and resistant towards cloxacillin and penicillin.

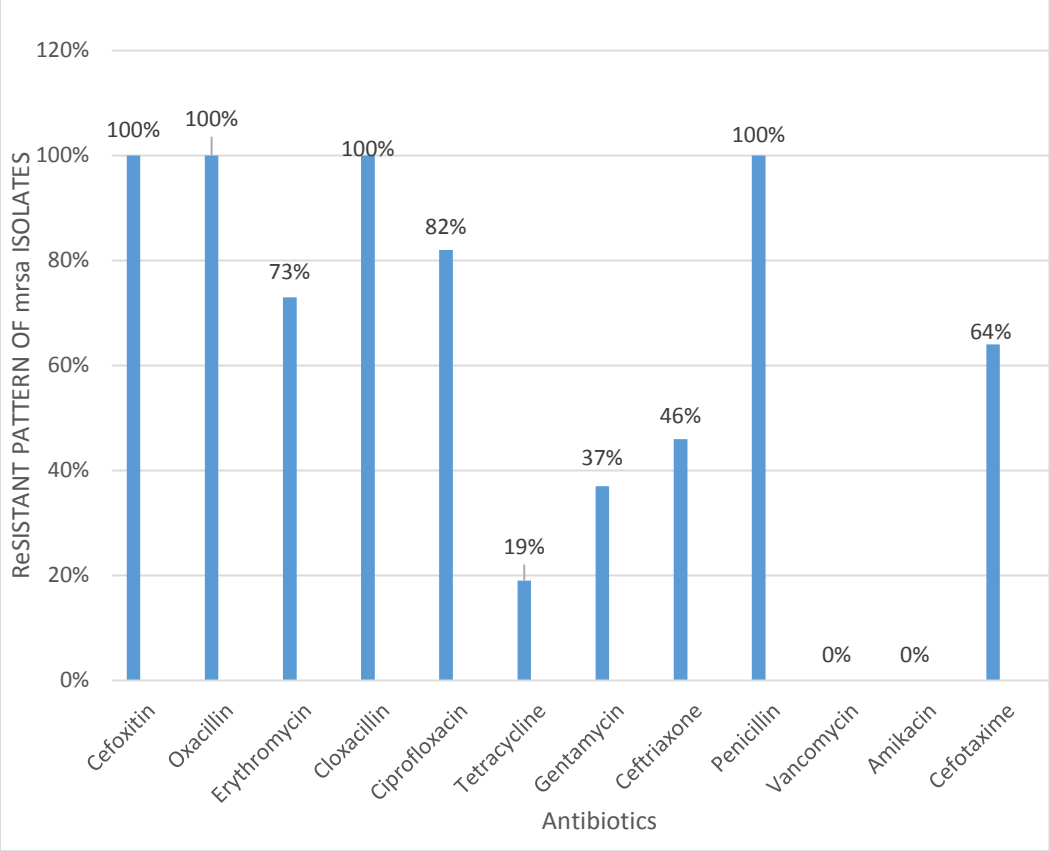


Figure 2: Antibiotic resistance pattern of MRSA

4.3.3 Multidrug resistant *S. aureus* (MDR)

The isolated strains of *S. aureus* were categorized into two groups: Methicillin Sensitive *S. aureus* (MSSA) and Methicillin Resistant *S. aureus* (MRSA). Both the group of *S. aureus* showed marked variation in sensitivity pattern to common antibiotics.

Pan susceptible, monoresistant and multidrug resistant *S. aureus* (MDR) were identified by their antibiotic sensitivity pattern. The *S. aureus* susceptible to all antibiotics were pan susceptible and resistant towards only one antibiotic was considered as monoresistant and resistant to two or more were multidrug resistant. Out of 47 Methicillin sensitive *S. aureus*, only 8.51% were pan susceptible and 27.66% were mono resistant and 63.83% were MDR. In case of MRSA 100% isolates were MDR no isolates were pan susceptible and mono resistant.

Table 6: Drug susceptibility pattern of MSSA and MRSA

	MSSA	MRSA
Pan Susceptible	4 (8.51%)	-
Mono Resistant	13 (27.66%)	-
Multi Drug Resistant	30 (63.83%)	11(100%)
Total	47	11 (100%)

4.3.4 Gender wise distribution of MRSA

Out of 11 MRSA isolated from health care workers, 18.18 % were male and 19.15 % were female. Though MRSA is found to more in female than male, statistically there is no significant association of occurrence of MRSA in male and female (p= 0.723).

Table 7: Gender wise distribution of MRSA

Sex	MRSA	MSSA	*S. aureus* carrier	*p*-value
Male	2(18.18%)	9(81.82%)	11(18.97%)	
Female	9(19.15%)	38(80.85%)	47(81.03%)	0.723
Total	11(18.97%)	47(81.03%)	58(100%)	

4.3.5 Distribution of MRSA among different age group

In this study highest percentage of MRSA was found in age group 21-30 years (21.95%) which is followed by age group 31-40 years (13.33%). No MRSA carrier were found in age group ≥20 years, 41-50 years and 51-60 years. The association between MRSA colonization among different age group was not statistically significant (p=1.261).

Table 8: Distribution of MRSA among different age group

Age group	*S. aureus* carrier	MRSA	MSSA	*p*- value	*p*-value
≥ 20	0	-	-		
21-30	41	9(21.95%)	32(78.05%)	0.000	
31-40	15	2(13.33%)	13(86.33%)	0.000	1.261
41-50	2	-	2(100%)		
51-60	0	-	-		

4.3.6 Distribution of MRSA in different department

In this study, nasal carrier MRSA among hospital care worker were found, highest percentage of MRSA was found in paediatric and surgery department (50%) which is followed by laboratory (33.33%), ENT and post up (25%). Lowest percentage of MRSA was found in ICU (14%) and medicine (16.67%). No MRSA was found in emergency, gyane, maternity, NICU, orthopedic and PICU. The association between MRSA colonization among different department was not statistically significant (p= 0.420).

Table 9: Distribution of MRSA in different department

Department	Total *S. aureus* carrier	MRSA	MSSA	*p*-value	*p*-value
Emergency	4	-	4		
ENT	8	2(25%)	6	0.066	
Gyane	3	-	3		
ICU	7	1(14%)	7	0.005	
Laboratory	6	2(33.33%)	4	0.284	
Maternity	1	-	1		
Medicine	6	1(16.67%)	5	0.040	0.420
NICU	4	-	4		
Orthopaedic	4	-	4		
Paediatric	4	2(50%)	2	0.757	
PICU	3	-	3		
Post-up	4	1(25%)	3	0.243	
Surgery	4	2(50%)	2	0.757	

4.3.7 Distribution of MRSA among different professional group

Out of 58 S. aureus isolates, 11 MRSA were obtained from nasal swab of different professional group. Among 11 *S. aureus* isolates from doctors, 20% were MRSA similarly, among 36 *S. aureus* isolated from nurse, 22.22 % were MRSA and 5 *S. aureus* isolated from lab worker, 20% were MRSA. No MRSA was found from attendant. The association between MRSA colonization among different department was not statistically significant (p= 0.349).

Table 10: Distribution of MRSA among different professional group

Profession	*S. aureus* carrier		Total *S. aureus* carrier	*p*-value	*p*-value
	MRSA	MSSA			
Doctor	2(20%)	8(80%)	10	0.012	
Nurse	8(22.22%)	28(19.31%)	36	0.000	
Lab worker	1(20%)	4(80%)	5	0.103	0.349
Attendant	-	7(100%)	7		
Total	11(18.97%)	47(81.03%)	58(100%)		

CHAPTER-V

5. DISCUSSION

Staphylococcus aureus is one of the most common causes of hospital acquired infections. It has been found that nasal carriage of *S. aureus* is a well-defined risk factor for subsequent infection in nearly all categories of hospitalized patients (Sah et al 2013). *S. aureus* is a well-known pathogen with an alarmingly increasing level of developing resistance to most available antimicrobial agents. Nasal *S. aureus* have been implicated in community associated infections like soft tissue infections and hospital infections like bacteremia (Onanuga and Temedie 2011). *S. aureus* can colonize on anywhere of human bodies, particularly in the anterior nares. The carriage of *S. aureus*, including MRSA, is well known to be a significant risk factor for subsequent infection (Chang et al 2015). Health care workers (HCWs) colonized with MRSA may carry these virulent hospital strains in their nose and skin and may transmit these organisms to the community creating a more dreadful situation (Khatri et al 2017).

This study was carried out at Gandaki Medical College and Research Centre Pvt. Ltd. to determine the prevalence of nasal carrier *S. aureus* and MRSA among hospital care workers. A total of 288 nasal samples 22.57% were male and 77.43% were female.

In this study, nasal carrier of *S. aureus* were 21.08% female and male 16.92%. Similar findings has been reported by Sah et al (2013) which revealed that nasal carriage rate of *S. aureus* in female HCWs were 21.2% and in male 19% in National Medical College and Teaching Hospital. However, in this study, the association between sex and nasal carriage of *S. aureus* was not statistically significant (p=0.463). Onanuga and Temedie (2011) also found sex is not a risk factor for nasal colonization of *S. aureus* and there is no activity of any of the groups that predisposes them to *S. aureus* colonization or infection.

In this study, nasal carriage rate of *S. aureus* was 20.14% among HCWs which were higher than that 15.7% reported by Khanal et al (2015) from Universal Medical College, Bhairahawa. Similar results have been reported by Sah et al (2013) about 20.37% nasal carrier *S. aureus* among HCWs at National Medical College. In contrary, Pant and Rai (2008) findings revealed higher *S. aureus* nasal colonization rate were 43.8% in staffs of teaching hospital in Kathmandu. Al-Talib et al (2013) reported total prevalence of *S. aureus* nasal carriage among health care workers in Malaysia was 28.7%. Onanuga and Temedie (2011) found overall prevalence of 33.3% of nasal carrier *S. aureus* in Nigeria.

Highest nasal carrier of *S. aureus* was found in age group 31-40 year (23.82%) and lowest percentage of carrier were found in the age group 41-50 year (14.29%). While, no *S. aureus* nasal carrier was found in age group ≥ 20 year and 51-60 year. Similarly, a study conducted by Khatri et al (2017) in Kathmandu have been found higher percentage of nasal carrier were found in age group 36-45 year (33.3%) and lowest percentage was found among the age group of above 46 year (4.8%). This may be due to lowest number of samples were collected from the age group ≥ 20 year and 51-60 year.

Regarding the ward wise distribution of nasal carrier, higher prevalence of nasal carrier *S. aureus* was found in department of ENT 40%. The higher carrier rate among HCWs of ENT department could possibly by the high frequency of contact with patients of eye, nose and throat and maintenance of hygiene is poor. In post-operative department *S. aureus* carrier was 36.36 % and in medicine department was 28.57%. Similarly, Khatri et al (2017) also found the percentage of nasal carriage of *S. aureus* in post-operative ward was 35.7% and El Alia et al (2017) have been observed highest nasal carriage rate of *S. aureus* was found in medicine (44.8%). *S. aureus* nasal carriage among the staffs from post-operative suggests the possible transmission from wound infection caused by these organisms and also could be due to weak hygiene practice. Lowest percentage of *S. aureus* was found in maternity ward 4.75% and gynecology

and obstetrics 11.11%. Healthcare workers of different department are always exposed to patients with *S. aureus* infection so, they were more prone to be colonized.

In profession wise study highest nasal carriage rate of *S. aureus* was found in nurses 24.83%. Shrestha et al (2009) have been found similar result conducted in Nepal hospital, where 22.22% nurse were colonized by *S. aureus*. In contrary, Rashid et al (2013) had observed that the highest carriage rate 66% were in nurses. Nurses are regularly in contact with patients so, the carrier number might be higher. Likewise, in the present study, 16.41% doctors and 16% lab workers were colonized by *S. aureus*. Lowest percentage of *S. aureus* carrier was found in attendants (13.75%). While, Rashid et al (2012) studied showed higher nasal carriage of *S. aureus* among HCWs where, 66.6% nurses, 51.8% doctors and 59% sanitary workers. Staffs in the hospital tend to be colonized while working in the hospital and carrier rate may increase during their prolonged stay and can act as source of infection. It also indicates the need of the control in the frequency of their exposure with the vulnerable patients.

This study showed *S. aureus* were 100% sensitive towards vancomycin and amikacin. Same result were reported by Boncompion et al 2017 in Argentina and Khanal et al (2015) in Western Nepal and Khatri et al (2017). Tetracycline, cefoxitin, gentamicin, ceftriaxone and cloxacillin showed higher susceptibility with 94.83%, 81.03%, 75.86%, 75.86% and 72.41% respectively. The isolated strains showed highest resistant to penicillin and oxacillin 68.97% which is followed by erythromycin 50%, ciprofloxacin 46.55%. The least effectiveness of penicillin and oxacillin is probably due to indiscriminate and empirical use of these drugs leading to emergence of resistant strains. Furthermore, these drugs are relatively cheaper and easily available all over-the-counter in Nepal (Kumari et al 2008). El-Alia et al (2017) have been also found higher sensitivity with variable degrees, to gentamicin, ciprofloxacin and vancomycin were 96.7%, 88.7%, and 85.4% respectively. Ahmed et al 1998 reported that medical

persons were colonized with more antibiotic resistant isolates than non-medical persons because of lack of antimicrobial surveillance within hospital premises, ineffective hospital decontamination procedure and unhygienic practice has lead to spread of antibiotic resistant *S. aureus* strain.

In this study, cefoxitin (81.03%) was more susceptible than oxacillin (31.03%). Recently a number of studies proposed using cefoxitin to be superior in predicting the presence of mecA in *S. aureus* and coagulase negative staphylococci with a high degree of sensitivity and specificity. Cefoxitin was suggested to be used as a surrogate for the oxacillin disk diffusion test. In particular, cefoxitin would overcome the failure of routine oxacillin disk diffusion tests to detect heterogeneous MRSA populations, it is a good inductor of penicillin binding protein 2a production in *S. aureus* isolates that carry the *mecA* gene (El-Alia et al 2017).

All MRSA strain were susceptible towards vancomycin and amikacin and resistant towards cloxacillin and penicillin and low resistance towards tetracycline (18.18 %) and gentamicin (37%). MRSA isolates revealed high resistance towards ciprofloxacin (81.8%), erythromycin (72.73%), cefotaxime (63.64%) and ceftriaxone (45.45%). Khanal et al (2015) had also found revealed high MRSA strain resistance towards gentamycin and erythromycin and low resistance towards ciprofloxacin and all MRSA isolates were susceptible towards vancomycin and amikacin and resistant towards cloxacillin and penicillin same as this study.

Out of 47 Methicillin sensitive *S. aureus*, 63.83% were MDR only 8.51% were pan susceptible and 27.66% were mono resistant. In case of MRSA 100% isolates were MDR no isolates were pan susceptible and mono resistant. Many studies had also shown the emergence of MDR *S. aureus* in hospitals (Shah et al 2002; Anupurba et al 2003). These studies clearly indicate about the

appropriate steps to be taken to reduce MRSA and MDR strains in hospital settings to minimize nosocomial infections.

The present study revealed that the total identified MRSA carrier was 3.81%. The result of the present study correlates with the findings of study conducted by Khanal et al (2015) where a prevalence of Methicillin-resistant *S. aureus* was 3.4%. The findings of this study was lower than Askarian et al (2009) where the researchers found 5.3% of all HCWs in Namazi hospital Shiraz Iran and from National Medical college, Birgung, Nepal (Shakya et al 2010) where 10% of them nasal carrier of MRSA. Chen and Huang (2005) 13.6% in Taiwan and Olonitola et al (2007) reported 14.85% in Zaria, Nigeria. The observed MRSA is higher than the reports of Gorwitz et al (2008) reported 1.5% in U.S.A, Hogan et al (2016) reported 1.3% in Madagascar. This may indicate cross-contamination of MRSA between health care personnel and patients.

Out of 288 HCWs, 19.15% female and 18.18 % male were carrier of MRSA. Hogan et al (2016) in Madagascar also found higher prevalence of MRSA among female HCWs than male HCWs. Another study conducted by Khanal et al (2015) in Universal medical college found 19.5% male and 11.5% female were carrier of MRSA. While on contrary, Shibabaw et al (2013) found MRSA prevalence rate for male HCWs were two-times more likely to be MRSA carriers than females. This could be because more number of sample were collected from female HCWs and female HCWs might lack good hygiene practice along with less immune power.

In this study highest percentage of MRSA was found in age group 21-30 years (21.95%) followed by age group 31-40 years (13.33%) and no MRSA was found in the age group ≥20 year, 41-50 year and 51-60 year. Similarly, Shibabaw et al (2013) also observed highest rate of MRSA carriers in the age group 20 to 29 years (5.9%). While on contrary, Boncompion et al (2017) found

carriage of MRSA Strains were higher in the oldest group > 40 year (9.6%) in Argentina. However, there is no association between age and being MRSA carrier (p=1.261). The higher prevalence among the younger HCWs may be due to their lack of knowledge with regard to infection control policies and their missing experience in taking care of MRSA infected patients.

In this study, highest percentage of MRSA was found in paediatric and surgery (50%) which is followed by laboratory (33.33%), ENT and post up (25%). Lowest percentage of MRSA was found in ICU (14%) and medicine (16.67%). Askarian et al (2008) found 43.8% of the MRSA carriers working in several surgical units in Namazi hospital. Shibabaw et al (2013) had also found highest rate of MRSA carriers were working in surgical ward. Similarly, highest prevalence (40%) of nasal carriage of MRSA were in the surgical wards of Universal Medical College (Khanal et al 2015). The poor sanitation of the different departments and the poor hygiene practice of the health care workers in different departments may be the reasons behind the higher prevalence of carriage rates in staffs from different departments.

According to their profession, most of the MRSA were isolated from nurses was 22.22% followed by lab workers 20% and doctors 20% and no MRSA was found from attendant. Khanal et al (2015) had found MRSA carriage rate was highest among nurses 7.8 %. Another study conducted by Shibabaw et al (2013) in Ethiopia found the MRSA carriage was particularly high among nurses (21.2%), doctors (12.5%) and laboratory technicians (12.5%). El Aila (2017) also reported highest MRSA carrier (30.4%) among nurses. Similarly Khatri et al (2017) also reported the higher percentage of MRSA was found among lab personnel (10.5%) followed by nurses (9.9%) and doctors (6.4%). Another study conducted by Rashid et al (2013) also observed highest carriage rate of MRSA was found among nurses (27.3%) followed by physicians (13.6%). The higher MRSA rate among nurses, doctors and lab workers could possibly be explained by the high frequency of patient contact among these professionals. The nasal carriage of MRSA among HCWs has indicated the chances of transmission of the organism to patients during patient-care.

CHAPTER VI

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

This study highlights that health care worker might have acquired nasal carrier, drug resistant *Staphylococcus aureus* (MRSA) from patients during their care and are also responsible for cross contamination. It is necessary to follow the proper hand washing protocols and other protective measures to protect both the health care worker and patients.

Even though this study revealed that the prevalence of nasal carriage *S. aureus* and MRSA among HCWs were comparatively lower than other studies conducted in our country and internationally. But still nasal carriage *S. aureus* and MRSA among HCWs necessitates the need of control in the frequency of their exposure with the vulnerable patients. The basic infection control measures, screening program and treatment of MRSA- positive HCWs can help as an effective measure to control MRSA infections.

Multi drug resistance strains are the biggest problem for hospitals because these are usually resistant to most of the common antibiotics. Higher percentage of MDR strain emphasize the need to discourage antibiotic's abuse. It also supports the need to implement strategies for elimination of nasal carriage of *S. aureus*, so as to prevent severe multi-drug resistant *S. aureus* transmission.

6.2 Recommendations

1. All hospital staffs can be source of transmission, so they should follow universal barrier precautions. Their expose to patients' particularly vulnerable patients should be limited during patient care and their nasal decolonization should be done.
2. Sanitary protocols and the antibacterial guidelines of the health professionals should be followed strictly to prevent nosocomial infections.
3. Simple preventive measures like hand washing, using sterile mask and gown and avoiding touching one's nose during work, should be reinforced in all health care settings.
4. Hospital care workers nasal swab should be examined frequently to control transmission of diseases.
5. The research can be extended to molecular level by using different molecular techniques like PCR and RFLP in order to reveal the epidemiology of the MRSA isolated.

REFERENCES

Abudu L, Blair I, Fraise A and Cheng KK (2001). Methicillin resistant *Staphylococcus aureus* (MRSA): a community based prevalence survey. J Epidemiol Infect **126:** 351–356.

Al-Abdli NA, and Baiu SH (2014). Nasal carriage of *Staphylococcus* in health care workers in Benghazi hospitals. American Journal of Microbiological Research **2**: 110-112

Al-Talib H, Yean CY, Hasan H, Nik Zuraina NMN and Ravichandran M (2013). Methicillin resistant *Staphylococcus aureus* nasal carriage among patients and healthcare workers in a hospital in Kelatan. Malaysia. J Pol Microbiol **62**: 109-12.

Anatanarayan and Panikar (2009). Textbook of Microbiology. 8th edition, Hyderbad, India, Unive Chang C-J, Chen N-C, Lao C-K, Huang Y-Crsities Press Private Limited. pp 199-207.

Anwar MS, Jaffrey G, Rehman Bhatti KU, Ayyib M and Bokhari SR (2004). *Staphylococcus aureus* and MRSA nasal carriage in general population. J Coll Physicians Surg Pak **14**: 661-664.

Appenbulum PC (2007). Microbiology of antibiotic resistance in *Staphylococcus aureus*. J Clinical Infectious Diseases **45**: 165-170.

Arch G, Mainous AG and Hueston WJ (2006). Nasal carriage of *Staphylococcus aureus* and methicillin resistant in United States, 2001-2002. J Ann Family Med **4**: pp 132-137.

Askarian M, Zeinalzadeh A, Japoni A, Alborzi A and Memish ZA (2009). Prevalence of nasal carriage of methicillin resistant *Staphylococcus aureus* and its susceptibility pattern in healthcare workers at Namazi Hospital, Shiraz, Iran. J Infect Dis **13**: 241-247.

Atlas R (1995). Principles of Microbiology. 1st edition, Mosby-Year Book, Inc; 11830 Westline Industrial Drive, St. Louis, Missouri 63146, pp 681-682.

Bastola K (2017). Prevalence of methicillin resistant and multi-drug resistant *Staphylococcus aureus* in various clinical samples. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. pp 1-36.

Bektas S, Obradovic A, Aljicevic M, Numanovic F, Hodzic D and Sporisevic L (2016). The frequency of community acquired methicillin resistant *Staphylococcus aureus* among samples in institute for public health in canton Sarajevo. J Materia Socio-Medica **28**: 61-65.

Bhatiani A, Yadav S, Pal N and Chandra A (2017). Prevalence of carriage of methicillin resistance *Staphylococus aureus* among health care workers in a tertiary care center, Kanpur U.P. J Curr Microbiol App Sci **5**: 739-746.

Boncompain CA, Suarez CA and Morbidoni HR (2017). *Staphylococcus aureus* nasal carriage in health care workers: First report from a major public hospital in Argentina. J Rev Argent Microbiol **49**(2): 125-131.

Borer A, Gilad J, Yagupsky P, Pelled N, Porat N, Trefler R, Shprecherlevy H, Riesenberg K, Shipman M and Schlaeffer F (2002). Community acquired methicillin resistant *Staphylococcus aureus* in Institutionalized adults with developmental disabilities. J Emerging Infectious Diseases **8**: 966-970.

Borriello G, Murray P and Funke G (2009). Topley Wilson's Microbiology and Microbial infections Bacteriology. 10th edition. UK, John Wiley and sons Limited
pp 771-882.

Boyce JM (1989). Methicillin resistant *Staphylococcus aureus*: detection epidemiology and control measures. J Infect Dis Clin North Am **3**: 901-913.

Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ and Wren MW (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother **56**: 1000-1018.

Brooks GF, Butel JS and Morse SA (2004). Medical Microbiology. 23rd edition, New York: Mc Graw Hill pp 223-247.

Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GI, Towner KJ and Wren MW (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother **56**: 1000-1051.

Brumfitt W and Hamilton MJ (1989). Methicillin resistant *Staphylococcus aureus*. New England Journal of Medicine **320**(18): 1188-1196.

Campbell KM, Vaughn AF, Russell KL, Smith B, Jimenez DI, Barrozo CP, Minarci JR, Crum NF and Ryan MA (2004). Risk factors for community-associated methicillin-resistant *Staphylococcus aureus* infections in an outbreak of disease among military trainees in San Diego, California in 2002. J Clin Microbiol **42:**4050-4053.

Campanile F, Bongiorno D, Perez M, Mongelli G, Sessa I, Benvenuto S, Gona F, Varaldo PE and Stefani S (2015). Epidemiology of *Staphylococcus aureus* in Italy: first nationwide Survey, 2012. J Glob Antimicrob Resist **3:** 247-254.

Carvalho MS, Andrade DF, Sousa AF, Valle AR, Freitas DR, Nascimento GC, Andrade D and Watanabe E (2016). Nasal colonization with *Staphylococcus aureus* in nursing students ground for monitoring. Rev Bras Enferm, **69**:1046-1051.

Centers for Disease Control (CDC) (2006). Management of multi-drug resistant organisms in Health care settings. CDC, Atlanta USA. pp 1-74.

Chakrabroty P (2007). A Textbook of Microbiology. 2nd edition, New Central Book Agency (P) Ltd. 8/1 Chintamoni Das Lane, Kolkata 700 009 India. pp 188-210.

Cheesbrough M (2000). District laboratory practice in tropical countries. Part 2. Cambridge University Press. pp 157-165.

Chambers HF, Hartman BJ and Tomasz A (1985). Increased amounts of a novel penicillin-binding protein in a strain of methicillin resistant *Staphylococcus aureus* exposed to nafcillin. Journal of Clinical Investigation **76**: pp 325-331.

Chang CJ, Chen NC, Lao CK and Huang YC (2015). Nasal *Staphylococcus aureus* and Methicillin-Resistant *S. aureus* Carriage among Janitors Working in Hospitals in northern Taiwan. Plos One **10**(9).

Cheesbrough M (2000). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press, pp 157-165.

Chen CJ, Hsu KH, Lin TY, Hwang KP, Chen PY and Huang YC (2011). Factors associated with nasal colonization of methicillin resistant *Staphylococcus aureus* among healthy children in Taiwan. Journal of Clinical Microbiology **49**: 325-331.

Chen C, Huang Y (2005). Community- acquired methicillin resistant *S. aureus* in Taiwan. J Microbial Immunol Infect **38**: 376-382.

CLSI (2015). Performance standards for Antimicrobial susceptibility testing. CLSI document M100-S25. Wayne PA: clinical laboratory standard institute.

Collee JG, Fraser SA, Narmion BP and Simmons (2006). McCartney Practical Medical Microbiology. 14th edition, Churchil Livingstone pp 245-258.

Cosgrove SE, Qi Y and Kaye KS (2005). The impact of methicillin-resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay and hospital charges. Infect Control Hosp Epidemiol **26**: 166-174.

Criswell D (2004). The evolution of antibiotic reistance. Institute of Cresation Research.

Dar JA, Thoker MA, Khan JA, Ali A, Khan MA, Rizwan M, Bhat KH, Dar MJ, Ahmed N and Shamim A (2006).Molecular epidemiology of clinical and carrier strains of methicillin resistant *Staphylococcus aureus* (MRSA) in the hospital settings of north India. BioMed Central Ltd. **5**: 22.

Das H (2010). Textbook of Microbiology. 4th edition, Wiley Pvt. Ltd. India, pp 559.

Deleo FR, Otto M, Kreiswirth BN and Chambers HF (2010) Community-associated Methicillin resistant *Staphylococcus aureus*. Lancet **375**: 1557-1568.

Derek FJB, David IE, Peter MH, Donald M, Geoffrey LR, Kevin JT and Michael WDW (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy **56**: 1000–1018.

Elalia NA, Allaham Na and Ayesh BM (2017). Nasal carriage of methicillin resistant *Staphylococcus aureus* among health care workers at AL Shifa hospital in Gaza Strip. BMC Infect Dis **17**: 28.

Farzana K, Rashid Z and Akthtar N (2008) Nasal Carriage of Staphylococci In health care workers: Antimicrobial Susceptibility Profile. Pak J Pharm Sci **21:** 290-294.

Franklin DL, Allison EA, Bhat M, Vicki D, Johnson L, Mei-Ho L, Earl B, Lester NW, Glenny V and Elaine LL (2007). *Staphylococcus aureus* Colonization and Infection in New York State Prisons. The Journal of Infectious Diseases **196**: 911–918.

Gorwitz RJ, Kruszon-Moran D, Mcallister Sk, Mcquillan G, Mcdougal LK, Fosheim GE, Jensen BJ, KillgoreG and Tenovar FC (2008). Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. The Journal of Diseases **197**: 1226-1234.

Geha DJ, Uhl JR, Gustaferro CA and Persing DH (1994). Multiplex PCR for identification of methicillin resistant Staphylococci in the clinical laboratory. Journal of Clincal Microbiology **32**: 1768-1772.

Giri B. (2012). Prevalence of methicillin reistant *Staphylococcus aureus* (MRSA) isolated from different clinical samples in Western Regional Hospital Pokhara. M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. pp 4-35.

Gorwitz RJ, Kruszon-Moran D, Mcallister SK, Mcquillan G, Mcdougal LK and Foseim GE (2008). Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J Infect Dis pp **197**. 1226-1234

Grennwood D, Slack RCB Peutherer JF (2002).Medical Microbiology-a guide to microbial pathogenesis, immunity, laboratory diagnosis and control. 16th edition, Edinburgh: Elsevier Science Limited. pp 622-629.

Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T and Tenovar FC (1997). Methicillin resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother **40**: 135-139.

Hogan B, Rakotozandrindrainy R, Al-Emran H, Dekker D, Hahn A, Poppert S, Frickman H, Hagen RM, Michel V, Crusius S, Hernianana JN,

Rakotondrainiarivelo JP and Schwarz NG (2016). Prevalence of nasal colonization by methicillin sensitive and methicillin resistant *Staphylococcus aureus* among healthcare workers and students in Madagaskar. J BMC Infect Dis **16**: 016-1733.

Hugo WB and Russell AD (1993). Pharmaceutical Microbiology. 5th edition, Blackwell Scientific Publication, UK. pp 152-164.

Huh K and Chung DR (2016). Changing epidemiology of community-associated methicillin resistant *Staphylococcus aureus* in the Asia-Pacific region. Expert Rev Anti Infect Ther **14**: 1007-1022.

Ibrahim A, Al-Zahrani, Hamson C, Edge D, Collins J, John D, Perry, Muhammad R, Gould K and Harwood CR (2011). Small restriction site- based multiplex PCR for typing of hospital and community acquired methicillin resistant *Staphylococcus aureus*. Journal of Clinical Microbiology **49**: 3820-3828.

Ike B, Ugwu MC, Ikegbunam MN, Nwobodo D, Ejikeugwu C, Gugu T and Esimone CO (2016). prevalence, antibiogram, and molecular characterization of community acquired methicillin resistant *Staphylococcus aureus* in AWKA, Anambra Nigeria. J Open Microbiol **10**: 211-221.

Jevons MP, Coe AW and Paniker MT (1963). Methicillin resistance in Staphylococci. J Lancet. **1**: 904-908.

Khalili MB, Sharifi-Yazdi MK, Dargahi H and Sadeghian HA (2009). Nasal colonization rate of *Staphylococcus aureus* strains among health care service employees of teaching university hospitals in Yazd. J Acta Med Iran **47**: 315-317.

Khanal LK and Jha BK (2010). Prevalence of methicillin resistant *Staphylococcus aureus* among skin infection cases at a hospital in Chitwan, Nepal. J Nepal Med Coll **12**: 224-228.

Khanal R, Sah P, Lamichhane P, Lamsal A, Upadhaya S and Pahwa VK (2015). Nasal carriage of methicillin resistant *Staphylococcus aureus* among health care workers at a tertiary care hospital in Western Nepal. J Antimicrobial Resistance and Infection Control **4**: 39.

Khatri S, Pant ND, Bhandari R, Shrestha KL, Shrestha CD, Adhikari N and Poudel A (2017). Nasal carriage rate of methicillin reistant *Staphylococcus aureus* among health care workers at tertiary care hospital in Kathmandu, Nepal. J Nepal Health Res Counc **15**: 26-30.

Kilic A, Mert G, Senses Z, Bedir O, Ayogan H, Basustaoglu AC and Appenbulum PC (2008). Molecular characterization of methicillin resistant *Staphylococcus aureus* nasal isolates from Turkey. J Antonie Van Leeuwenhoek **94:** 615-619.

Kluytamans J, Van BA and Verbrugh H (1997). Nasal carriage of *Staphylococcus aureus*: epideomioloy, underlying mechanisms and associated risks. J Clinical Microbiology Reviews **10**: 505-520.

Kumar P, Shukla I and Varshney S (2011). Nasal screening of healthcare workers for nasal carriage of coagulase positive MRSA and prevalence of nasal colonization with *Staphylococcus aureus*. J Biology and Medicine **3**: 182-186.

Kumari N, Malhotra TM and Singh YI (2008). Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in a tertiary care hospital in Nepal. Nepal Med Assoc **47**: 53-56.

Lamichhane R, Adhikari RP, Sherchand JB (1999). Study of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from different clinical samples. M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. pp 1-45.

Lee AS, Huttner B, Harbarth S (2011). Control of Methicillin-resistant *Staphylococcus aureus.* Infect Dis Clin N Am **25**:155-79.

Livermore DM (2012). Current epidemiology and growing resistance of gram negative pathogens. J The Korean Journal of Internal Medicine. **27**: pp 128-142.

Matthew JK, Deanna KM, Holly AH, Geraldine MQ, Sigrid KM, Gregory F, Linda KM, Jasmine C, Bette J, Scott KF, George K and Tenovar FC (2006). Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001-2002. The Journal of Infectious Diseases **193**: 172-179.

Mondal H, Gupta I, Nandi P,Ghosh P,Chattopadhyay S and Mitra GD (2016). Nasal screening of healthcare workers for nasal carriage of methicillin resistance *Staphylococcus aureus*, vancomycin resistance *Staphylococcus aureus* and prevalence of nasal colonization with *Staphylococcus aureus* in Burdwan Medical College and Hospital. Journal of Contemporary Medical Research **11**: 3342-3346.

Mulqueen J, Cafferty F, Cormican M, Keane JD and Rossney A (2007). Nasal carriage of methicillin resistant *Staphylococcus aureus* in GPs in the West of Ireland. J Gen Pract. **57**: 811-813.

Murray P, Barron E, Jorgensen J, Pfaller M and Yolkan R (2003). Manual of clinical microbiology. 8th edition, ASM press Washington D.C. USA. pp 383-387.

Niemeyer DM, Pucci MJ, Thanassi JA, Sharma VK and Archer GL (1996). Role of mecA transcriptional regulation in the phenotypic expression of methicillin resistant in *Staphylococcus aureus.* J Bacteriol **178**: 5464-5471.

Olonitola OS, Inabo HI, Olayika BO and Bugo ID (2007). Nasal carriage of methicillin resistant *Staphylococcus aureus* by primary school pupils in a

University staff school, Zaria, Nigeria. International journal of biological and chemical sciences **1**: 71-75.

Onanuga A and Temedie TC (2011). Nasal carriage of multi- drug resistant *Staphylococcus aureus* in healthy inhabitants of Amassoma in Niger delta region of Nigeria. J African Health Sciences **11**: 176-181.

Otto JA, French GL (2010).Molecular epidemiology of community-acquired methicillin resistant Staphylococcus aureus in Europe. The Lancet Infect Dis **10**: 227-239.

Pandey S, Raza MS and Bhatta CP (2012). Prevalence and antibiotic susceptibility pattern of methicillin resistant Staphylococcus aureus in Kathmandu Medical College- Teaching Hospital. Journal of Institute of Medicine **34**(1): 13-17.

Pant J and Rai SK (2007). Occurance of *Staphylococcus aureus* in hospital environment and staffs in teaching hospital in Kathmandu, Nepal. NAMLS **8**: 72-73.

Parija SC (2012). A textbook of microbiology and immunology. 2nd edition, Elsevier publication, India. pp: 181-190.

Pelczar MJ, Chan ECS and Krieg NR (2006). Microbiology. 5th edition. Tata Mc Graw Hill publishers. pp 285-298.

Pourakbari B, Khodabandeh M, Mahmoudi S, Sabouni F, Aziz-Ahari A, Bahador A, Keshavarz VS, Hosseinpour SR and Mamishi S (2017). Molecular epidemiology of *Staphylococcus aureus* nasal colonization among patients and their parents /guardian in an Iranian referral hospital. J Microb Patho **107:**75-80.

Quezada JNM, Diekema DJ, Perencevich EN, Bailey G, Winokur PL and Schweizer ML (2013). Long term risk for readmission, methicillin resistant *Staphylococcus aureus* (MRSA) infection and death among MRSA colonized veterans. J Antimicrob Agents Chemother **57**: 1169-1172.

Rai S, Nratuladhar and Shrestha H (1990). Methicillin resistant *Staphylococcus aureus* in a tertiary medical care center, Nepal. Ind J Med Microbial **8**: 108-109.

Rashid Z, Farzana K, Satter A and Murtaza G (2012). Prevalence of nasal *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in hospital personnel and associated risk factors. J Acta Pol Pharma **69**: 985-991.

Rijal RK, Pahari N, Shrestha BK, et al. (2008). Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara Nepal. J Nepal med coll **10**: 192-195.

Rocha LC, Carvalho MO, Nascimento VM, Dos S, Barros TF, Adorno EV, Reis JN, Guarda DA, Santiago RP and Goncalves MS (2017). Nasopharyngeal and Oropharyngeal colonization by *Staphylococcus aureus* and *Streptococcus pnumoniae* and prognostic markers in children with sickle cell disease from the Northeast of Brazil. J Front Microbiol, **8**: 217.

Sah P, Rijal KR, Shakya B, Tiwari BR and Ghimire P (2013). Nasal carriage rate of *Staphylococcus aureus* in hospital personnel of National Medical college and Teaching Hospital and their susceptibility pattern. J Health Appl Sci **3**: 21-23.

Sahm DF, Marsillo MK and Piazza G (2000). Antimicrobial resistance in key bloodstream bacterial isolates: electronic surveillance with the surveillance Network Databasse-USA. Clin Infect Dis **26**: 259-263.

Salmenlinna S, Lyytikainen O and Vuopio-Varkila J (2002). Community acquired methicillin resistant *Staphylococcus aureus*, Finland. J Emerg Infect Dis **8**: 602-607.

Sanjana RK, Shah R and Chaudhary N (2010). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* (MRSA) in CMS teaching hospital. J College Medical Sci Nepal **6**: 1-6.

Sanford MD, Widmer AF, Bale MJ, Jones RN and Wenzel RP (1994). Efficient detection and long term persistence of carriage of methicillin resistant *Staphylococcus aureus*. Clin Infect Dis **19**: 1123-1128.

Shakya B, Shrestha S and Mitra T (2010). Nasal carriage rate of methicillin resistant *Staphylococcus aureus* among at National medical college teaching hospital, Birgunj, Nepal. J Nepal Med Coll **12**: 26-29.

Sharan NK (2016). The rate of induciblr clindamycin resistance among *Staphylococcus* and *Streptococcus* isolates from various clinical specimens. M.Sc. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. pp 1-45.

Shrestha B, Pokhrel BM and Mohapatra TM (2009). *Staphylococcus aureus* nasal carriage among health care workers in a Nepal hospital. J Brazilian Infect Dis **13**: 322.

Shibabaw A, Abebe T and Mihret A (2013). Nasal carriage rate of methicillin resisant *Staphylococcus aureus* among Dessie Referral hospital health care workers, Dessie, northest Ethiopia. J Asntimicrob Resist Infect Control **2**: 25.

Soriano A, Martinez JA, Mensa J, Marco F, Almela M, Moreno-Martinez A, Sanchez F, Munoz I, Jimenez De Anta MT and Soriano E (2000). Pathogenic significance of methicillin resistance for patients with *Staphylococcus aureus* bacteremia. J Clin Infect Dis **30**: 368-373.

Stapleton PD and Taylor PW (2002). Methicillin resistant *Staphylococcus aureus*: mechanisms and modulation. J Science Progress **85**: 57-67.

Thapa KB (2011). Methicillin resistant *Staphylococcus aureus* (MRSA) in clinical samples and nasal screening for MRSA carriage among healthy carriers in hospital setting. Dissertation submitted to Central Department of Microbiology, Tribhuvan University. pp 2-40.

Tenovar FC (2006). Mechanisms of antimicrobial resistance in bacteria. The American Journal of Medicine. **119**: 3-10.

Tille P (2014). Bailey and Scott's Diagnostic Microbiology, 13th edition. Elsevier Mos by 3251 Riverport Lin St. Louis, Missouri 63043. pp 223.

Tiwari HK, Sapkota D and Sen MR (2008). High prevalence of multidrug resistant MRSA in a tertiary care hospital of northen India. J Infect Drug Resist **1**: 57-61.

Vonberg RP, Stamm-Balderjahn S, Zuschneid I, Ruden H and Behkne M (2006). How often do asymptomatic healthcare workers cause methicillin resistant *Staphylococcus aureus* outbreaks? A symptomatic evolution. J Infect Control Hosp Epidemiol **27**: 1123–1127.

WHO (2014). Antimicrobial resistance: global report on surveillance. World Health Organization, Geneva.

APPENDIX-A

Questionnaire sheet for nasal sample collection

For laboratory identification purposes:

S. No.

Laboratory code for sampling:

Date of sample collection:

Name:

Occupation:

Age:

Sex:

Education level:

Department/ward:

Medical History:

- Fever:
- Nasal surgery:
- Diabetes:
- Antimicrobial therapy:
- URTI:
- Hormonal medication used:

APPENDIX-B

Materials and Equipments

List of Materials

Glass wares

Beaker	Conical flask
Test tubes	Glass rod
Slides	Pipettes
Measuring cylinder	Micropipette
Micropipette tips	

Miscellaneous

Bacteriological loop	Labeling stickers
Bunsen burner	Sterile cotton swabs
Spirit lamp	Tube holder
Forceps	Gloves
Marker	Soaps
Tissue paper	

Equipments

Autoclave	Incubator
Water bath	Refrigerator
Hot air oven	Compound Microscope
Water distillation plant	

Chemical and Reagents

Crystal violet	Plasma
Gram's iodine	40% Potassium Hydroxide

Ethanol	1N Hydrochloride acid
Safranin	Distilled water
3% Hydrogen peroxide	MacFarland's Nephelometer Standard (0.5)
Physiological saline	Microscope oil
Paraffin oil	Lysol

Antibiotics (HiMedia Company)	**Media (Hi Media Company)**
Penicillin (10 units)	Nutrient Agar
Cefoxitin (30mcg)	Nutrient Broth
Amikacin (30mcg)	MacConkey Agar
Cloxacillin (10mcg)	Mannitol Salt Agar
Ceftriaxone (30mcg)	Muller Hinton Agar
Ciprofloxacillin (30mcg)	Peptone
Gentamicin (10mcg)	
Erythromycin (15mcg)	
Vancomycin (30mcg)	
Oxacillin (1mcg)	
Cefotaxime (30mcg)	
Tetracycline (30mcg)	

APPENDIX-C

Bacteriological media

Composition and preparation of different types of media

1. **Nutrient Agar (NA)**

Ingredients	**Gram/litre**
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final pH (at 25℃)	7.4 ±0.2

28 grams of media was dissolved in 1000 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15lbs at 121℃ for 15 minutes.

2. **MacConkey Agar (MA)**

Ingredients	**Gram/litre**
Pancreatic digest of gelatin	17.0
Peptone	3.0
Lactose	10.0
Sodium chloride	5.0
Agar	13.5
Neutral red	0.03
Crystal violet	0.001
Final pH	6.9-7.3

51.3 grams of the medium was suspended in 1000ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121℃ for 15 minutes.

3. **Mannitol Salt Agar (MSA)**

Ingredients	**Gram/litre**
Protease peptone	10.0
Sodium Chloride	75.0
D-Mannitol	10.0
Phenol red	0.025
Agar	15.0
Final pH	7.4±0.2

111 grams of the medium was suspended in 1000ml distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs at 121℃ for 15 minutes.

4. **Muller –Hinton Agar (MHA)**

Ingredients	**Gram/litre**
Beef extract	300.0
Casein acid hydrolysate	17.5
Starch	1.5
Agar	17.0
Final pH (at 25℃)	7.4±0.2

38 grams of the medium was suspended in 1000ml and boiled to dissolve completely. The medium was then autoclaved at 15 lbs at 121℃ for 15 minutes.

5. **Peptone water**

Ingredients	**Gram/litre**
Peptone	10.0
Sodium Chloride	5.0
Final pH (at 25℃)	7.2±0.2

1.5 grams of the medium was suspended in 100ml distilled water and dissolved completely. It was then sterilized by autoclaving at 15 lbs at 121℃ for 15 minutes.

APPENDIX-D

Reagents/Stain

i) Crystal violet stain

Solution A

Crystal violet	2.0 gm
95% ethyl alcohol	20.0 ml

Solution B

Ammonium oxalate	0.8 gm
Distilled water	30.0 ml

Crystal violet was dissolved in ethyl alcohol, and ammonium oxalate in distilled water. Then solution A and B were mixed properly.

ii) Gram's iodine solution

Iodine	1.0 gm
Potassium Iodide	2.0 gm
Distilled water	30.0 ml

iii) Ethyl alcohol (95%)

Absolute alcohol	95.0 ml
Distilled water	5.0 ml

iv) Safranin

Safranin (99% dye content	10.0 gm
Distiled water	1000.0 ml

2. Catalase reagent:

3% Hydrogen peroxide solution (100 ml)

Hydrogen peroxide	3 ml
Distilled water	97 ml

5. Physiological saline

Sodium Chloride	0.85 gm
Distilled water	100.0 ml

6. MacFarland Nephelometer Standards (0.5)

1% V/V solution of Sulphuric acid was prepared by adding 1ml of concentrated Sulphuric acid to 99 ml of distilled water. 1% W/V solution of barium chloride was prepared by dissolving 0.5 gram of dehydrate barium chloride in 50 ml of distilled water. Then to the 99.5ml of 1% Sulphuric acid solution, 0.5 ml of barium chloride solution was mixed and stirred continuously. Then the solution was transferred in to the clean screw capped tube and stored at dark place until use. The test tube for the broth preparation should be of same size as of McFarland tube. The tubes can be stored and used for six months.

APPENDIX-E

Gram's staining procedure

i. With the help of inoculating loop, isolated pure colonies from NA was touched at the tip and transferred to a clear and grease free slide containing a drop of distilled water.

ii. A uniform smear was made on the slide, which was first air dried and then heat fixed gently.

iii. After cooling, the smear was flooded with crystal violet solution for 1 minute and then rinsed with distilled water.

iv. The slide was flooded with Gram's iodine solution for 1 minute, after which it was rinsed off with water.

v. The slide was then decolorized with acetone alcohol for 10-15 seconds and rinsed off with water.

vi. The decolorized smear was again flooded with safranin for 1 minute and then washed with water.

vii. Finally, the slide was blot dried with absorbent paper and examined under oil immersion.

Observation of Gram positive cocci arranged in grape like clusters is indicative of Staphylococci (Chessbrough 2000 and Collee et al 2006).

APPENDIX-F

Further identification tests

S. aureus ATCC25923 was included as the standard organism in all tests.

Catalase test

a. A drop of 3% hydrogen peroxide was put in a clean slide.
b. The pure colony from NA plate was taken with the help of glass rod or plastic stick
c. The test organism was placed on the hydrogen peroxide drop.

The rapid evolution of gas bubbles is indicative of a positive test. The catalase positive organisms were tested for coagulase production for further confirmation of *S. aureus* (Chessbrough 2000).

Coagulase test

Positive coagulase test is the best single test to identify *S. aureus*. The following two tests were performed.

Slide coagulase test

i. A homogenous suspension of an isolated colony was made with a drop of physiological saline on a clean slide.
ii. A drop of plasma was added to the homogenous suspension.
iii. The suspension was mixed with plastic stick or glass rod.
iv. Clumping observed within 5- 10 seconds was indicative of positive test.
v. For positive as well negative test, further confirmation was done by tube coagulase test (Clumping factor test, Chessbrough 2000).

Tube coagulase test (Free coagulase test)

i. 1ml of 1:6 diluted plasma in saline (0.85% saline) was placed in small tube.
ii. A colony of the organism was emulsified in the plasma containing test tube.

iii. Control organism was inoculated in the same way as above and a tube containing only plasma was also kept.

iv. All the tubes were incubated at 37˚C and clot formation was examined at intervals of one hour for up to 4 hours, by tilting the tube. The negative tubes may keep for 24 hours

v. The negative tube was examined for auto -agglutition of plasma.

vi. Stiff gel formation or large clots floating in the tube was the indication of positive test (Collee et al 2006).

APPENDIX-G

Antibiotic susceptibility test

All *S. aureus* isolated from clinical samples and nasal screening process were subjected to in-vitro antimicrobial susceptibility test by Kirby-Bauer modified disc diffusion method as recommended by CLSI (formerly NCCLS). In this study the antibiotics used were Amoxycillin, Ciprofloxacillin, Cloxacillin, Erythromycin, Gentamicin, Methicillin, Oxacillin, Penicillin and Co-trimoxazole.

Procedure

i. Muller Hinton agar (MHA) was prepared, sterilized and poured into sterile petriplate to reach 4mm in depth.

ii. Pure culture of the test organism was transferred into a sterile nutrient broth tube.

iii. The inoculated culture was incubated at 37˚ C for up to 4 hours to obtain turbidity equivalent to the density of MacFarlands Nephelometer standard number 0.5.

iv. Sterile cotton swab was dipped into the culture tube of the organism and excess inoculum was removed by pressing and rotating the swab firmly against the tube wall.

v. Swabbing was performed uniformly all over the surface of the MHA plate by rotating the plate. With closed petriplate lid, it was kept at room temperature for 3-5 minutes for the surface of the agar to dry.

vi. Antibiotic discs were taken out from their respective vials with the help of sterile forcep and placed carefully on the surface of the swabbed medium, at least 15mm away from the edge of one disc to another.

vii. The discs were pressed lightly with the forceps to make complete contact with the surface of the medium.

viii. The plates left for 30 minutes at room temperature.

ix. The plates were then incubated at 35˚C for 24 hours.

x. After incubation, the diameter of the zone of inhibition of each disc was measured.

The organism was considered as resistant, intermediate or sensitive based on the standard interpretative chart updated according to the current CLSI standard (HiMedia Company, 2015). The standard zone size at which the organism is considered resistant, intermediate and susceptible is given in the zone-size interpretative chart (Appendix-H).

APPENDIX-H

Zone Size Interpretative Chart (CLSI 2015)

Antibiotics	Symbol	Disc content	Diameter of zone of inhibition (mm)			*S. aureus* ATCC 25923
			Resistant	intermediate	sensitive	
Penicillin G	P	10 units	≤28	-	≥29	26-37
Cloxacillin	COX	10mcg				23-34
Tetracycline	TE	30mcg	≤14	15-18	≥19	24-30
Oxacillin	OX	1mcg	≤10	11-12	≥13	18-24
Cefoxitin	CX	30mcg	≤21		≥22	23-29
Gentamicin	GEN	10mcg	≤12	13-14	≥15	19-27
Erythromycin	E	15mcg	≤13	14-22	≥23	22-30
Ciprofloxacin	CIP	5mcg	≤15	16-20	≥21	22-30
Ceftriaxone	CTR	30mcg	≤13	14-20	≥21	22-28
Vancomycin	VA	30mcg	-	-	≥15	17-21
Amikacin	AK	30mcg	≤17	15-16	≥14	20-26
Cefotaxime	CTX	30mcg	≤14	15-22	≥23	25-31

Note: CLSI =Clinical Laboratory Standard Institute

Source: Hi Media Company 2015

Appendix I

1. Statistical analysis Association of *S. aureus* among gender

Sex	*S. aureus* carrier	Non carrier	Total	χ2-value	df	*p*-value	Remarks
Male	11	54	65	0.540	1	0.598	Insignificant association
Female	47	176	233				
Total	58	230	288				

2. Association of *S. aureus* among age group

Age group	*S. aureus* carrier	Non carrier	Total	χ2-value	df	*p*-value	Remarks
≥ 20 year	0	6	6	3.356	4	0.5	Insignificant association
21-30 year	41	160	201				
31-40 year	15	48	63				
41-50 year	2	12	14				
51-60 year	0	4	4				
Total	58	230	288				

3. Association of *S. aureus* among professional group

Profession	*S. aureus* carrier	Non carrier	Total	χ2-value	df	*p*-value	Remarks
Doctor	10	57	67	4.413	3	0.220	Insignificant association
Nurse	36	109	145				
Lab worker	5	20	25				
Attendant	7	44	51				
Total	58	230	288				

4. Association of *S. aureus* among different department

Department	*S. aureus* carrier	Non carrier	Total	χ2-value	df	*p*-value	Remarks
Emergency	3	13	16	13.055	12	0.365	Insignificant Association
ENT	8	12	20				
Gynecology	3	24	27				
ICU	7	30	37				
Laboratory	6	24	30				
Maternity	1	20	21				
Medicine	6	15	21				
NICU	4	11	15				
Orthopedic	5	27	32				
Paediatric	4	19	23				
PICU	3	12	15				
Post-up	4	7	11				
Surgery	4	16	20				
Total	58	230	288				

5. Association of MRSA among gender

Sex	MRSA carrier	Non carrier	Total	$\chi 2$-value	df	*p*- value	Remarks
Male	2	63	65	0.126	1	0.723	Insignificant association
Female	9	214	233				
Total	11	277	288				

6. Association among age group and MRSA

Age group	MRSA carrier	Non carrier	Total	$\chi 2$-value	df	*p*- value	Remarks
$\geq$ 20 years	0	6	6	1.261	4	0.868	Insignificant association
21-30 years	9	192	201				
31-40 years	2	61	63				
41-50 years	0	14	14				
51-60 years	0	4	4				
Total	11	277	288				

7. Association of MRSA and profession

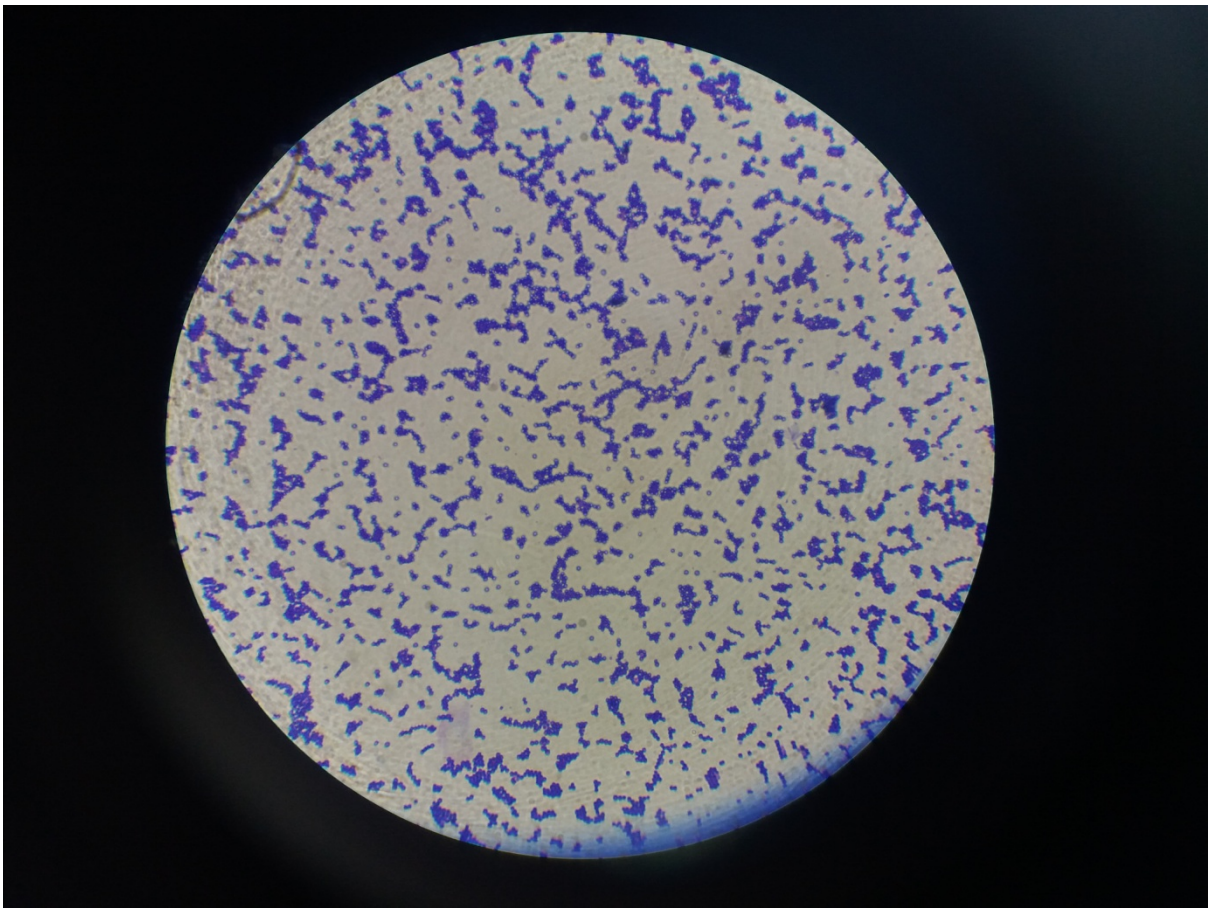
Profession	MRSA carriers	Non carriers	Total	$\chi 2$-value	df	*p*- value	Remarks
Doctor	2	65	67	3.292	3	0.349	Insignificant association
Nurse	8	137	145				
Lab worker	1	24	25				
Attendant	0	51	51				
Total	11	277	288				

8. Association of MRSA among different department

Department	MRSA carrier	Non carrier	Total	$\chi 2$- value	df	*p*-value	Remarks
Emergency	0	16	16	12.322	12	0.420	Insignificant association
ENT	2	18	20				
Gynecology	0	27	27				
ICU	1	36	37				
Laboratory	2	28	30				
Maternity	0	21	21				
Medicine	1	20	21				
NICU	0	15	15				
Orthopedic	0	32	32				
Paediatric	2	21	23				
PICU	0	15	15				
Post-up	1	10	11				
Surgery	2	18	20				
Total	11	277	288				

Photograph 3: Golden yellow colony on MSA (# 56)

Photograph 4: Antibiotic susceptibility pattern of *S. aureus* (# 201)



Photograph 1: Microscopic View of *Staphylococcus aureus* (# 246)

Photograph 2: Coagulase positive (# 85)

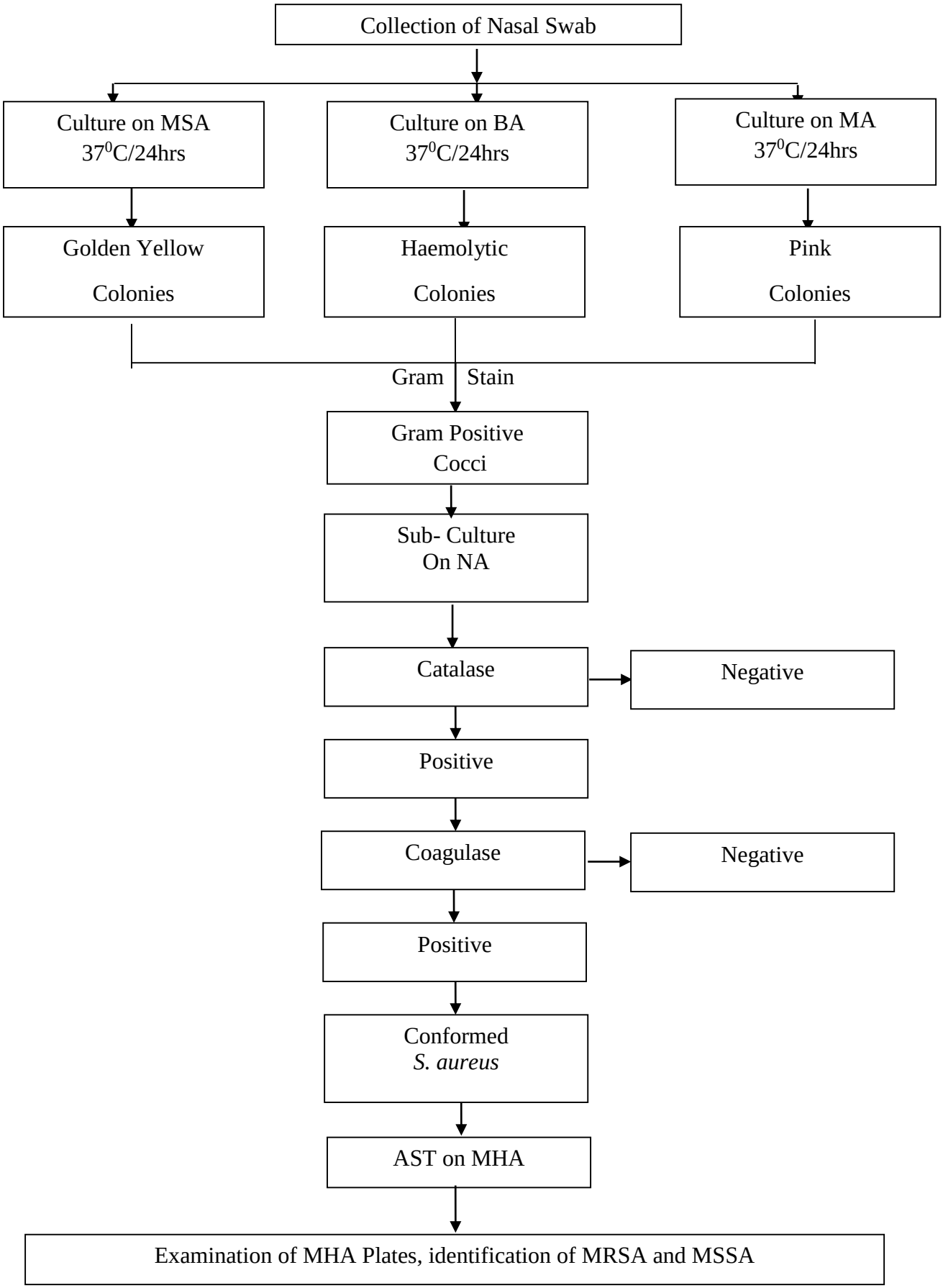


Figure 1: Flow chart showing summary of laboratory protocols.