ANTIBIOTIC SUSCEPTIBILITY TEST OF *Staphylococcus aureus* FROM CLINICAL SAMPLES OF PATIENTS VISITING A TERTIARY CARE CHILDREN HOSPITAL OF KATHMANDU, NEPAL

A Dissertation Submitted to the Department of Microbiology/Central Campus of Technology Tribhuvan University, Kathmandu, Nepal, In Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Microbiology
(Medical)

by

Mrs. Lata Chalise
T.U. Reg. No.:5-2-003-0088-2011
Exam Roll no.: MB 436/072

2021

CERTIFICATE OF APPROVAL

On the recommendation of **Asst. Professor Mr. Hemanta Khanal** and **Dr. Shovana Thapa** this dissertation work of **Mrs. Lata Chalise**, entitled **"Antibiotic susceptibility test of *Staphylococcus aureus* from clinical samples of patients visiting a tertiary care children Hospital, Kathmandu, Nepal",** a study carried out in Microbiology Laboratory, "International Friendship Children's Hospital**",** has been approved for the examination and is submitted to Tribhuvan University in partial fulfillment of the requirements for M. Sc. degree in Microbiology (Medical).

…… ……..

Dr. Kamana Sahani
Teaching Assistant
Department of M.sc Micribiology
Central Campus of Technology
Hattisar, Dharan, Sunsari
Nepal

Date: - ……

BOARD OF EXAMINER

Recommended by:

.....……
(Supervisor)
Hemanta Khanal
Assistant professor
Department of Microbiology
Central Campus of Technology,
Hattisar, Dharan, Sunsari
Nepal

.....……
(Supervisor)
Dr. Shovana Thapa
Senior Pathologist
International Friendship
Children′s Hospital
Maharajgunj, Kathmandu

Approved by:

…….
(Program Co-Ordinator)
Dr. Kamana Sahani
Teaching Assistance
Department of MSc
Microbiology
Central Campus of Technology
Hattisar, Dharan, Sunsari
Nepal

Examiners:

…….
(External Examiner)
Dr. Reshma Tuladhar
Assistant Professor
Central Department of
Microbiology
Tribhuvan University
Kritipur, Kathmandu
Nepal

.....……...
(Internal Examiner)
Shiva Nandan Shah
Department of Microbiology
Central Campus of Technology
Hattisar, Dharan, Sunsari
Nepal

Date:

RECOMMENDATION

This is to certify that Mrs. Lata Chalise has completed this dissertation work entitled **"Antibiotic susceptibility test of *Staphylococcus aureus* from clinical samples of patients visiting a tertiary care children Hospital of Kathmandu, Nepal"** as a partial fulfillment of M. Sc. degree in Microbiology (Medical) under our supervision. The study was carried out in Microbiology Laboratory, International Friendship Children′s Hospital. To the best of our knowledge, this is an original research work and has not been submitted for any other degree.

Supervisor Mr. Hemanta Khanal Asst. Professor Department of Microbiology Central Campus of Technology Dharan Nepal	Supervisor Dr. Shovana Thapa Senior Pathologist International Friendship Children′s Hospital Maharajgunj, Kathmandu

Date: ……..

ACKNOWLEDGEMENT

I would like to extend my sincere gratitude and earnest compliment to my respected supervisors Asst. Professor Mr. Hemanta Khanal and Dr. Shovana Thapa, MD, Pathologist, International Friendship Children′s Hospital, Kathmandu for their continuous guidance, kind encouragement and support for the completion of this thesis work. Without his valuable help, it would not have been possible to complete this dissertation work successfully.

Additionally, I am incredibly thankful to all the teachers especially Asst. Professor Suman Rai and librarian Mr. Om Khatiwada and library staff for their great cooperation and help.

My sincere appreciation goes to all the staffs of Pathology Department of International Friendship Children′s Hospital, especially to Mr. Sudarshan Pandey, Bishnu Khakurel and Dipa Gurung for their invaluable support during the research period. I am thankful to all the staffs of Department of Microbiology, central campus of technology for their kind support and familial environment they provided throughout the college days.

Finally, I would like to express my sincere gratitude to my entire family members and friends for motivating and supporting me during the thesis work.

…..……

Lata Chalise

Date: …….

ABSTRACT

Staphylococcus aureus is one of the most important and common cause of community-acquired as well as hospital-acquired infections. Moreover, methicillin resistant strains of *S. aureus*, usually being resistant to several antibiotics, are now presenting the major threat in many different countries throughout the world. The aim of this study was to determine the prevalence of infection caused by *S. aureus* as well as MRSA strains and to determine their antimicrobial susceptibility pattern. A cross-sectional study was carried out from September to December 2018 at IFCH, Kathmandu, Nepal, in which 227 *S. aureus* isolated from 961 clinical specimens. Methicillin-resistant *Staphylococcus aureus* (MRSA) identified by using the Cefoxitin (30 μg) disc diffusion method followed by the Clinical and Laboratory Standards Institute (CLSI 2012) guidelines. Among 227 isolates, 55.9% (n=127) were from inpatients and 44.1% (n=100) were from outpatients. Likewise, 62.5% (n=142) were from male patients and 37.4% (n=85) were from female patients. Overall, the highest percentage of *S. aureus* isolation (32.2%) was found in toddler's age group. Antibiogram of all 227 *S. aureus* strains showed chloramphenicol (78.4%) was most effective drug, followed by meropenem (76.2%), clindamycin (74%) and the least effective drug was found to be erythromycin (37.4%). Prevalence of MRSA, using cefoxitin discs, was found to be 48% (n=109) whereas, vancomycin was found to be 100% effective. Out of 109 MRSA strains, the maximum number of strains (n=62) were isolated from the inpatients. Similarly, in overall, the highest number of MRSA isolates (n=33) was found in the patients of toddlers age group. High prevalence of staphylococcal infection and the infection due to MRSA in the hospital patients showed the need of regular surveillance. The study also showed the need of evaluation of antibiotic disks before the study in Nepal.

Key words: *S. aureus*, antibiogram, MRSA, cefoxitin, International Friendship Children′s Hospital.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

µM	:	Micrometer
AMR	:	Antimicrobial Resistance
ATCC	:	American Type Culture Collection
CA-MRSA	:	Community Acquired-Methicillin Resistant *S. aureus*
CDC	:	Centre for Disease Control
CFU	:	Colony Forming Unit
CLSI	:	Clinical Laboratory Standard Institute
CONS	:	Coagulase Negative Staphylococci
DNA	:	Deoxyribonucleic acid
HA-MRSA	:	Hospital Acquired-Methicillin Resistant *S. aureus*
NB	:	Nutrient Broth
MDR	:	Multidrug Resistance
MIC	:	Minimum Inhibitory Concentration
MSA	:	Mannitol Salt Agar
PBP	:	Penicillin Binding Protein
VISA	:	Vancomycin-intermediate *S. aureus*
VRSA	:	Vancomycin-resistant *S. aureus*
VSSA	:	Vancomycin-susceptible *S. aureus*

LIST OF TABLES

LIST OF FIGURES

LIST OF PHOTOGRAPHS

LIST OF APPENDICES

CHAPTER I

INTRODUCTION AND OBJECTIVES

1.1 Background

Staphylococci are spherical shaped, Gram positive bacteria belonging to the family Micrococcaceae. Micrococcaceae cells may occur singly or as irregular clusters (Atlas 1995). 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 (Rajaduraipandi et al 2006). *S. aureus* can be differentiated from other species of staphylococci by a unique characteristic of it to produce coagulase, an enzyme that converts fibrinogen to fibrin and clots the plasma (Brooks et al 2004).

Staphylococcus aureus is Gram positive coccus appears as grape like clusters, non-sporing, nonmotile, and usually non capsulated bacteria (Chakraborty et al 2005). They are facultative anaerobes and most strains ferment mannitol aerobically. They are catalase and coagulase positive and produce an extracellular cell clumping factor, and some strains produce capsule (Brown et al 2005). *Staphylococcus aureus* has the capacity to produce a wide range of virulence factors, causes various pyogenic infections, food poisoning and toxic shock syndrome. *Staphylococcus aureus* capable of invading intact normal skin are rare, most able of cause infection, only when they enter through breaks in the skin. *Staphylococcus aureus* causes pyogenic infections like breast abscess, post-operative wound infections, folliculitis, impetigo, furuncles, septic arthritis, lung abscess and etc. Disseminated infections are septicemia often consequent metastatic secondary foci and toxin mediated infections are toxic shock syndrome, staphylococcal food poisoning, and staphylococcal scalded skin syndrome (Collee et al 2006).

Infection due to *S. aureus* caused several deaths before the discovery of penicillin, a beta-lactam drug. After the discovery of this antibiotic, the frequency of staphylococcal infections reduced to a minimum level, however, soon after a few years of its discovery, penicillin-resistant strains of *S. aureus*

developed. These organisms produced beta-lactamase enzyme, which is plasmid encoded, and caused the disruption of the beta-lactam ring, hence, no effect of this antibiotic appeared against these organisms. Later, a semi-synthetic drug, methicillin was introduced against those beta-lactamase producers and proved to be successful (Chambers 2001). However, once again, soon after its discovery, methicillin resistant strains of *S. aureus* appeared in 1961. Since its first report, the strain has been progressively causing increased mortality, morbidity, and health care costs with skin and soft tissue infections, ventilator-associated pneumonia, catheter associated bacteraemia, and many other infections in hospitals and communities (Shanson 1981; Maple et al 1989).

When MRSA strains first appeared, they occurred predominantly in the healthcare setting. However, methicillin resistance is now increasingly recognized in the community (Chambers 2001). Healthcare-associated MRSA (HA-MRSA) is particularly efficient at developing resistance to antimicrobial agents. Treatment of infections caused by Methicillin resistance *Staphylococcus aureus* strain became more difficult since *Staphylococcus aureus* became resistant not only to usual penicillin related antibiotics but also most other structurally unrelated antibiotics such as chloramphenicol, rifampicin (Cosgrove et al 2005).

Drug resistance is mostly seen in hospital acquired infection 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 includes *E. coli, S. aureus* etc. (Parija 2013).

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 resistance is defined as resistance to two or more antibiotics belonging to different structural classes (CDC 2006). Multidrug resistance is one of the biggest

problems facing global public health. Antibiotic available without prescription, without benefit of guardiance from a clinician or even a pharmacist, their indiscriminate usage without regard for specific symptoms has favoured the increasing trend of antibiotic resistance as shown by various studies (ASM 2009). In the study of antibiotic resistance pattern of *S. aureus* conducted at Manipal Teaching Hospital, of 117 *S. aureus* isolates tested 15.4% were found to be MRSA with fourteen (77.8%) of the methicillin resistant isolates resistant to all agents tested (Subedi et al 2005).

The resistance to antimicrobial agents among *Staphylococci* is an increasing problem which has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLSB) antibiotics to treat staphylococcal infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties (Delialioglu et al 2005; Deotale et al 2010). These three antibiotics are chemically distinct but their mode of action is similar (Gadepalli et al 2006; Leclercq and Courvalin, 1991). The MLS antibiotics have three different mechanisms of resistance such as; target site modification, enzymatic antibiotic inactivation and macrolide efflux pumps (Jadhav et al 2011).

Staphylococcal resistance to oxacillin/methicillin occurs when an isolate produces an altered penicillin-binding protein, PBP2a, which is encoded by the mecA gene. The variant penicillin-binding protein binds beta-lactams with lower avidity, which results in resistance to this class of antimicrobial agents. In 1961, the first methicillin resistant *Staphylococcus aureus* strains were identified (Grundmann et al 2006). Methicillin resistance is genetically and biochemically complex, mediated by staphylococcal cassette chromosome (SCCmec), a mobile genetic element encoding for an altered penicillin-binding protein (PBP2a, mecA) with decreases affinity to β-lactams (Gorden et al 2008). MRSA infections account for 20–80% of all nosocomial *S. aureus* infections in many centers across the world (Fomda et al 2014; Fluit et al 2001; Krishnamurthy et al 2014). MRSA may transmit from person to person by physical contact and rarely by air. The nasopharynx is the main ecological

niche of the *S. aureus* (Burian et al 2010), although it is found in almost all parts of body.

MRSA strains are difficult to eradicate as they are multidrug-resistant leaving glycopeptides antibiotics such as vancomycin, as the drugs of choice. Since, the emergence of vancomycin resistance in enterococci in 1988 and its in vitro demonstration that its resistance gene (Van A and Van B) are transmissible to other bacterial species including *S. aureus*, emergence of vancomycin resistance in clinical Staphylococococci has become a great concern. The treatment of suspected *S. aureus* infections is becoming more complicated and clinical significance of these strains requires further investigation (CDC 2002). The indiscriminate use of antibiotics, prolonged hospital stay, lack of awareness, receipt of antibiotics before coming to hospital etc. increases the chance of emergence and spread of MRSA (McDonald et al 1997). The rise of drug resistant MRSA is a serious problem in the treatment and control of staphylococcal infection.

The prevalence of MRSA has varied from hospital to hospital in various countries. Several researches and studies conducted in our country also show the range of percent isolates. In a study carried out by Kumari et al (2008), 26.14% MRSA strains were isolated in a tertiary-care hospital in Eastern Nepal. Lamichhane et al in 1999 reported 11.76% MRSA strains were isolated from 17 *S. aureus* samples collected in TUTH whereas 31.43% MRSA strains were isolated from 35 *S. aureus* in Kanti Children's Hospital. Likewise, Rajbhandari et al (2002) in 2002 found that 54.9% of *S. aureus* isolates were resistant to methicillin.

The prime focus of this study is on the frequency of infections caused by *S. aureus* in the patients visiting hospitals as well as on its antibiotic sensitivity pattern. The study will also demonstrate the present scenario of MRSA and the sensitivity pattern of different antibiotics used against it. This study is really useful for the future planning and policy making in healthcare centers and hospitals in order to combat with the spreading.

1.2 OBJECTIVES

1.2.1 General objective

- To study antimicrobial susceptibility pattern of *Staphylococcus aureus* isolated from clinical samples.

1.2.2 Specific objectives

- To isolate and identify *Staphylococcus aureus* from clinical samples.
- To determine Methicillin resistant *Staphylococcus aureus.*
- To determine the distribution of *Staphylococcus aureus* and MRSA according to the ward, age and gender of patients.
- To assess the Multi-drug resistance (MDR) pattern among the isolates,
- To determine Inducible Clindamycin resistance.

CHAPTER II

LITERATURE REVIEW

2.1 General Characteristics of Staphylococci

Staphylococci are Gram positive spherical cells, that occurs usually in grape-like clusters (Brooks et al 2004). The term Staphylococcus is derived from the greek word (staphyle, meaning bunch; kokkus, meaning berry) (Chakraborty et al 2005). Staphylococci are gram positive cocci and non-motile, non-spore forming, occasionally capsulate, catalase positive in nature (Cheesbrough 2008). Staphylococci are widespread in nature, their normal habitats being the skin and mucous membranes of man and birds. Most important pathogenicstrains are *S. aureus*, which can cause both superficial and deep pyogenic infections as well as a number of toxin-mediated illnesses. Common species of staphylococci that are found on human skin include *S. epidermidis, S. haemolyticus, S. hominis, S. warneri, S. capitis, S. lugdunensis and S. simlulans*. All of these are opportunistic pathogens, especially in patients with intravascular catheters, or implanted prosthetic devices, or who are immunosuppressed (Colley et al 2006). The pathogenic staphylococci often hemolyze blood, coagulase plasma, and produce a variety of toxins and an extracellular enzyme (Brooks et al 2004). Staphylococci are also transmitted from person to person (Forbes et al 2007).

2.2 Classification of Staphylococcus

Staphylococci can be classified in various ways depending on their cultural characteristics, colony morphology, biochemical characteristics, pathogenicity and cell wall structure.

2.2.1 Classification on the basis of pigment production

1. *S. aureu* producing golden yellow colonies and are pathogenic.
2. *S. albus* producing white colonies and are non-pathogenic.
3. *S. citreus* producing yellow colonies and are non-pathogenic. (Ananthanarayan and panikar, 1986).

2.3 *Staphylococcus aureus*

Staphylococcus aureus is a spherical shaped Gram-positive bacterium and gets its name from the golden colour of its pigmentation produced on agar media. These bacteria are catalase and coagulase positive and relatively resistant to reduced water potential and tolerate drying and high salt fairly well (Brock 2003). It is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin. *Staphylococcus aureus*, a worldwide pathogen, causes a variety of infections ranging from minor skin infections such as impetigo, boils, cellulitis, folliculitis, scalded skin syndrome and abscesses to life-threatening diseases such as osteomyelitis, meningitis, endocarditis, toxic shock syndrome and bacteremia (Tenoverfag 2006). *Staphylococcus aureus* also produce several different toxins, includes staphylococcal enterotoxin, exfoliatin toxin, toxic shock syndrome toxin, alpha toxin and leucocidin (Salyersaaw 2002).

2.3.1 Morphological and Cultural Characteristics

Staphylococcus aureus is approximately 1μm in diameter, non motile, nonsporing, and non capsulated. However, they contain a microcapsule, which can be visualized by electron microscope only but not light microscope (parija 2013). *S. aureus* can usually grow in basic media like nutrient agar within the temperature range of 12-44°C. The optimum temperature and pH for the growth is 37°C and 7.5 respectively.

S. aureus can produce round, convex, smooth, opaque colonies having diameter of 1-3mm in nutrient agar at aerobic incubation of 37°C for 24 hours. Most strain produce golden yellow pigment. Some strain may produce orange or yellow pigment and a few are non-pigment producer. Pigment production is best seen when the cultures are grown aerobically at 22°C (Chakraborty et al 2005).

On nutrient broth, most strain give moderate to dense turbidity with powdery deposit at the bottom. No pigmentation is produced in liquid broth (Baired-parker 1997).

S. aureus show medium to large, slightly raised, smooth, translucent colonies with yellow pigmentation and show beta-hemolysis in 5% sheep blood agar (Forbes et al 2002).

S. aureus is able to grow on MSA media with 8-10% sodium chloride (Ananthanarayan and panikar, 1986). Most bacteria are inhibited on MSA while *S. aureus* is tolerant to sodium chloride incorporated into the media and produce yellow colonies having 1mm diameter, surrounded by yellow medium due to acid production from mannitol fermentation (Collee et al 1996).

2.3.2 Biochemical Characteristics

S. aureus is coagulase positive that can ferment sugars namely glucose, lactose, sucrose, lactose and mannitol with the production of acid but no gas. They are catalase positive, MR/VP test positive and indole test negative. The organism can hydrolyze urea, reduces nitrates to nitrites and liquefy gelatin and produce phosphatase (Parija 2013). Urease and esterase production and lactose fermentation are variable characters useful in the differentiation of methicillin resistant strains. It also produces a deoxyribonuclease (DNase) and thermonuclease, TNase (Forbes et al 2002). Though coagulase test is of diagnostic value in detecting *S. aureus*, some other Staphylococcus strains also give positive coagulase test such as *S. intermedius, S. hyicus* (Ananthanarayan and Panikar 1986).

2.4 Virulence factors

The wide range of infections caused by *S. aureus* is related to a number of virulence factors that allow it to adhere to surface, invade or avoid the immune system, and cause harmful toxic effects to the host (Bien et al 2013).

2.4.1 Cell-associated polymers

2.4.1.1 Capsule

Capsular polysaccharide surrounding the cell wall inhibits opsonization (Kumar 2012).

2.4.1.2 Peptidoglycan

The cell wall polysaccharide peptidoglycan confers rigidity and structural integrity to the bacterial cell. It gives rigidity to the cell and represents 50% of cell wall weight. The peptidoglycan can stimulate macrophages to produce cytokines and can activate the complement and induces release of inflammatory cytokines (Chakraborty et al 2005).

2.4.1.3 Teichoic acid

Teichoic acid, an antigenic component of the cell wall facilitates adhesion of the cocci to the host cell surface and protects them from complement-mediated opsonisation (Kumar 2012). It mediates adherence of Staphylococci to mucosal cell (Parija 2013).

2.4.2 Cell Surface Proteins

2.4.2.1 Protein A

Protein A is a surface protein covalently bound to the peptidoglycan layer and found in more than 90% of *S. aureus* strains. It is absent in both Coagulase negative Staphylococci (CNS) and Micrococci. Protein A has many biological properties including chemotactic, anticomplementary and antiphagocytic and also induce platelet damage and hypersensitivity. It binds to the Fc portion of the IgG molecules except IgG3, leaving the Fab region free to combine with its specific antigen. When suspension of such sensitized cells is treated with homologousb (test) antigen, the antigen combines with free Fab sites of IgG attached to Staphylococcal cells. This is knows as Co-agglutination has many applications in immunochemical and cell-surface structural studies (Ananthanarayan and Panikar 1986).

2.4.2.2Fibrinonectin-binding protein

It promotes adhesion of staphylococci to mucosal cells and tissue matrices (Mongodin et al 2002).

2.4.2.3 Clumping Factor (Bound Coagulase)

It is a surface associated protein also known as bound coagulase, which reacts with fibrinogen (Ananthanarayan and Panikar 1986). This clumping factor directly reacts with plasma, converts it to insoluble fibrin, causing the Staphylococci to clump or aggregate (Kumar 2012).

2.4.3 Super-antigen exotoxins

2.4.3.1 Enterotoxins

There are six antigenic types of enterotoxins (named SE-A, B, C, D, E and G). Enterotoxins cause diarrhea and vomiting when ingested and is responsible for staphylococcal food poisoning. These toxic proteins are relatively heat stable and resisting 100°C for several minutes (Chakraborty et al 2005).

2.4.3.2 Toxic shock syndrome toxin (TSST)

TSST-1 is expressed systemically and is the cause of toxic shock syndrome. Both enterotoxins and TSST-1 are the superantigens. Superantigens stimulate T cells non-specifically without normal antigenic recognition. IL-1, IL-2 and tumor necrosis factor (TNF) are released in large amounts, causing the symptoms of TSS (Ananthanarayan and Panikar 1986).

2.4.3.3 Exofoliatin (Exfoliative toxin)

The exfoliatin toxin, associated with scalded skin syndrome, causes separation within the epidermis, between the living layers and the superficial dead layers (Chakraborty et al 2005).

2.4.4 Membrane-damaging toxins that lyse eukaryotic cell membranes

2.4.4.1 Alpha toxin (alpha-hemolysin)

It is most potent membrane-damaging toxin of *S. aureus.* It has lethal effects on a wide variety of cell types. Alpha toxin is also called alpha-hemolysin because it can lyse red blood cells.

Some strains of *S. aureus* also produce other toxins- beta toxin, gamma toxin, delta toxin. These toxins can damage membranes of cells other than red cells and may well have a role similar to alpha toxin (Salyers et al 2002).

2.4.4.2 Leukocidin

Leukocidin is a multicomponent protein toxin produced as separate components which at together to damage membranes. Leukocidinsinclude alpha-lysin, pantop-valentine-leukocidin (PV-leukocidin) and leukolysin (Parija 2013).

2.4.5 Extracellular enzymes

Staphylococcus aureus produce numerous extracellular enzymes including coagulase, hyaluronidase, lipase, staphylokinase, deoxyribonuclease, and phosphatase (Parija 2013).

2.4.5.1 Hyaluronidase

This enzyme acts mainly on the hyaluronic acid and breaks down the connective tissue of host, which helps the organism to spread from the localized part to surrounding tissues. It is also called spreading factor (Chakraborty et al 2005).

2.4.5.2 Staphylokinase

Many strains of *S. aureus* express a plasminogen activator called staphylokinase. This factor lyses fibrin, hence, also called fibrinolysin. As it forms a complex and causes dissolution of fibrin clots by its proteolytic activity, it serves as a spreading factor (Chakraborty et al 2005).

2.4.5.3 Lipase

This enzyme degrades lipid of the skin tissues which helps them in its spread. Lipase degradation facilitates *S. aureus* to colonize the sebaceous glands (Chakraborty et al 2005).

2.4.5.4 Deoxyribonuclease

This enzyme degradeshosts DNA (Parija 2013).

2.4.5.5 Coagulase

Coagulase is an enzyme that acts along with a coagulase reacting factor (CRF) present in plasma, binding to prothrombin and converting fibrinogen to fibrin. It is of two types; free coagulase and bound coagulase. About 97% of *S. aureus* produce both forms of coagulase (Maranan et al 1997; Langone 1982).

2.4.5.6 Phosphatase

This enzyme breaks down phospholipid of the host cell (Chakraborty et al 2005).

2.5 Staphylococcal Diseases

Staphylococcal infections are the most common bacterial infection that range from the trivial to the fatal. Staphylococcal infections are characteristically localized pyogenic lesion, in contrast to the spreading nature of streptococcal infections. *Staphylococcus aureus* causes diseases through the direct invasion and destruction of tissue or through the production of several toxins (Bailey and Scotts et al 2007).

2.5.1 Cutaneous infections

Staphylococcal infections cause a variety of cutaneous infections including impetigo, cellulites, wound infections, and abscesses (Lwatsuki et al 2006).

2.5.1.1 Impetigo

This contagious infection usually occurs on the face, especially around the mouth**.** Impetigo presents in 2 basic forms; simple or crusted lesions are formed when vesicles develop, burst and discharge copious amount of senous fluid. This forms the characteristic ‘honey coloredstuck-on crusts’ (Bailey and Scotts et al 2007).

2.5.1.2 Cellulitis

This is a deeper infection of the cells. It may first appear as a red, swollen area that feels hot and tender to the touch (Bailey and Scotts et al 2007).

2.5.1.3Wound infections

Any skin wound that can be infected with *Staphyloccus aureus*, resulting in an abscess, cellulitis or both. When a sutured post-surgical wound becomes infected, it must be reported and treated (Bailey and Scotts et al 2007).

2.5.1.4 Abscesses

This can occur in any organ when the organism circulates in the bloodstream. These are usually called metastatic abscesses because they occur by the spread of bacteria from original site (Bailey and Scotts et al 2007).

2.5.2 Deep infections

These infections include osteoyelitis, arthritis, pneumonia, septicemia, meningitis, endocarditis, breast abscess, renal abscesses and abscesses in other organs (Bailey and Scotts et al 2007).

2.5.2.1 Osteoyelitis

This bone infection particularly occurs in children. The infection is usually due to hematogenous spread of organism, presenting locally with warm, swollen tissue over the bone and with systematic fever and shakes (Oryanetal 2014).

2.5.2.2 Pneumonia

Staphylococcus aureus is rare but severe cause of community acquired bacterial. Pneumonia is more common In hospitalized patients (Strohlb et al 2002). Community-acquired staphylococcal pneumonia is usually seen in patients that are recovering from respiratory infection especially by influenza virus. The violent, destructive, necrotizing pneumonia frequently causes effusions and ermphysema. In community, pneumonia cases primarily

occurred in children, but older age groups may also be affected (Francis et al 2005).

2.5.2.3 Septic arthritis

Invasion of the synovial membrane by *Staphylococcus aureus* resulting in joint inflammation. Symptoms typically include redness, fever, weakness, heat, headache and pain in a single joint associated with a decreased ability to move the joint. Onset is usually rapid (Horowitz et al 2011).

2.5.2.4 Acute Endocarditis

S. aureus typically causes acute endocarditis with damage to cardiac valves with the sudden onset of high fever, chills and myalgia, embolisation of vegetation to extracardiac sites and progresses to death within weeks if left untreated (Mohiyiddeen et al 2008).

2.5.2.5 Meningitis, Cerebritis and brain abscess

Patient with these disease show symptoms like high fever, severe headache, stiff neck, come and focal neurological signs (Levinson and Jawetz 1996).

2.5.2.6 Septicemia

It can be originated from any localized lesion, especially wound infection or as a result of intravenous drug abuse (Parija 2013).

2.5.3 Toxin-Mediated Diseases

2.5.3.1 Food Poisoning

Staphylococcal food poisoning is gastrointestinal illness caused by eating foods in which *Staphylococcus aureus* has multiplied and formed enterotoxin, characterized by a sudden start of nausea, vomiting, stomach cramps and diarrhea. The types of fish, meat, milk and milk products. The illness is usually self-limited, with recovery in a day or so. The illness is rarely fatal (Hennekinne et al 2012).

2.5.3.2 Toxic Shock Syndrome (TSS)

The toxin responsible for the disease is referred as toxic shock syndrome toxin (TSST-1). The disease is characterized by syndrome of high fever, confusion, headache, conjunctival reddening, subcutaneous oedema, vomiting and diarrhoea, and profound hypotensive shock in children and adults (Davis et al 1980). A strong association is found with the use of highly absorbent intravaginal tampons during menstruation, especially when these are used continuously and changed infrequently (Shands et al 1980).

2.5.3.3 Staphylococcal scalded skin syndrome (SSSS)

It is the cutaneous manifestation of infection with an exfoliatin-producing strain of *Staphylococcus aureus.* The disease is characterized by separation of the superficial layers of the skin by sideways pressure or the formation of bullae (blisters) as a result of the action of epidermolytic toxins. Scalded skin syndrome is commonly seen in young children (Kumar 2012).

2.6 Methicillin resistance *Staphylococcus aureus*

Methicillin resistant *Staphylococcus aureus* (MRSA) are strains of the *Staphylococcus aureus* that are resistant to the action of methicillin and related beta-lactam antibiotics (e.g. penicillin, oxacillin etc.) (Otto 2012). MRSA isolates are often multiple-resistant to commonly used antibiotimicrobial agents including aminoglycosides, chloramphenicol, ciprofloxacin, erythromycin and tetracycline (Greenwood et al 2004; Collier et al 1998; Ali et al 2007). Methicillin-resistant *Staphylococcus aureus* (MRSA) was first emerged as nosocomial pathogens in the early 1960s are of great concern to public health and highly reported in human clinical samples (Robinson et al 2009).

In recent years, strains of *S. aureus* have emerged that are resistant to virtually all antibiotics except Vancomycin. These have been called methicillin resistant *Staphylococcus aureus* (MRSA) strains, but the description “multiple resistant *S. aureus*” strains would be more appropriate because many MRSA strains are also resistant to macrolids, tetracycline, lineosamides, fluroquinolones, and aminoglycosides. Resistance to trimethoprim/sulfomethoxazole is also is also

becoming common in MRSA strains. In some hospitals, nearly 90% of *S. aureus* isolates are MRSA (Kaur et al 2015).

Methicillin resistant *Staphylococcus aureus* strains are not only a problem in hospital as distinct strains have emerged in community too. CA-MRSA strains have spread in community settings and have also entered healthcare facilities. Healthcare workers who are at interface between the hospital and the community may serve as agents of cross contamination of Hospital acquired MRSA (HA-MRSA) and Community acquired MRSA (CA-MRSA) (Khanal et al 2015).

2.7 Classification of MRSA

Taxonomy	Name
Domain:	Bacteria
Kingdom:	Eubacteria
Phylum:	Firmicutes
Class:	Baccilli
Order:	Bacillales
Family:	Staphylococcaceae
Genus:	*Staphylococcus*
Species*:*	*Staphylococcus aureus*
Subspecies:	Methicillin-resistant *staphylococcus aureus*

(Source: Bergey′s Manual of Determinative Bacterioloby, 1939)

2.8Types of MRSA

2.8.1 Community acquired MRSA (CA-MRSA), and

2.8.2 Hospital acquired MRSA (HA-MRSA).

2.8.1 Community acquired MRSA (CA-MRSA)

CA-MRSA is acquired by persons who have not been recently (within the past year) hospitalized nor had a medical procedure (such as dialysis, surgery, catheters). These infections manifest usually as skin infections, such as pimples and boils and occur in otherwise healthy people (Buckingham et al 2004). About 75 percent of CA-MRSA infections are localized to skin and soft tissue and usually can be treated effectively. However, CA-MRSA strains display enhanced virulence, spread more rapidly and cause more severe illness than traditional HA-MRSA infections, and can affect vital organs leading to widespread infection (sepsis), toxic shock syndrome and pneumonia (Parija 2013).

Community-associated methicillin-resistant *Staphylococcus aureus* has become an important threat to public health (Huh and Chung 2016). The growing number of community acquired infections caused by methicillin-resistant *Staphylococcus aureus* in children and healthy adult is a major problem (Rocha et al 2017). Outbreaks of community acquired MRSA infection are extremely rare (Borer et al 2002).

2.8.2 Hospital acquired MRSA (HA-MRSA)

Hospital-acquired MRSA (HA-MRSA) infection is acquired by persons admitted to hospitals for more than 48 hours or those have medical history of MRSA infections or colonization during previous admission. Common sites of HA-MRSA are surgical wound infections, urinary tract infections, and pneumonia. A number of factors have been found to associated with a higher risk for nosocomial acquisition of MRSA includes prolonged hospitalization, prolonged antimicrobial therapy using broad spectrum antibiotics, care in an intensive care unit, surgical procedures, having a surgical wound and intravenous (IV) line, severe underlying illness and close proximity to other ill patients who infected or colonized with MRSA (Boyce et al 2005).

In case of HA-MRSA, patients who already have an MRSA infection or patients as well as employee who carry the bacteria on their bodies but do not have symptoms (are colonized) are the most common sources of transmission (Tambic et al 1997). The main mode of transmission to other patients is through human hands, especially healthcare workers' hands. Hands may become contaminated with MRSA bacteria by contact with infected or colonized patients (Armin 2007).

2.9 Source and transmission of MRSA

MRSA is primarily transmitted from person to person by direct contact, usually from hand to hand of an infected or colonized individual. It can also be transmitted by sharing towels, cloths, athletic equipment, personal hygiene items, and public used bath and used equipment (Bassim et al 2005).

Droplet infection is another type of transmission which causes pneumonia and in such a case; the patient is infectious through droplet infections to the surrounding patients and health care workers. MRSA can colonize the skin, nose, blood, urine and throat (Bradley 2015).

2.10 Cell wall structure and molecular basis of methicillin resistance *Staphylococcus aureus*

The Staphylococcal cell is surrounded by peptidoglycan, that is composed of a series of short glycan chains of approximately 20 alternating N-acetylmuramic acid and beta-1, 4-N-acetylglucosamine residues. Attached to each N-acetylmuramic acid residue is a pentapeptide chain referred to as the stem peptide. The glycan chains in peptidoglycan are linked together via the last glycine residue of a pentaglycine cross-bridge attached to the L-lysine residue (position 3) on one stem peptide and the D-Ala residue (position 4) an another ((Rao 2009). Pentaglycine cross-bridges are performed in the cytoplasm by the FemX, FemA and FemB proteins, which attach the glycine residues to the L-lysine residue of the stem peptides of an adjacent glycan strand. The cross-linking or transpeptidation reactions take place on the external surface of the cytoplasmic membrane in a reaction catalyzed by penicillin-binding proteins

(PBPs). There are four PBPs in *S. aureus,* PBP1, PBP2, PBP3, and PBP4 (fuda et al 2005). The transglycosylase and transpeptidase domains are spatially well separated. Carboxypeptidases, also members of the PBP family, remove the terminal D-Ala of the peptidoglycan peptide stems. PBPs have two protein domains, one involved in transpeptidation (cross-linking) the other involved in transglycosylation (extending the glycan chain). The beta-lactam antibiotics, which resemble the terminal D-alanyl-D-alanine bond of the stem peptide, inhibit the transpeptidation domain of PBPs (and carboxypeptidase activity of low molecular weight PBPs) thus interfering with the cross-linking reaction. Without cross-linking of the peptidoglycan, the cell wall becomes mechanically weak, some of the cytoplasmic contents are released and the cell dies (Koch et al 2003; Waxman et al 1983).

2.11 Prevalence of MRSA

In a study carried out at the Department of Microbiology and Department of Pathology, Iran, out of 175 strains of *S. aureus,* 53 were found to be resistant to methicillin using E-test where as disk diffusion method using oxacillin or cefoxitin showed 52 strains to be methicillin resistant (Rahbar et al 2006).

In the study carried out at the department of microbiology, B.P. Koirala Institute of health Science, Dharan, 78 out of 300 strains of *S. aureus* were found to be Methicillin using disc diffusion method (Baral et al 2011).

In another study, out of 210 various clinical samples collected and analyzed, 65 were found to be *S. aureus* and among which again, 19 strains were found to be methicillin using Kirby bauer disc diffusion method (Thapa et al 2004).

In contest of India, various degree of methicillin resistance strains of *S. aureus* has been obtained. In a study, out of 13975 isolates of *S. aureus,* 5864 were MRSA and out of 12335 isolates, 5133 were MRSA in 2008 and 2009 respectively (Joshi et al 2013).

In another study done at tertiary care hospital in Agra, North India showed 379 strains to be MRSA from 1163 *S. aureus* isolates collected handling altogether 11496 different clinical samples (Goyal et al 2013).

In another study, out of 783 S. *aureus* strains, 301 showed methicillin resistant and among which again, 217 strains were found to be multidrug- resistance (Tiwari et al 2008)

In the study carried out at the clinical microbiology lab of KMC Teaching Hospital, 29 out of 111strains of *S. aureus* were found to be MRSA. Out of 29 MRSA isolates, 22 were found to be MDR strains (Pandey et al 2012).

2.12 Treatment

Most β-lactam antibiotics are ineffective against HA-MRSA. linezolid, tetracycline, trimethoprim-sulfamethoxazole, or Vancomycin exhibit an excellent anti staphylococcal activity.

Currently, the number of effective antibiotics used to treat MRSA infectios is dwinding. Treatment options can vary depending on the types of infection and other patient factor. Overall, the standard antibiotic used to treat MRSA infection is vancomycin (Gould et al 2012).

2.13 Multi-Drug Resistance

Multi drug resistance bacteria are those, which shows resistance to two or more classes of antimicrobial agents (CDC 2006). Multi-drug resistance is defined as resistance to at least two antibiotics of different classes including aminoglycosides, chloramphenicol, tetracyclines, and/or erythromycin (Pandey et al 2012).

Multi-drug resistance to antibiotics represents a global health challenge that resulting increase morbidity and mortility. methicillin-resistant *Staphylococcus aureus* (MRSA) are problematic because they apart from their SCCmec genotype often carry resistance determinants to other important antibiotics for treatment of *Staphylococcus aureus* infections (Dosler et al 2011). Methicillin resistance *Staphylococcus aureus* is resistant not only to methicillin (which was developed to fight against penicillinase-producing *S. aureus*) but usually also to aminoglycosides, macrolides, tetracycline,

chloramphenicol, and lincosamides (lencastre et al 2007).For these reasons Treatment of methicillin resistance *Staphylococcus aureus* infections usually include treatment with glycopeptides antibiotics (vancomycin) and oxazolidinones such as linezolid. Vancomycin resistance was first reported in enterococci in 1988 and the First vancomycin resistance *Staphylococcus aureus* (VRSA) was characterised in 2002 (Weigel et al 2007).

Multidrug resistance in bacteria may be generated by one of two mechanisms. First, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Second, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs (Nikaido 2009).

2.14 D test

Erythromycin and Clindamycin represent two distinct classes of antimicrobial agents that act by binding to the 50s ribosomal subunit of bacteria to inhibit its protein synthesis. Erythromycin resistance in *Staphylococcus aureus* is by diverse mechanisms. The resistance to macrolide can aries by efflux mechanism, classically mediated by msrA gene or via erm gene encoding for streptogramin (MLSB resistance) (Laclercq 2002)). This resistance mechanism can be constitutive, where rRNA methylase is always produced ($_{c}MLS_{B}$) or can be inducible where methylase is produced only in the presence of an inducing agent ($_{i}MLS_{B}$). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive *in vitro* when not placed adjacent to each other. In vitro *Staphylococcus aureus* isolates with constitutive resistance are resistant to both erythromycin and clindamycin (Drinkovic et al 2001; Deotale et al 2010). D-test is a simple disc diffusion test which is used to study the macrolide lincosamide streptogramin resistance (MLSB), both constitutive and inducible as well as macrolide streptogramin resistance (MSB) in *Staphylococcus aureus* (Shrestha and Rana 2014).

In the study conducted by Sah et al (2015) reported the prevalence of inducible and constitutive clindamycin resistance was found 12.1% and 7.9% respectively. Govindan et al (2014) found 11.6% *Staphylococcus aureus* strains were showing inducible clindamycin resistance and other 88.4% strains were not showing positive D test. In North india, Gupta et al (2009) reported a higher percentage of inducible and constitutive clindamycin resistance in MRSA (20% and 46%) compared to MSSA (17.3% and 10%) respectively. Azap et al (2005) in Turkey reported higher inducible clindamycin resistance in MRSA (5.7%) than MSSA (3.6%) respectively.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

A complete list of materials, equipment, media, chemicals, reagents and antibiotics used in this study are listed in Appendices IV.

3.2 Methods

A cross-sectional study was carried out from September to December 2018 at IFCH, Kathmandu, Nepal, in which 227 *Staphylococcus aureus* isolated from 961 various clinical specimens. The antibiotic sensitivity test was done by using Kirby-Bauer disc diffusion method and the prevalence of MRSA was found using cefoxitin disc.

3.2.1 Sample collection

Most of the clinical samples such as pus/swab from wound, throat as well as blood were collected aseptically by experienced medical officers, nurses or laboratory technicians. 1ml (neonates) and 5ml (children) of blood was collected and it was inoculated into Brain Heart Infusion (BHI) broth at the ratio of 1:10 (blood: broth). Urine was collected by the patients themselves.

All the samples were labeled appropriately with patient's identification number. The samples were processed immediately as soon as possible. In case of delay, they were stored at the refrigerated temperature.

3.2.2 Sample processing

The *Staphylococcus aureus* isolates were identified based on Cheesbrough 2012. After receiving and labeling the samples, the specimens were processed (i.e., Gram staining for microscopic observation and microbial culture) in the microbiology laboratory within 2 hour of the collection. Blood specimens were transferred aseptically in BACTEC^{TM} blood bottles. All the bottles were incubated at 37ºC in the BACTEC^{TM} system for three consecutive days.

During the incubation periods, any bottles detected as positive by the BACTEC^{TM} system were removed and only positive sample blood were sub cultured on blood agar and incubated at 37ºC for 24 hours. Samples such as pus/wound swab, urine were inoculated directly into blood agar and incubated at 37ºC for 24 hours. The growth obtained in BA plates were further cultured into Mannitol Salt Agar (MSA). Colonies formed in MSA were picked and processed for Gram staining. Only cocci were processed for the identification of *S. aureus*. For conformational identification of *S. aureus,* catalase test, coagulase (slide and tube) test were performed.

3.2.3 Bacteriological identification of *S. aureus*

S. aureus colonies were identified and confirmed on the basis of colony morphology and gram′s staining and biochemical properties like catalase production test and coagulase production test by slide and tube methods. The colonies with golden yellow pigmentation on mannitol salt agar and β-hemolytic on blood agar; Gram-positive cocci in grape-like cluster in Gram staining and catalase and coagulase tests positive were identified as *S. aureus* (Forbes et al 2007).

The procedures for Gram′s staining, Catalase test, Coagulase test for the the confirmatory identification of *Staphylococcus aureus* are listed in Appendices VII.

3.2.4Antibiotic susceptibility testing

All identified *S. aureus* isolates from different clinical samples were subjected to *in-vitro* antibiotic susceptibility test by Kirby-Bauer disc diffusion method as recommended by CLSI guidelines (2012). Fresh colonies were selected and transferred into NB to obtain turbidity equivalent to 0.5 Mcfarland barium sulfate standards ($1.5x10^{8}$CFU/ml). MHA plates were inoculated with sterile cotton swabs then antibiotics were placed with sterile forceps and allowed to stand at room temperature for 15 minutes for pre-diffusion then incubated at 37ºC for 16-18 hours. The zone of inhibition was interpreted as susceptible, intermediate and resistant according to CLSI ″Diffusion Supplemental Table". The antibiotics used in this study were amikacin (30µg), cefoxitin (30µg),

ciprofloxacin (5µg), chloramphenicol (30µg), co-trimoxazole (25µg), clindamycin (2µg), erythromycin (15µg), gentamicin (10µg), meropenem (10µg), tetracycline (30µg), vancomycin (30µg), nitrofurantoin (300µg) and erythromycin (15µg), clindamycin (2µg) discs (Hi-media-India) at 15mm apart were also used on same plate for the detection of inducible clindamycin resistance as per CLSI guidelines. Isolates resistant to three or more classes of antibiotics were considered MDR (Nair et al. 2013).

Clindamycin resistance was detected as:

1. Inducible resistance phenotypes ($_{i}MLS_{B}$): *Staphylococcal* isolate showing resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D-shaped zone.
2. Constitutive resistance phenotypes ($_{c}MLS_{B}$): this phenotype was labeled for those *Staphylococcal* isolates, which showed resistant to both erythromycin and clindamycin
3. MS phenotype: *Staphylococcal* isolate exhibiting resistance to erythromycin (zone size ≤13 mm) while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin was labeled as having this phenotype.

The standard zone size at which the organism is considered resistant, intermediate or susceptible is given in the zone-size interpretative chart (Appendix-VIII).

3.2.5 Quality control for test

Quality control for all the tests is the most important factor for the data to be reliable. Hence, the quality control was maintained throughout this study.

To maintain quality control of chemical reagents, antibiotics and media, they were prepared and stored as per instructions provided by the respective companies. Similarly, antibiotics were also stored as recommended by the

manufacturing company. Antibiotic discs were stored at refrigerator temperature.

3.2.7 Data analysis

All the raw data collected in the microbiology laboratory was documented and tabulated. The statistical analysis software (SPSS) was used to calculate a p-value by using Chi-square test. A p-value of less than or equal to 0.05 was considered to be statistically significant ($p \leq 0.05$)

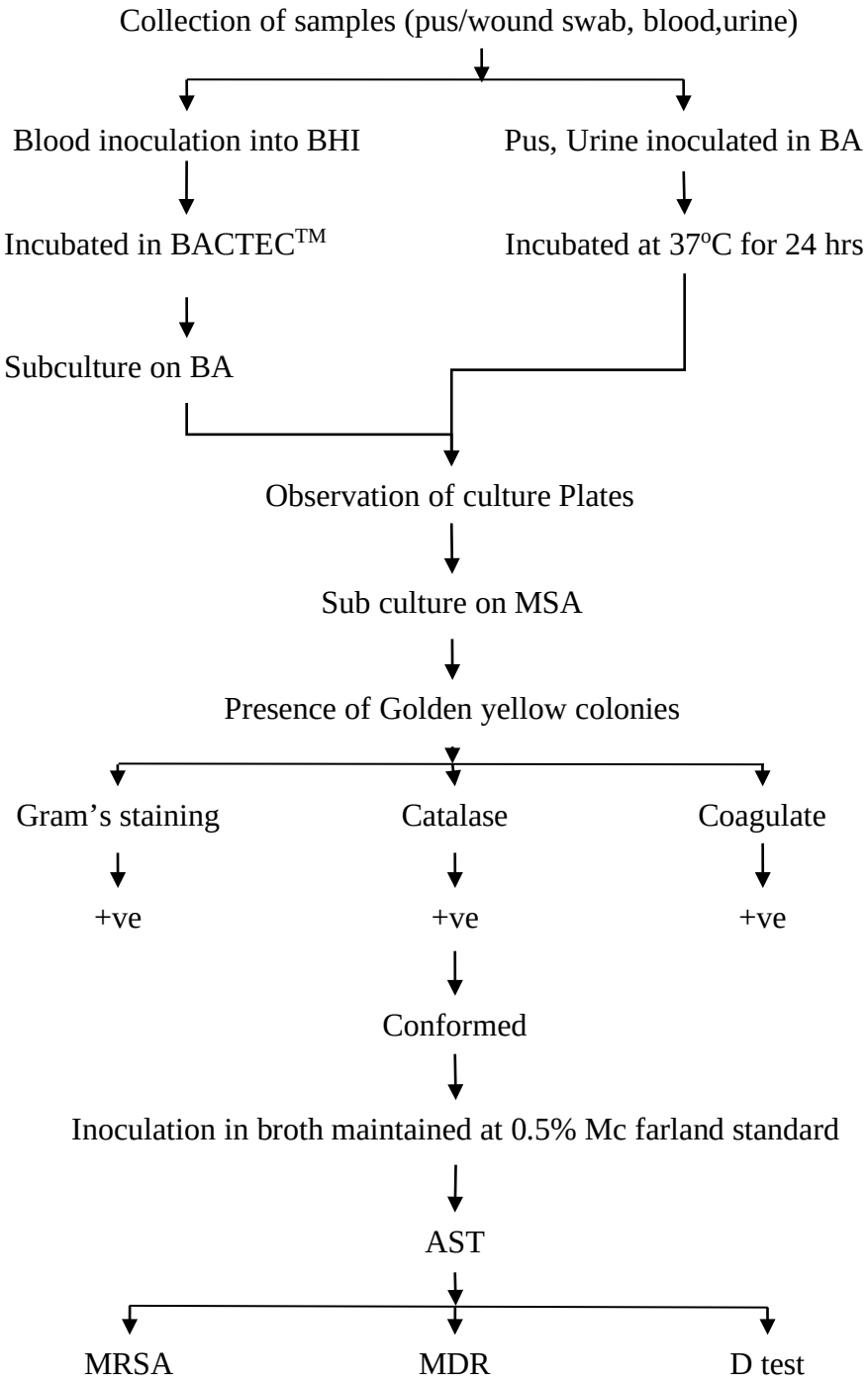


Fig: flow chart of isolation and identification of Staphylococcus aureus from clinical specimens (Cheesbrough 2012)

CHAPTER IV

RESULTS

4.1 Study population

The study was done in Microbiology Lab of International Friendship Children′s Hospital; study period was from September to December 2018. Out of total 961 different clinical samples, 573 (59.62%) samples were from male and 388 (40.37%) samples were from female.

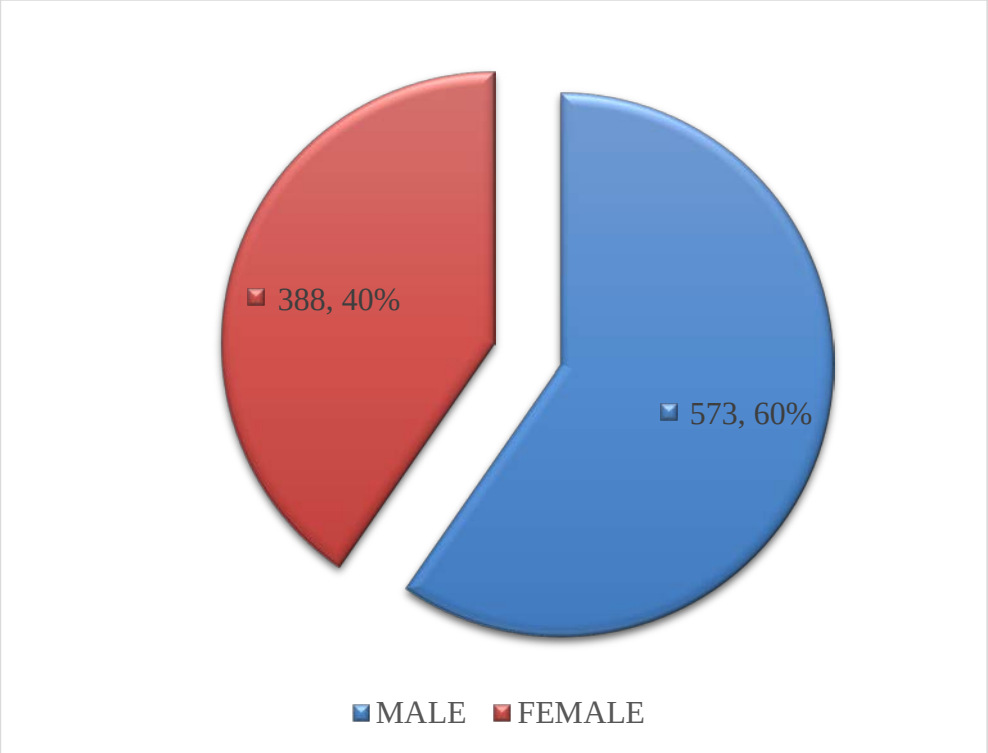


Figure 1: Study population

4.2 Number of *S. aureus* isolated from different clinical specimens

Out of 227 *S. aureus*, highest number of isolates was from pus and wound swab (72.7%), followed by blood (18.9%) and least number from urine (8.4%).

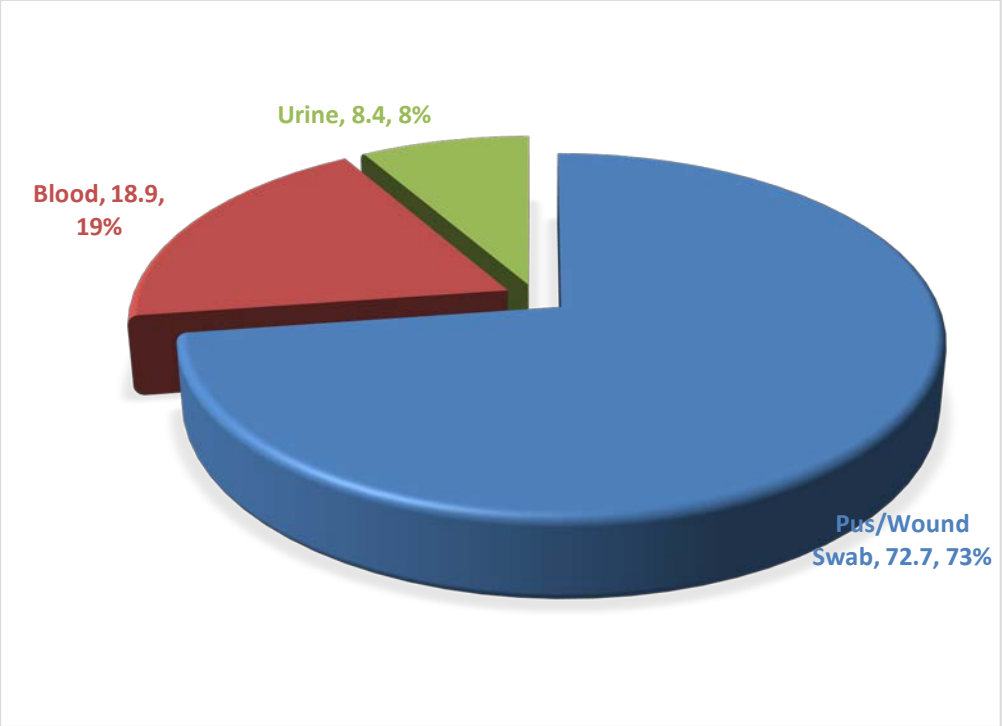


Figure 2: Percentage of *S. aureus* isolated from different clinical specimens

4.3 Distribution of *S. aureus* in different age groups and types of patients.

Out of 961 clinical samples processed, bacterial growth was detected in 418(43.4%) samples only. Among all bacterial isolates, *S. aureus* were isolated in 227 samples, comprising 127 (55.9%) isolates from inpatients and 100(44.1%) from outpatients. Among outpatients, 29 strains (12.8%) were isolated from age group 1 year- below 3 years, which was the highest in number. Likewise, maximum number of *S. aureus* strain isolated from admitted patients was isolated from the age group of 1 year –below 3 years. The distribution of *S. aureus* in different age group and types of patients was statistically insignificant (p=0.308).

Table 1: Distribution of *S. aureus* in different age groups and types of patients

AGE	AGE RANGES	TYPE OF PATIENTS						
		Outpatient		**Inpatients**		**Total**		**P-value**
		No	**%**	**No**	**%**	**No**	**%**	
NEONATE	Newborn up to first 28 days	13	5.7	18	7.9	31	13.7	
INFANT	28 days-below 1 year	16	7.0	22	9.7	38	16.7	
TODDLERS	1 year-below 3 years	29	12.8	44	19.4	73	32.2	
PRESCHOOL	3 years-below 5 years	25	11.0	18	7.9	43	18.9	0.308
SCHOOL	6 years-10 years	14	6.2	16	7.0	30	13.2	
ADOLESCENT	11years-15years	3	1.3	9	4.0	12	5.3	
TOTAL		100	44.1	127	55.9	227	100	

4.4 Distribution of *S. aureus* according to gender of patients

Out of 961 samples taken, 227 were *Staphylococcus aureus* positive isolates whereas 142 isolates were obtained from male and 85 isolates were obtained from female respectively.

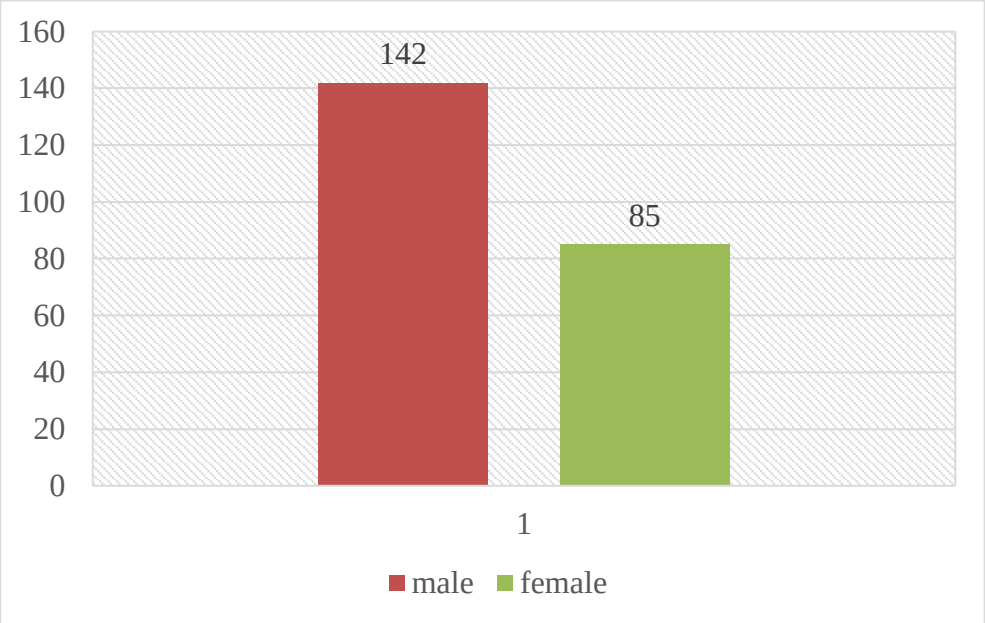


Figure 3: Distribution of *Staphylococcus aureus* according to gender of patients.

4.5 Distribution of MRSA in clinical specimens

In this study, higher number of MRSA was isolated from pus/wound swab samples (86.2%) and least number was from urine samples (4.6%) respectively. The prevalence of MRSA isolates from blood samples was 9.2%. However, the association between the MRSA occurence and clinical specimens was found to be statistically significant (p=0.000).

Table 2: Distribution of MRSA in clinical specimens

Samples	MRSA		MSSA		Total		p-value
Pus/wound swab	94	86.2%	71	60.2%	165	72.7%	
Blood	10	9.2%	33	28%	43	18.9%	0.000
Urine	5	4.6%	14	11.9%	19	8.4%	
Total	109	100%	118	100%	227	100%	

4.6 Distribution of MRSA in different age group and gender

Out of 227 *S. aureus* isolates, 109 were MRSA positive isolates out of which 62 (56.7%) were from males and 47 (43.1%) were from females. In this study, highest percentage of MRSA was found in toddlers' group (28.4%) which is followed by infant age group (21.1%) and preschool age group (19.3%) respectively. The prevelence of MRSA in different age group and gender was statistically significant (p=0.000).

Table 3: Distribution of MRSA in different age group and gender

AGE	MALE		FEMALE		TOTAL		P-VALUE
	NO.	%	NO.	%	NO.	%	
NEONATE	15	24.2%	0	0.0%	15	13.8%	
INFANT	18	29.0%	5	10.6	23	21.15	
TODDLERS	11	17.7%	20	42.6%	31	28.4%	0.000
PRESCHOOL	12	19.4%	9	19.1%	21	19.3%	
SCHOOL	5	8%	8	17.0%	13	11.9%	
ADOLESCENT	1	1.6%	5	10.6%	6	5.5%	
TOTAL	62	100%	47	100%	109	100%	

4.7 Ward- wise distribution of MRSA

The cefoxitin disc diffusion test showed that out of 227 *S. aureus* isolates, 109 (48%) and 118 (52%) were identified as MRSA and MSSA respectively. Moreover, we found that among these 47 (43.1%) and 53 (44.9%) of MRSA and MSSA respectively, were isolated from outpatients. The analysis further showed that the incidence of MRSA and MSSA isolations (56.9%) and (55.1%), respectively, were slightly higher in inpatient than in outpatient samples. However, the association between the MRSA occurrence and inpatients was found statistically insignificant ($p = 0.783$). These data clearly show that frequency of MRSA in inpatients is higher compared with outpatient.

Table 4: Ward- wise distribution of MRSA and MSSA

Methicillin Susceptibility	Outpatients	Inpatients	Total	P-Value
MRSA	47 (43.1%)	62 (56.9%)	109 (100%)	
MSSA	53 (44.9%)	65 (55.1%)	118 (100%)	0.785
TOTAL	100 (44.1%)	127 (55.9%)	227 (100%)	

4.8 Antibiogram of *S. aureus*

All the total 227 strains of *S. aureus* isolated were tested with specific antibiotics by using Kirby Bauer disc diffusion method. Antibiotic resistance pattern of those 227 *S. aureus* strains showed that the highest number of isolates was resistant to erythromycin (62.6%) followed by co-trimoxazole (49.8%). Likewise, highest number of strains was susceptible to chloramphenicol (78.4%), followed by meropenem (76.2%) and clindamycin (74%).

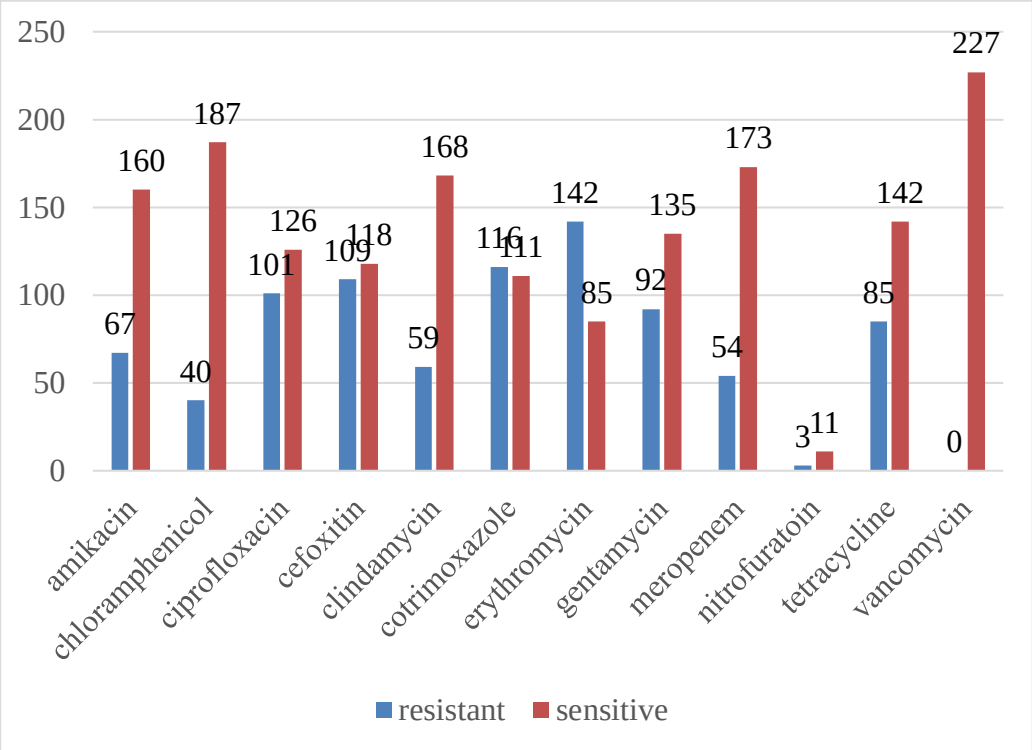


Fig 4: Antibiotic susceptibility pattern of *Staphylococcus aureus*

4.8.1 Antibiogram of methicillin-resistant *Staphylococcus aureus* and methicillin sensitive *Staphylococcus aureus*

The antimicrobial resistance (AMR) patterns of MRSA and MSSA isolates against antimicrobial agents are summarized in Table 4.5. More than 25% of MRSA isolates were resistant to erythromycin (71.6%), ciprofloxacin (59.6%), cotrimoxazole (56.9%), gentamycin (47.7%), tetracycline (45%), clindamycin (34.9%) and meropenem (32.1%). While MSSA isolates showed resistance against erythromycin (54.2%). The rest of the antibiotic showed less than 50% resistance toward the isolated MSSA. All the organisms were sensitive to Vancomycin.

Table 5: Antibiogram of methicillin-resistant Staphylococcus aureus and methicillin sensitive Staphylococcus aureus

Antibiotics	MRSA				MSSA				P-Value
	Sensitive		Resistant		Sensitive		Resistant		
	No	%	No.	%	No.	%	No	%	
Amikacin	88	80	21	19.3	72	61	46	39	0.001
Chloramphenicol	86	78.9	23	21.1	92	78	26	22	0.864
Cefoxitin	0	0	109	100	118	100	0	0	0.000
Ciprofloxacin	44	40.4	65	59.6	90	76.3	28	23.7	0.000
Clindamycin	71	65.1	38	34.9	97	82.2	21	17.8	0.003
Cotrimoxazole	47	43.1	62	56.9	67	56.8	51	43.2	0.040
Erythromycin	31	28.4	78	71.6	54	45.8	64	54.2	0.007
Gentamicin	57	52.3	52	47.7	78	66.1	40	33.9	0.034
Meropenem	74	67.9	35	32.1	99	83.9	19	16.1	0.005
Nitrofuratoine	3	75	1	25	8	80	2	20	0.837
Tetracycline	60	55	49	45	82	69.5	36	30.5	0.025
Vancomycin	109	100	0	0.0	118	0	0	0	-

4.8.2 Multidrug resistant of *S. aureus* (MDR)

82 (75.2%) MRSA isolates were found to be MDR strains. Only 21 (17.6%) among 118 MSSA strains were MDR strains.

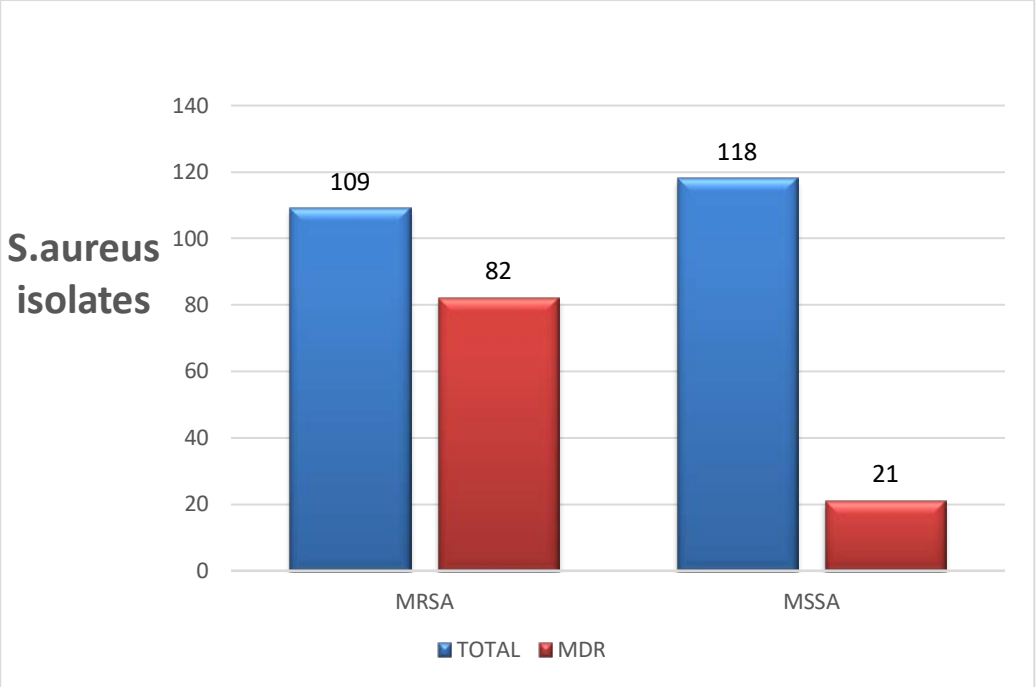


Fig 5: Multidrug resistant of *S. aureus*

4.9 Inducible clindamycin resistance

Among 227 *S. aureus* isolates, inducible macrolide-lincosamide-streptogramin B (MLS_B) resistance, constitutive MLS_B and MS_B was found thirthy three, fifty-nine, fifty respectively. Of the 109 MRSA isolates, 20.2% (n = 22/109) had inducible MLS_B resistance. Constitutive MLS_B was observed in 34.9% (n = 38/109) of MRSA isolates. Both $_iMLS_B$ and $_cMLS_B$ phenotypes predominated in MRSA strains (p value = 0.004)

Table 6: Inducible clindamycin resistance

Phenotype	Erythromycin	Clindamycin	D-test	MRSA		MSSA		P-value
$_iMLS_B$	R	S	+ve	22	20.2%	11	9.3%	0.004
$_cMLS_B$	R	R	-ve	38	34.9%	21	17.8%	
MS	R	S	-	18	16.5%	32	27.1%	

CHAPTER V

DISCUSSION

Staphylococcus aureus is one of the most common pathogens isolated in most microbiology laboratories (Ansari et al 2014). It remains as the normal flora on different parts of the body of human beings throughout the life but may cause a variety of diseases ranging from benign local skin infections such as folliculitis, pustules, boils, carbuncules, impetigos and infections of wound to the life-threatening diseases like pneumonia, meningitis, osteomyelitis, endocarditis and bacteraemia in immunocompromised patients. It may cause several toxin mediated infections and diseases such as food poisoning, skin scalded syndrome, and toxic shock syndrome (Forbes et al 2007). *S. aureus* is one of the common hospitals acquired organisms which accounts for the most of the infections (Sah et al 2013).

MRSA is an important group of multi drug resistant organisms which are responsible for increasing the rate of morbidity and mortality (Wolk et al 2009). *S. aureus* infections are a significant clinical problem in medical practice as they show resistance to the commonly used first line drugs. In this study, the occurrence of *S. aureus* was studied among the pediatric patients visiting IFCH, using various types of sample. All the samples with clinically detected *S. aureus* may serve as a reservoir of MRSA, which may transmit the infection in a community. Thus, there is a chance of a rapid increase in the development of community-acquired MRSA infection.

In the present study, 227(43.8%) *S. aureus* isolates were found out of 518 culture positive samples. In most of the study, the frequency of infection caused by *S. aureus* reaches the peak. The results are similar to those reported by Regmi et al (2020), with growth positivity of 33.8%. Kumari et al (2008), in their report, have mentioned that out of total 98-gram positive isolates, *S. aureus* occupied 83.67%. Similar results were obtained in a study by Karkee *et al* (2008). The results are accordance to those reported by Mukhiya et al (2012) with growth positivity of 17.4%.

In our study, highest percentage of *S. aureus* strains were isolated from pus/wound swabs 165 (76.7%) followed by blood 43 (18.9%) and urine 19 (8.4%) respectively. Antibiogram of total 227 *S. aureus* strains showed 48% (n=109) were methicillin resistant and 118 (52%) were methicillin sensitive S. aureus by the use of cefoxitin disc test. Highest percentage of MSSA were isolated from pus/wound swabs (60.1%), followed by blood (28%) and urine (11.9%) respectively. In a study by Kumari et al (2008) in a tertiary-care hospital in Eastern Nepal also found the highest number of *S. aureus* isolates (64%) from pus and wound swab samples. our findings also correlate with the findings of Sapkota et al (2006) who reported 53.4% of *S. aureus* isolates were from pus swab from wound. Here, the result shows that children are more prone to *S. aureus*-associated infection. This might be due to their low immunity or high chances of contact with an infectious agent. Our findings also show that *S. aureus* is a major cause of pyogenic infections. The skin and soft tissue (SST) was the most common site for the infection, likely because this bacterium normally inhabits the skin. Baral et al (2011) also found the higher percentage of S. aureus isolate from pus (74%) followed by blood (14%) and urine (2.66%). Likewise,

In context to *S. aureus* isolates, in overall, the highest positivity of 32.2% (73/227) was observed in the age group of 1 year-below 3years followed by 18.9% in the age group of 3 years –below 5 years and 16.7% was observed in age group of 28 days- below 1 years respectively. Lowest positivity of 5.3% was observed in age group of 11-15 years. The prevalence of *S. aureus* among different age group was not statistically significant, p=0.308.

In our study, higher prevalence of *S. aureus* was isolated from male 142 than in female 85. The prevalence of *S. aureus* in two gender was not found statistically significant, p=0.090.

In our study, when comparing the number and percentages of *S. aureus* in the Inpatient Department and Outpatient Department, the rate of *S. aureus* was higher in inpatients (55.9%) compared with outpatients (44.1%). Our result is in accordance with Baral et al (2011) in Nepal. They also reported the higher prevalence of *S. aureus* (75%) among the inpatient setting as compared with

(23%) in outpatients. Likewise, if we look at the distribution of MRSA isolates in different types of our patients, we found them to be 62 (27.31%) inpatients and 47 (20.70%) outpatients out of a total 109. Sanjana et al (2010) also reported the higher prevalence of MRSA among the inpatient setting, accounting for 86 (62.3%) as compared with 52 (37.7%) in outpatients. This higher occurrence of MRSA among inpatients could be due to various hospital associated risk factors such as prolonged hospital stay, hospital environment, antibiotic treatment, underlying immune-compromised condition, instrumentation and other invasive procedure, which predispose patients to MRSA acquisition. Our report indicates high prevalence of MRSA among inpatients compared to out-patients which contrasts with findings of Khanal et al (2013) who reported higher incidence of *S. aureus* infection in the outpatients' patients as compared to inpatients. The prevalence of MRSA in inpatients and outpatients was not statistically significant, p=0.783.

In this study out of 109 MRSA, majority of them (86.2%) were isolated from pus/wound swabs followed by blood (9.2%) and urine (4.6%) respectively. our finding is also in agreement with the reports by Saikia et al (2009) and Goyal *et al* (2002) from India, who found 46.67% and 66.03% MRSA from pus and wound swab respectively. The prevalence of MRSA among different clinical sample was statistically significant, p=0.000.

In our study higher frequency of MRSA was isolated from males than in females, 62 (56.77%) from males and 47(43.11%) were from females. The present study showed the opposite variation with the study conducted by Lama et al. (2018) showed female patients (44.8%) were more predisposed than males (40.3%). Study conducted by Boucher and Corey (2008) showed high incidence of MRSA from males (64.4%) and females (35.6%). Prevalence of MRSA in male was higher than in female according to previous study done by Khanal et al 2010. Hence, higher prevalence of MRSA was seen in males as compared with females.

In male, prevalence of MRSA was highest with 18 (29%) in age group 28 days-below 1 years followed by 15 (24.2%) from new born upto first 28 days. In female, the prevalence of MRSA was highest in the age group of 1-below 3

years of age with 20 (42.6%) positive samples. However, the prevalence of MRSA among different age group and gender was statistically significant, p=0.000.

The present study reports the MRSA prevalence rate of 48%, which was similar with the findings by Rajbhandari et al 54.9% MRSA isolates at Bir Hospital. The prevalence of MRSA in Nepal ranges from 39% to 69% (Kumari et al 2008: Rijal et al 2008; Tiwari et al 2009; Khanal et al 2010; Mukhiya et al 2012) which shows similar result to our study. Lower prevalence was also reported by Subedi and Brahmadathan (15.4%) and Baral et al (26%). The difference in rates of isolation of MRSA in different studies might be due to different detection method, difference in hygienic conditions maintained in different hospitals, healthcare facilities, efficacy of infection control practices and antibiotic usage that vary from hospital to hospital (Kshetry et al 2016; Baral et al 2011).

In this study, Among 227 *S. aureus* isolates, inducible macrolide-lincosamide-streptogramin B (MLS_B) resistance, constitutive MLS_B, and MS_B was found thirty three, fifty-nine, fifty respectively. Of the 109 MRSA isolates, 20.2% (n = 22/109) had inducible MLS_B resistance. Constitutive MLS_B was observed in 34.9% (n = 38/109) of MRSA isolates. In this study, both the percentage of inducible and constitutive clindamycin resistance were significantly higher in MRSA (20.2% and 34.9%) than MSSA (9.3% and 17.8%) respectively (p=0.000). The result of the present study correlates with the finding of the study conducted by Ansari et al (2014) where an inducible clindamycin resistance was observed in 12.4% of the isolates. The finding of this study was higher than Adhikari et al (2017) where the inducible clindamycin resistance was found in 10% of *S. aureus* isolates. Gadepalli et al (2006) found that constitutive resistance is significantly higher than inducible resistance in both MRSA and MSSA. In MRSA constitutive and inducible resistance was 38% and 30% whereas in MSSA constitutive and inducible resistance was 15% and 10% respectively. Clindamycin, one of the drugs of choice for some *Staphylococcal* infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients (Drinkovic et al 2001).

As use of clindamycin for treatment of Staphylococcal infection may result in treatment failure, clindamycin should not be used for treatment of such infection; rather it should be used only for treatment of the infections caused by bacteria which are negative for inducible resistance (D-test positive) and it has been suggested that inducible clindamycin resistant strains should be reported as clindamycin resistant (Prabhu et al 2011; CLSI 2013). Avoiding the use of clindamycin for the treatment of infections caused by erythromycin resistant strains also omits the chance of treatment failure (Fiebelkorn et al 2003).

Over the course of time, *S. aureus* has developed resistance to different, conventionally used antibiotics. All the MRSA isolates were resistant to more antibiotics as compared with MSSA isolates. Significant difference (P-value <0.05) was observed in case of amikacin, cefoxitin, ciprofloxacin, clindamycin, co-trimoxazole, erythromycin, gentamicin, meropenem and tetracycline. However, the difference observed in case of chloramphenicol and nitrofuratoin was statistically insignificant (P-value > 0.05).

Antimicrobials such as chloramphenicol and Meropenem with resistance less than 25% could be used against of Staphylococcal infection. This study showed *S. aureus* were 100% sensitive towards Vancomycin same results were reported by Boncompion et al (2017) in Argentina and Khanal et al (2015) in Western Nepal and Khatri et al (2017). However, Vancomycin-intermediate and Vancomycin-resistant *S. aureus* (VISA and VRSA) strains have been reported from various part of the world (Menezes et al 2008; Tiwari et al 2006).

MRSA strains were more resistant to all antibiotics than MSSA strains except for vancomycin Because of the resistance of MRSA to all commonly used antibiotics, it is necessary to test newer group of antibiotics such as vancomycin and teicoplanin routinely MRSA strains are often multidrug resistance. Antibiotic susceptibility pattern of Methicillin resistant *S. aureus* showed that Vancomycin was 100% sensitive followed by amikacin (80.7%), chloramphenicol (78.9%), nitrofuratoin (75%), meropenem (67.9%),

clindamycin (65.1%), tetracycline (55%), and gentamicin (52.3%). Nitrofuratoin is used only in urine samples.

MRSA isolates revealed high resistance towards erythromycin (71.6%). Resistance to quinolones (ciprofloxacin) was also high (59.6%) in this study. In the study reported by Saikia et al (2009), the resistant rate was also high (87.5%) in Assam but the same study showed a high resistance pattern of erythromycin (72.7%) and ciprofloxacin (45.4%). 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. The rapid emergence of ciprofloxacin is probably due to the indiscriminate and empirical use of these drugs.

The multi-drug resistant is a particular characteristic of the methicillin-resistant *S. aureus* strains. It has added to the burden of hospital personnel to control infection associated with MDR-MRSA. Present study showed high rate of MDR strain among MRSA isolates (75.2%). Only 7.6% among 118 MSSA strains were MDR strains. Studies conducted In Nepal, 78% and > 65% of multi drug resistant MRSA strains have been reported in two different regions by Subedi et al (2005) and Kumari et al (2008) respectively. whereas in the neighboring country India, the burden of such strains has ranged from 23.2%, to 32%, to 63.6% (Majumder et al 2001; Anupurba et al 2003; Rajaduraipandi et al 2006). Though these MDR strains are not found with additional virulence properties, their characteristic multidrug resistance restricts the options available to treat infections caused by this organism (Voss et al 1995).

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

The occurrence of MRSA was found to be 109 (48%) out of a total 227 *S. aureus* isolates. The highest numbers of MRSA isolates were found in the toddler age group and inpatients. Methicillin susceptibility of *S. aureus* was found higher in males than females.

From this study, it could be concluded that 75.2% of MRSA strains and 7.6% of MSSA strains were multi drug resistant (MDR), which is the significant public health problem in context of Nepal, indicating the high risk of staphylococcal infections in our context. MRSA shows resistance to antibiotics except than Vancomycin. These studies clearly indicated about the appropriate steps to be taken to reduce MRSA and MDR strains in hospital settings to minimize the staphylococcal infection

The study showed the MRSA occurrence is prevalent in pediatric patients. This corroborates the findings of previous researchers as discussed. MRSA infection is still one of the most life-threatening infections in hospitals. Therefore, regular surveillance of MRSA should be carried out in all hospital settings. In addition, restriction of the indiscriminate use of such antibiotics may be an effective strategy to control AMR. We should discourage empirical therapy practices, instead considering microbiological test report. Present study conclusively shows that vancomycin remains the first choice of treatment for MRSA infection. To preserve its value, use of vancomycin should be limited to those cases where there are clearly needed

6.2 Recommendations

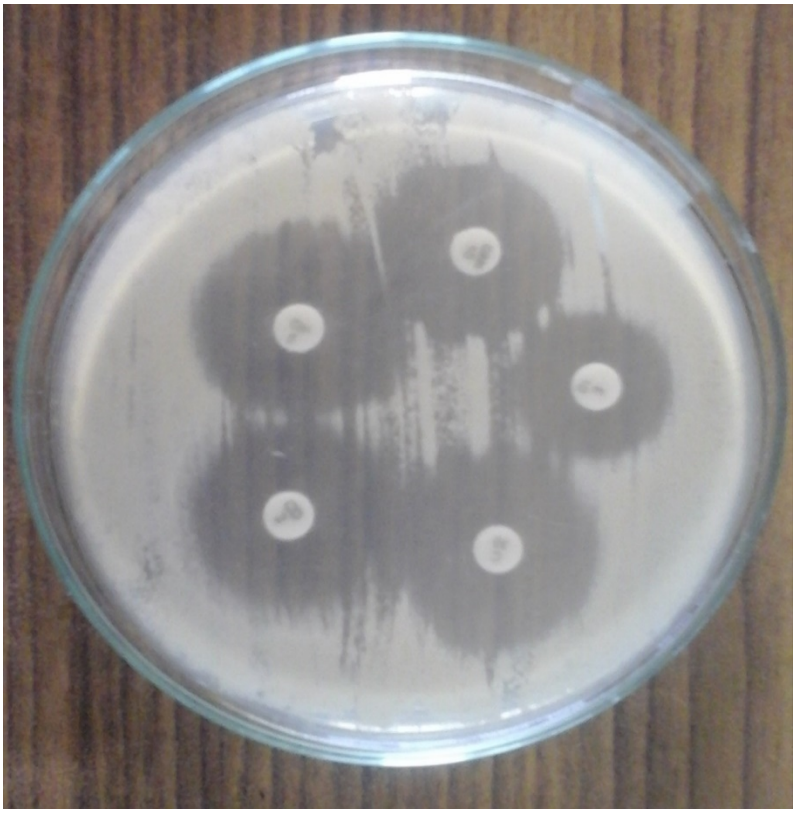
- The regular surveillance in MRSA transmission is required to prevent MRSA infections.
- Unauthorized distribution of antibiotics without prescription of doctor has increased the prevalence of MDR isolates. Hence, such irrational use of antibiotics should be banned.
- MIC value for methicillin antibiotics should be determined which was not done due to lack of resources.
- To make clinical therapy effective, drugs should be given only on the basis of culture and antibiotic sensitivity reports.
- The research should be extended to molecular level by using different molecular techniques like PCR and RFLP in order to reveal the epidemiology of the MRSA strains isolated.

PHOTOGRAPHS

Photograph 1: *Staphylococcus aureus* in mannitol salt agar

Photograph 2: Methicilllin resistant *Staphylococcus aureus in* MHA

Photograph 3: Sample processing in microbiology lab.



Photograph 4**:** Antibiotic susceptibility test of MRSA

Reference

Adhikari RP, Shrestha S, Barakoti A and Amatya R (2017). Inducible clindamycin and methicillin resistant Staphylococcus aureus in a tertiary care hospital, Kathmandu, Nepal. BMC Infectious Disease.

Ali MA, Abbasi S, Arif S and Mirza IA (2007). Nosocomial infections due to Methicillin Resistant *Staphylococcus aureus* in hospitalized patients. Pak J Med Sci Vol. 23 No. 4.

Anathanarayan R, Paniker CJK (1986). Textbook of Microbiology. 3rd edition, Orient Longman Ltd. Chennai, India

Ansari, S., H.P. Nepal, R. Gautam, N. Rayamajhi, S. Shrestha, G. Upadhyay, A. Acharya and M.L. Chapagain (2014). "Threat of drug resistant Staphylococcus aureus to health in Nepal". BMC infectious diseases 14(1): 157.

Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK and Mohapatra TM (2003). Prevalence of methicillin resistant *Staphylococcus aureus* in a Tertiary care Referral Hospital in Eastern Uttar Pradesh. Indian J Med Microbiol 21: 49–51.

Armin S, Karimi AE, Fahimzad S, Falah F, Shamshiri A (2007) Staphylococcal nasal colonization in Mofid children hospital staff; carrier state and antibiotic susceptibility.

Arora S, Devi P, Aror a U and Devi B (2010). Prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) in a Tertiary Care Hospital in Northern India. J Lab Physicians, 2(2): 78-81, DOI: 10.4103/0974-2727.72154.

ASM (2009). A report from the American Academy of Microbiology Antibiotic Resistance: An etiological Perspective of an Old problem. ASM Press, Wasghington D.C.

Atlas R (1995). Principles of Microbiology. 1st edition, Mosby-Year Book,Inc; 11830 Westline Industrial Drive, St. Louis, Missouri 63146, pp 681-6.

Azap OK, Arslan H, Timurkaynak F, Yapar G, Oruc E, and Gagir U (2005). Incidence of inducible clindamycin resistance in erythromycin-resistant isolates of *Staphylococcus aureus*. J Clin Microbiol. 43: 1716-21.

Baird-Parker AC (1997). Methods for identifying Staphylococci and Micrococci. The Society for Applied Microbiology Technicals Series, No. 14. Academic Press, London.

Baral R, Khanal B and Acharya A (2011). Antimicrobial susceptibility patterns of clinical isolates of *Staphylococcus aureus* in Eastern Nepal. Vol 9 (2): 78-82.

Bassim H and Maghraby ME (2005). Methicillin Resistant *Staphylococcus aureus* (MRSA) A Challenge for Infection Control. ASJOG 2: 277-278.

Bergey, D. H., R. S. Breed, E.G. D. Murray and A.P. Hitchens (1939). "Manual of determinative bacteriology" manual of determinative bacteriology, Fifth Edn.

Bien J, Sokolova P and Bozko P (2011). Characterization of Virulence Factors of *Staphylococcus aureus*: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. J of Pathogens, DOI: 10.4061/2011/601905.

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.

Boucher HW and Corey GR (2008). Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 46: 344-349

Boyce JM, Cookson B, Christiansen K (2005). Methicillin resistant *Staphylococcus aureus*. The Lancet Infect Dis; 5(10): 653-63.

Bradley S. F. (2015). MRSA colonization (eradicating colonization in people without active invasive infection.

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

Brown DF, Edwards DI, H awkey PM, Morrison D, Ridway GL, Towner KJ, and Wren MW (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant *Staphylococcus aureus* (MRSA). J AntimicrobChemother 56: 1000-1051.

Buckingham S, McDougal L, Cathey L, et al. (2004) Emergence of Community-Associated Methicillin-Resistant *Staphylococcus aureus* at a Memphis, Tennessee Children's Hospital. Ped Infections Dis J. 23: 619-24.

Burian M, Wolz C and Goerke C (2010). Regulatory adaptation of *Staphylococcus aureus* during nasal colonization of humans. *PLoS ONE* 5(4), e10040

CDC (2002) *Staphylococcus aureus* Resistant to Vancomycin-United States, Morbidity and Mortality Weekly Report 51: 565-67.

Chakrabroty P (2005). A textbook of Microbiology. 1stedition. New Central Book Agency (P) Ltd, India.

Chambers HF (2001).The changing epidemiology of *Staphylococcus aureus Emerg. Infect. Dis.* 7(2), 178–182.

Centers for Disease Control (CDC) (2006). Management of multi-drug resistant organisms in Health care settings. CDC, Atlanta USA. Pp1-74.

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

CLSI (2012). Performance standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. M100-S22.

CLSI (2006). Performance Standards for Antimicrobial Susceptibility Testing, Fifteenth Informational Supplement, CLSI document M100-S24.

Collee JG, Fraser AG, Marmion BP (1996), Mackie and McCartney Practical Mediacal Microbiology, 14thedition, Churchill Livingstone, Pp. 1210-15.

Colley JG, Fraser A, Marmion BP and Simmons A (2006) Mackie and McCartney Practical Mediacal Microbiology. 14th edition, Churchill Livingstone. Pp. 245-258.

Collier L, Balows A and Sussman M (1998). Topley and Wilson′s Microbiology and Microbial Infections. Volume 3: Bacterial Infections, 9th Edition; 231-48.

Cosgrove SE, Qi Y, Kaye KS, Harbarth S, Karchmer AW, Carmeli Y (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(2), 166–174.

David MZ, Daum RS (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. Clinical microbiology reviews 23: 616-687.

Delialioglu N, Aslam G, Ozturk C, Baki V, Sen S, Emekdas G (2005). Inducible clindamycin resistance in staphylococci isolates from clinical samples. Jpn J Infect Dis., 58:104-6.

Deotale V, Mendiratta DK, Raut V, Narang P (2010). Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Indian J Med Microbiol, 28:124-6.

Drinkovic D, Fuller ER, Shore KP, Holland DJ and Ellis-Pegler R (2001). Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J Antimicrob Chemother, 48: 315-6.

Fluit AC, Wielders CL, Verhoef J, Schmitz FJ (2001). Epidemology and susceptibility of 3,051 *Staphylococcus aureus* isolates from 25 university hospitals participating in the European SENTRY study. J Clin Microbiol 39: 3727-32

Fomda BA, Thokar MA, Khan A, Bhat JA, Zahoor D, Bashir G, Majid A and Ray P (2014). Nasal carriage of methicillin-resistant *Staphylococcus aureus* among healthy population of Kashmir, India. Indian J. Med. Microbiol. 32(1), 39–43.

Forbes B, Sahm DF, Weissfeld AS (2002). Bailey and Scott′s diagnostic microbiology, eleventh edition.

Forbes BA, Sahm DF and Weissfeld AS (2007) Bailey and Scott's Diagnostic Microbiology. 12th edition, Mosby Inc. US. Pp. 254-261

Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G, Ross T, Cai M, Hansel NN, PerlTicehurst JR, Carroll K, Thomas DL, Nuermberger E, Bartlett JG. (2005). Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. Clin Infect Dis; 40:100-7.

Fuda CCS, Fisher JF, and Mobashery S (2005). Beta-lactam resistance in *Staphylococcus aureus*: the adaptive resistance of a plastic genome, Cell. Mol. Life sci., 62:2617-2633.

Gadepalli R, Dhawan B, Mohanty S, Kapil A, Das BK, Chaudhary R (2006). Inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus*. Indian J. Med. Res. 123(1): 571-573.

Greenwood D, Slack RCB and Peutherer JF (2002). Medical Microbiology, 16th Edition; Churchill Livingstone, UK.

Grundmann H, Aires-De-Sousa M, Boyce J and Tiemersma E (2006). Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet* 368(9538), 874–885.

Gorden RJ, Lowy FD (2008). Pathogenesis of Methicillin –resistant *Staphylococcus aureus* infection. Clin Infection Dis; 46: 350-9.

Gould IM, David MZ, Esposito S, Garau J et al (2012). New insights into meticillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. Int J Antimicrob Agents 39(2): 96–104.

Govindan S, Mohammed AC and Bairy I (2014). Inducible Clindamycin Resistance among the *Staphylococcus aureus* Colonizing the Anterior Nares of School Children of Udupi Taluk. Vol 4, No. 1.

Goyal R, Das S, Mathur M (2002). Colonisation of methicillin resistant *S. aureus* among health care workers in a tertiary care hospital of Delhi. Indian J Med Sci. 56: 321–4.

Gupta V, Datta P, Rani H and Chander J (2009). Inducible clindamycin resistance in *Staphylococcus aureus*: A study from North India. Int J of Sci and Research. 55(3): 176-179.

Gurung RR, Maharjan P and Chhetri GG (2020). Antibiotic resistance pattern of *Staphylococcus aureus* with reference with MRSA isolates from pediatric patients. Future Sci OA. 6(4):FS0464; Doi: 10.2144/fsoa-2019-0122.

Hennekinne, J.A., M.L. De Buyser and S. Dragacci (2012).″ *Staphylococcus aureus* and its Food poisoning toxins: characterization and outbreak investigation.″ FEMS microbiology reviews 36(4):815-836.

Horowitz DL, Katzap E., Horowitz S., and Labarca MLB (2011). Approach to septic arthritis, 84(6):653-60.

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.

Jadhav SV, Gandham NR, Sharma M, Kaur M, MIsra RN, Matnani GB, Ujagare MT, Saikat B, Kumar A (2011). Prevalence of inducible Clindamycin resistance among community-and hospital-associated Staphylococcus aureus isolates in a tertiary care hospital in India. Biomed. Res.22 (4):465-469.

Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Gautam V, Goswami P, Gupta V, Harish BN, Kagal A, Kapil A, Rao R, Rodrigues C, Sardana R, Devi KS, Sharma A and Balaji V (2013). Methicillin resistant *Staphylococcus aureus* (MRSA) in India: prevalence & susceptibility pattern. Indian J Med Res. 137(2): 363–9.

Kaur DC and Chate SS (2015) Study of Antibiotic Resistance Pattern in Methicillin Resistant *Staphylococcus Aureus* with Special Reference to Newer Antibiotic, Journal of Global Infectious Diseases, 7:78-84

Khanal R, Sah P, Lamichhane P, Lamsal A, Upadhya S, Pahwa VK (2015). Nsal carriage of MRSA among health care workers at a tertiary care hospital in Western Nepal. Antimicrob Resist Infect Control; 4:39.

Khanal, L. and Jha B (2010). Prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) among skin infection cases at a hospital in Chitwan, Nepal.

Koch AL, (2003). Bacterial wall as target for attack: past, present and future research, Clin. Microbiol. Rev. 16:673-687.

Krishnamurthy V, Saha A, Renushri BV, Nagaraj ER (2014). Methicillin resistant *Staphylococcus aureus* carriage, antibiotic resistance and molecular pathogenicity among healthy individuals exposed and not exposed to hospital environment. J. Clin. Diagn. Res. 8(7), 1–5.

Kshetry AO, Pant ND, Bhandari R et al (2016). Minimum inhibitory concentration of vancomycin to MRSA isolated from different clinical samples at a tertiary care hospital in Nepal. Antimicrob Resist Infect Control. 5:27.

Lama U, Shah D and Shrestha UT (2018). Vancomycin Resistant *Staphylococcus aureus* Reported from Tertiary Care Hospital in Nepal. 4:63.

Lamichhane R, Adhikari RP and Sherchand JB (1999) Study of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from different clinical samples. M. Sc. Dissertation, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Langone JJ (1982). ProteinA of *Staphylococcus aureus* and related immunoglobulin receptors produced by Streptococci and Pneumococci. Adv Immunol; 32:157-252.

Leclercq R (2002). Mechanism of Resistance to Macrolides and Lincosamides: Nature of the Resistance Elements and Their Clinical Implications. Clin Infect Dis, 34(4): 482-492.

Leclercq R and Courvalin R (1991). Bacterial resistance to macrolide, lincosamide and streptogramin antibiotics by target modification. Antimicrobial Agent and Chemotherapy. 35(7): 1267-1272, DOI: 10.1128/aac.35.7.1267.

Lencastre HD, Oliveira D, and Tomasz A. (2007) Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power, current opinion in microbiology, vol. 10, page no. 428-435.

Levinson, W. and E. Jawetz (1996). Medical microbiology and immunology: examination and board review, Appleton and Lange.

Lwatsuki K., Yamasaki O., Morizen S., and Oono T. (2006) Staphylococcal cutaneous infections: Invasion, evasion and aggression, Journal of dermatological science, 42(3) p. 203-214.

Madigan MT, Martinko JM and Parker J (2003) Brock Biology of Microorganisms. 10th edition, Pearson Education, Inc. USA. Pp. 399-400.

Majumder D, Bordoloi JN, Phukan AC and Mahanta J (2001). Antimicrobial susceptibility pattern among methicillin resistant Staphylococcus isolates in Assam. Indian J Med Microbiol 19: 138–140.

Maple PAC, Hammilton-Miller JMT and Brumit W (1989). Worldwide antibiotic resistance in methicillin resistant *Staphylococcus aureus*. Lancet.VOL.1, pp. 537-40.

MCDonald M (1997). "The epidemiology of methicillin resistant *Staphylococcus aureus*: Surgical relevance 20 years on Aust N Z J Surg". 67:682-5.

Mohiyiddeen G, Brett I, and Jude E (2008) Infective endocarditis caused by *Staphylococcus aureus* in a patient with atopic dermatitis: a case, J Med Case Reports, 2: 143, DOI: 10.1186/1752-1947-2-143.

Mongodin, E., O. Bajolet, J. Cutrona, N. Bonnet, F. Dupuit, E. Puchelle and S. De Bentzmann (2002). "Fibronectin-binding proteins of *Staphylococcus aureus* are involved in adherence to human airway epithelium." Infection and immunity 70(2): 620-630.

Mukhiya RK, Shrestha A, Rai SK, Singh RN, Rai G and Prajapati A (2012). Prevalence of methicillin-resistant *Staphylococcus aureus* in hospitals of Kathmandu valley. NJST 13(2), 185–190.

Nikaido H (2009) Multidrug resistance in bacteria, HHS public access, 78:119-146.

Oryan A, Alidadi S, Moshiri A, and Maffulli N. (*2014).* Bone regenerative medicine: classic options, novel strategies, and future directions, J OrthopSurg, 9:18, DOI: 10.1186/1749-799X-9-18.

Otto M (2012). MRSA virulence and spread. Cell Microbiol. 14(10): 1513–1521

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

Prabhu K, Rao S and Rao V (2011). Inducible Clindamycin Resistance in *Staphylococcus aureus* Isolated from Clinical Samples. J Lab Physicians, 3(1): 25–27. DOI: 10.4103/0974-2727.78558.

Rahbar M and Safadel N (2006). Evaluation of Cefoxitin Disc Diffusion Test for Routine Detection of Methicillin Resistant *Staphylococcus aureus.* Iranian J of Pathology 1: 145-148

Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P (2006). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: A multicentre study. Ind J Med Microbiol 24: 34–38.

Rajbhandari R (2002). Prevalence and Antibiotic Sensitivity Pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Bir Hospital. M. Sc. Dissertation. Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Raut S, Bajracharya K, Adhikari J, Pant SS and Adhikari B (2017). Prevalence of methicillin resistant *Staphylococcus aureus* in Lumbini Medical College and Teaching Hospital, Palpa, Western Nepal.

Regmi S, Amatya J, Labh SN (2020). Antimicrobial Resistance Pattern of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Strains Isolated from Clinical Specimens in Kathmandu, Nepal. Arch Clin Microbiol Vol. 11 No. 4:116. Doi:10.36648/1989-8436.11.4.116.

Rijal K, Pahari N, Shrestha B, Nepal A, Paudel B, Mahato P and N. Skalko-Basnet (2008). ″Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara.″ Nepal med coll J 10(3): 192-195.

Robinson JO, Pearson JC, Christiansen KJ, Coombs GW, Murray RJ (2009). Community-associated versus healthcare-associated methicillin-resistant *Staphylococcus aureus* bacteraemia: a 10-year retrospective review. Eur J Clin Microbiol Infect Dis. 28: 353–61

Rocha LC, Carvalho MO, Nascimento VM, Dos S. Barron TF, Adorno EV, Reis JN, Guarda DA, Santiago RP and Goncalves CMS (2017). Nasopharyngeal and Oropharyngeal colonization by *Staphylococcus aureus* and *Streptococcus pneumonia* and prognostic markers in children with sickle cell disease from 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.

Sah P, Khanal R, Lamichane P, Upadhaya S, Lamsal A and Pahwa VK (2015). Inducible and constitutive clindamycin resistance in *Staphylococcus aureus*: An experience from Western Nepal. Int J Biomed Res. 6(5): 316-9.

Saikai L, Nath R, Choudhary BC and Sarkar B (2009). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* in Assam. Indian J of Crit Care Med, 13:156-8.

Salyers AA and Whitt DD (2002) Bacterial Pathogenesis: A Molecular Approach. 2nd edition. ASM Press, Washington, D.C.Pp. 216-228.

Salyersaw DD (2002). Bacterial pathogenesis, a molecular approach, 2nd edn. ASM PRESS, Washington DC.

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.

Sapkota K (2006) Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Clinical Specimens from Patients and Screening of Nasal Carriage of MRSA from Medical Staffs of Bir Hospital. M. Sc. Dissertation, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Shanson DC (1981). Antibiotic resistance *Staphylococcus aureus*. J Hosp. Infect., pp 2-11.

Shrestha B, Pokhrel BM and Mohapatra TM (2009). Phenotypic Characterization of nosocomial isolates of Staphylococcus *aureus* with refrence to MRSA. J Infect Dev C tries, 3(7):554-560.

Subedi S and Brahmadathan K.N. (2005). Antimicrobial susceptibility patterns of clinical isolates of *Staphylococcus aureus* in Nepal. Clin Microbiol Infect, 11: 235-237, Doi: 10.1111/J.1469-069.2004. 01056.

Tambic A, Power EGM, Talsania H, (1997). Analysis of an outbreak of non-phage-typeable Methicillin-resistant *Staphylococcus aureus* by using a randomly amplified polymorphic DNA assy. J Clin Microbiology; 35: 3092-97.

Tenoverfag RJ (2006). The epidemiology of Staphylococcus infections. In Gram-positive pathogens. Fiscetti 5th edn. Washington, DC, pp. 526-34.

Thapa S (2004). Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Children Visiting Kanti Children′s Hospital. M. Sc. Dissertation, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.

Tiwari HK, Sapkota D and Sen MR (2008). High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. Infection and Drug Resistance 1: 57-61.

Tiwari HK, Das A, Sapkota D, Sivraja K, Pahwa V (2009). Methicillin Resistant *Staphylococcus aureus*: Prevalence and antibiogram in a tertiary care hospital in western Nepal. J Infect Dev Ctries 3:681-684.

Voss A, Doebbeling BN (1995). The worldwide prevalence of methicillin resistant *Staphylococcus aureus.* Int J Antimicrob Agents. 5:101- 106.

Waxman D.J. and Strominger J.L. (1983) Penicillin –binding proteins and the mechanism of action of beta-lactam antibiotics. Annu. Rev. Biochem. 52:825-869.

Wolk DM, Struelens MJ, Pancholi P, Davis T, Della-Latta P, Fuller D, Picton E, Dickenson R, Denis O, Johnson D and Chapin K (2009). Rapid detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays. J Clin Microbiol. 47(3):823–6. DOI: 10.1128/JCM.01884-08.

APPENDICSES

APPENDIX I: NHRC APPROVAL

Government of Nepal

Nepal Health Research Council (NHRC)

Estd. 1991

Ref. No.: 534

10 September 2018

Ms. Lata Chalise
Principal Investigator
Central Campus of Technology
Hattisar, Dharan

Ref: **Approval of thesis proposal** entitled **Antibiogram of Staphylococcus aureus isolated from various clinical samples of patients visiting a tertiary care hospital of central region Kathmandu, Nepal**

Dear Ms. Chalise,

It is my pleasure to inform you that the above-mentioned proposal submitted on **29 July 2018** (**Reg. no. 480/2018**) has been approved by Nepal Health Research Council (NHRC) National Ethical Guidelines for Health Research in Nepal, Standard Operating Procedures Section 'C' point no. 6.3 through Expedited Review Procedures.

As per NHRC rules and regulations, the investigator has to strictly follow the protocol stipulated in the proposal. Any change in objective(s), problem statement, research question or hypothesis, methodology, implementation procedure, data management and budget that may be necessary in course of the implementation of the research proposal can only be made so and implemented after prior approval from this council. Thus, it is compulsory to submit the detail of such changes intended or desired with justification prior to actual change in the protocol. Expiration date of this proposal is **February 2019.**

If the researcher requires transfer of the bio samples to other countries, the investigator should apply to the NHRC for the permission. The researchers will not be allowed to ship any raw/crude human biomaterial outside the country; only extracted and amplified samples can be taken to labs outside of Nepal for further study, as per the protocol submitted and approved by the NHRC. The remaining samples of the lab should be destroyed as per standard operating procedure, the process documented, and the NHRC informed.

Further, the researchers are directed to strictly abide by the National Ethical Guidelines published by NHRC during the implementation of their research proposal and **submit progress report in between and full or summary report upon completion.**

As per your thesis proposal, the total research budget is **NRs 51,000** and accordingly the processing fee amounts to **NRs 1,000.** It is acknowledged that the above-mentioned processing fee has been received at NHRC.

If you have any questions, please contact the Ethical Review M & E Section at NHRC.

Thanking you,

Prof. Dr. Anjani Kumar Jha
Executive Chairperson

Tel: +977 1 4254220, Fax: +977 1 4262469, Ramshah Path, PO Box: 7626, Kathmandu, Nepal
Website: http://www.nhrc.gov.np, E-mail: nhrc@nhrc.gov.np

APPENDIX: II

Questionnaire

नाम :

उमेर :

मोबाईल नं :

लिङ्ग :

संलग्नता नं :

<u>प्रश्न :</u>

१. एन्टिबायोटिक सेवन गर्नु हुन्छ ? छ छैन

२. यदि छ भने सेवन गरेको एन्टिबायोटिकको नाम ?

३. रोगको लक्ष्यणहरु

४. के कुपोषण सम्बन्धि समस्या छ ?

५. अस्पतालमा भर्ना हुनुभएको छ या छैन ?

६. छ भने कति समय भयो ?

APPENDIX: III

ASSENT FORM

साधारण जानकारी

प्रमुख अन्बेषकको नाम : Lata Chalise (Msc Microbiology fourth semester)

सम्बन्धित संस्थानको नाम : केन्द्रिय प्राबिधिक क्याम्पस, धरान १४

सोध अध्ययन शीर्षक : Antibiogram of *Staphylococcus aureus* from various clinical from various clinical samples of patients visiting a tertiary care hospital of central region, Nepal

यस मन्जुरीनाम फरममा दुई भागहरु छन ।

भाग १ : जानकारी पत्र - तपाईंलाई अध्ययन सम्बन्धि जानकारी गराउनको लागि

भाग २ : मंजुरिनामाको प्रमाणपत्र (तपाइको सहभागिताको स्वीकृति जनाउन सहिछाप गर्ने फारम)

भाग १ : जानकारी पत्र

मानिसको शरीरमा (धेरैजसो नाक , स्वश्प्रस्वाश नली र छाला) पाइने Staphylococcus aureus नामक bacteria ले स्वस्थमा संक्रमण निम्त्याउन सक्छ । सुरुमा साधारण देखिएपनि येस्ता संक्रमणले भबिस्य जटिल समस्या उत्पन्न गराउन सक्छ । येस्तो संक्रमण काठमाडौँमा बढीहुने गर्दछ र यसको उपचारमा कठिनाई आइरहेकोले गर्दा अध्ययनको लागि मैले यो बिषयलाइ छनोट गरेकोछु । म यस बिषय अध्ययन गर्ने व्यक्ति हुनाले तपाइलाई यस अध्ययन सम्बन्धि जानकारी दिदै यस अनुसन्धानमा सहभागी हुनाकोलागी अनुरोध गर्दछु । तपाइले यस अध्ययनमा सहभागी हुने नहुने निर्णय गर्नुपुर्ब सहज लाग्ने व्यक्तिसंग सरसल्लाह गर्न सक्नुहुनेछ । यस अध्ययन संग सम्बन्धित कुनै पनि प्रस्न तपाइले कुनैपनि बेला मलाई वा यो अध्ययन संग संलग्न अन्य व्यक्ति संग सोध्न सक्नुहुनेछ । सर्बप्रथम तपाइलाई चिकित्सकले परिक्षण गर्नेछन र संक्रमण आएको संका लागेमा चिकित्सकले प्रयोगशालामा रगत , पिसाब , पीप परिक्षण गर्न पठाउनेछन । जहाँ तालिमप्राप्त स्वस्थाकर्मी द्वरा संकलित नमुना परिक्षण गरिनेछ । यस क्रममा तपाइलाई केहि प्रस्न सोधिनेछन । यस अध्ययनमा सहभागी हुदा तपाइलाई कुनै हानी हुने छैन साथै उपचार बिधिमा कुनैपनि प्रत्यक्ष्य वा अप्रत्यक्ष्य असर पर्नेछैन । तपाईबाट प्राप्त जानकारी गोप्य राखिनेछ । तपाइको व्यक्तिगत जानकारी नामबाट नभई कोडबाट गरिनेछ । प्रयोग्सालाबाट तपाइको नमुनाको जानकारी टिपोट गरिसकेपछि त्यसलाई सुरक्षित तरिकाले हटाइनेछ । जानकारी गोप्य राखिनेछ र नतिजा प्रस्तुति परिचय नखुलाई गरिनेछ । तपाइलाई यो मंजुरिनामा फारमको एक प्रति उपलब्ध गराइनेछ । तपाइलाई इच्छा नलागेमा कुनैपनि बेला सहभागिता परित्याग गर्नसक्नुहुनेछ , यसकारणले गर्दा तपाइले यस अस्पताल बाट प्राप्त गर्ने सेवा सुबिधामा कुनैपनि

असर पर्ने छैन । यस अध्ययन सम्बन्धित तपाइको कुनैपनि प्रस्न भएमा तपाइले यस अध्ययन संग सम्बन्धित व्यक्तिलाई सम्पर्क गर्न सक्नुहुनेछ ।

लता चालिसे पराजुली ९८४२२४८४११

सुमन राई ९८४२०४४५९५

<u>भाग २ : मंजुरिनामाको प्रमाणपत्र</u>

मैले *Staphylococcus aureus* नामक bacteria ले मानिसलाइ धेरै प्रकारको रोग (संक्रमण) लगाउछ र यसको निदान का लागि प्रयोग गरिने antibiotics को असर शिर्षक अध्ययनमा सहभागी हुन अनुरोध गरियो र मलाई नबुझेको कुरा सोध्ने पुरा अवसर दिईयो र सोधिएका प्रस्नहरु बुझ्नेगरी सन्तोषजनक जवाफ दिईयो । मैले यस अध्ययनमा सहभागी स्वेच्छाले मन्जुरीमा दिएको छु । प्रस्तुत शिर्षकमा मलाई पूर्ण जानकारी दिईयो । मेरो सन्तान १८ वर्ष मुनिको भएकाले उसको प्रतिनिधि भइ मैले उक्त बिषयको पूर्ण जानकारी प्राप्त गरेको छु । उक्त बिषयमा अध्ययन गर्न , मेरो स्वेच्छा ले अनुमति दिन चाहन्छु ।

प्रतिनिधिको नाम : हस्ताक्षर मिति

सहिछाप

निरक्षरहरुले यो फारममा भएको प्रतिनिधिले पढेर सुनाएपछि तलको कोस्ठमा दाहिने औठाको छाप लगाउनु पर्छ । अन्वेषकले यो फारम प्रस्ट भाषामा पढेर सुनाएको तथा सहभागीलाई नबुझेको कुरा सोध्ने पूर्ण अवसर दिएको सत्य हो । त्यसैले सहभागीले स्वेच्छाले यो मंजुरिनामा दिएको प्रमाणित गर्दछु ।

साक्षीको नाम : साक्षीको हस्ताक्षर

मिति

<u>मंजुरिनामा लिने अन्वेषक / प्रतिनिधिको अभिव्यक्ति</u>

यो फारम प्रस्ट भाषामा बुझ्नेगरी पढेर सुनाएको तथा सहभागीलाई नबुझेको कुरा सोध्ने पूर्ण अवसर दिएको सत्य हो । म यो पनि प्रमाणित गर्दछु कि सहभागीलाई कुनै पनि किसिमको मंजुरिनामा पार्न बाध्य पारिएको छैन र यो मंजुरिनामा स्वतन्त्र र स्वयेछित तवरले दिईएको छ जसको एक प्रति सहभागीलाई पनि उपलब्ध गराईएको छ ।

मंजुरिनामा लिने अन्वेषक / प्रतिनिधिको नाम : हस्ताक्षर

मिति

APPENDIX: IV

Materials and Equipment's

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	

Equipment's

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 (0.5)	MacFarland's Nephelometer Standard

Physiological saline

Microscope oil

Lysol

Antibiotics (HiMedia Company) Media (Hi Media Company)

Amikacin (30mcg)

Cefoxitin (30mcg)

Chloramphenicol (30mcg)

Ciprofloxacillin (5mcg)

Co-trimoxazole (25mcg)

Clindamycin (2mcg)

Erythromycin (15mcg)

Gentamicin (10mcg)

Meropenem (10mcg)

Nitrofurantoin (300mcg)

Tetracycline (30mcg

Vancomycin (30mcg)

Blood Agar

Brain-Heart Infusion Broth

Nutrient Broth

Muller Hinton Agar

Peptone

Mannitol Salt Agar

APPENDIX: V

Bacteriological media

Composition and preparation of different types of media

1. Blood agar (BA)

Blood agar base (infusion agar) +5-10% sheep blood

Ingredients	Gram/litre
Beef heart infusion	500.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	15.0
Final pH (at 25ºC)	7.3±0.2

About 42.5 gram of the blood agar base medium was suspended in 1000ml distilled water and sterilized by autoclaving at 121ºC (15lbs pressure for15 minutes. After cooling to 40-50ºC, 50ml sterile defibrinated sheep blood was added aseptically and mixed well before pouring.

2. Brain Heart Infusion Broth (BHI)

Ingredients	Gram/litre
HM infusion powder	12.5
BHI powder	5.0
Proteose peptone	10.0
Dextrose (Glucose)	2.0
Sodium chloride	5.0
Disodium hydrogen phosphate	2.5
Final pH (at 25°C)	7.4±0.2

Suspend 37.0 grams in 1000 ml purified/distilled water. Dispense into bottles or tubes and sterilize by autoclaving at15 lbs pressure (121°C) 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
pH (at 25^0 C)	*7.4±0.2*

111 grams of the medium was suspended in 1000 ml distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs at 121^0C 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
pH (at 25^0 C)	*7.4±0.2*

38 grams of the medium was suspended in 1000ml water and boiled to dissolve completely. The media was then autoclaved at 15 lbs at 121^0C for 15 minutes.

5. Nutrient Broth (NB)

Ingredients	Gram/litre
Peptone	5.0
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5

pH (at 25^0 C)	*7.4±0.2*

13 gram of medium was dissolved in 1000 ml of distilled water and heated to dissolve the media. The media was autoclaved at 15 lbs at 121^0C for 15 minutes.

APPENDIX: VI

Composition and Preparation of different Reagents

1. Gram's Stain Reagent

I Crystal Violet Solution

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 was dissolved in distilled water. Then, solution A and solution B were mixed

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

Safranine (99% dye content)	10 gm
Distilled water	1000 ml

2 Catalase Reagent

3% Hydrogen peroxide solution (100ml)

Hydrogen peroxide	3 ml

Distilled water	97 ml

1 N Hydrochloric acid (1 mol/litre)

Concentrated hydrochloric acid	8.6 ml
Distilled water	100 ml

3 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: VII

Procedure of different biochemical tests

1. **Gram′s strain**: (Mackie and McCartney Vol.2, 14th edition)

Isolated colony selected for staining:

1 Smear was made from pure culture by emulsifying a colony in normal saline and heat fixed.

2 Smear flooded with crystal violet for 1 minute.

3 Wash with water.

4 Add Gram′s iodine for 1 minute.

5 Wash with water.

6 Decolorize with absolute alcohol for 10-15 seconds.

7 Wash with water.

8 Flood with saffron for 1 minute.

9 Wash with water, blot dry and examine under oil immersion objective of the microscope.

Gram positive cocci seen in grape-like clusters were an indicative of Staphylococci.

2. Catalase test

1. A small amount of isolated colony form pure culture was transferred to the surface of clean and grease free glass slide.
2. A drop of 3% H_2O_2 was placed onto the inoculum.
3. The evolution of oxygen bubbles was recorded immediately.
4. The slide was then discarded into a disinfectant.

3. Coagulation test

I slide test (to detect bound coagulase)

1. A drop of physiological saline was placed on on each end of a slide and colony of test organism was emulsified in eah of the drop to make two thick suspensions.
2. Add a drop of plasma to one of the suspensions, and mix gently.
3. It was looked for clumping of the organisms within 10 seconds.

4. No plasma is added to the second suspension to differentiate any granular appearance of the organism from true coagulase clumping.

II. Tube test (to detect free coagulase)

1. The plasma was diluted 1 in 10 physiological salines (mixing 0.2ml of plasma with 1.8 ml of saline),
2. 3 tubes were taken and labeled as:
   T= test organism (18–24-hour broth culture),
   P=positive control (*S. aureus* broth culture),
   N= negative control (sterile broth),
3. 0.5ml of diluted plasma was pipetted into each tube.
4. About 5-5 drops each of test organism, *S. aureus* culture, and sterile broth was added to the tubes labeled ′t′, ′P′ and ′N′ respectively.
5. After mixing gently, 3 tubes were incubated at 37ºC. It was examined for clotting after 1 hour. If no clotting occurs tubes were examined at 30 minutes intervals for up to 6 hours.

APPENDIX: VIII

A. Antibiotic Susceptibility Test (Kirby-Bauer′s Disc Diffusion Method)

In vitro susceptibility of the pure bacterial species to fifteen different antibiotics was determined using Kirby-Bauer disk diffusion technique using Muller-Hinton agar and antibiotic disc as described by the National Committee for Clinical Laboratory Standards (CLSI, 2006). One ml of each bacterial isolates prepared directly from an overnight agar plates adjusted to 0.5 McFarland Standard was inoculated using sterile swab into each of the petri discs containing Muellar-Hinton Agar and were allowed to stand for 30 minute for pre-diffusion of the inoculated organisms.

Antibiotic disc were seeded into the petri dishes containing Mueller-Hinton agar (MHA) for each bacterial isolates. The AST of the isolates towards various antimicrobial disc was done by modifide Kirby-Bauer Disc Diffusion method as recommended by Clinical Laboratory Standard Institute (CLSI) using MHA as follows:

1. MHA was prepared and sterilized as instructed by the manufacturer.
2. The PH of the medium was adjusted to 7.2-7.4 and the depth of the medium at 4mm (about 25ml per plate) was maintained in petri dish.
3. A single isolated colony whose susceptibility pattern is to be determined was touched and inoculated into nutrient broth with the help of sterile wire loop and incubated at 37ºC for 24 hours.
4. After incubation, the turbidity of the suspension was matched with the McFarland standard tube number 0.5 (which is equivalent to 10 to power 4 organisms)
5. Sterile cotton swab was then dipped into the tube and excess inoculum was removed by pressing and rotating the swab firmly against the wall of the tube.
6. Swabbing was done evenly over the surface of the MHA plate by rotating the plates.
7. The petri dish was closed with its lid and then kept at room temperature for 3-5 minutes for the surface of agar to dry.
8. Appropriate antibiotic discs were taken out of the respective vials with the help of sterile forceps and placed carefully on the agar surface. The disc were placed at the considerable distance apart from each other on a 90mm petri-dish. Then the plate was incubated at 37ºc for 24 hours.
9. After incubation, the plates were observed for the zone of inhibition and the diameters of inhibition zones were measured in millimeters (mm). The measurement was interpreted as sensitive and resistant according to

the manufacture′s standard zone size interpretative manual of CLSI (2006).

The percentage resistance was calculated using the formula PR=a/b×100, where ‘PR’ was percentage resistance, ‘a’ was the number of resistant isolates and ‘b’ was the number of isolates tested with the antibiotic. The percentage sensitivity was calculated using the formula PS=c/d×100, where ‘PS’ was percentage sensitivity, ‘c’ was the number of sensitive isolates and ‘d’ was the number of isolates tested with the antibiotics.

B.

Antibiotics used	Symbol	Strength (mcg)	Resistant	Intermediate	Sensitive
Amikacin	AK	30	≤14	15-16	≥17
Cefoxitin	CX	30	≤21	-	≥22
Chloramphenicol	C	30	≤12	13-17	≥18
Ciprofloxacin	CIP	5	≤15	16-20	≥21
Clindamycin	CD	2	≤14	15-20	≥21
Co-trimoxazole	COT	25	≤10	11-15	≥16
Erythromycin	E	15	≤13	14-22	≥23
Gentamicin	GEN	10	≤12	13-14	≥15
Meropenem	MRP	10	≤13	14-15	≥16
Nitrofurantoin	NIT	300	≤14	15-16	≥17
Tetracycline	TE	30	≤14	15-18	≥19
Vancomycin	VA	30	≤13	14-16	≥17

(HI Media Laboratory Limited, India)

APPENDIX: IX

Chi-square data

1. Sample and prevalence of *Staphylococcus aureus*

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	19.445[a]	2	.000
Likelihood Ratio	20.267	2	.000
Linear-by-Linear Association	15.804	1	.000
N of Valid Cases	227		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 9.12.

Test is statistically significant

2. Age and ward and prevalence of *Staphylococcus aureus*

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	5.982[a]	5	.308
Likelihood Ratio	6.064	5	.300
Linear-by-Linear Association	.075	1	.784
N of Valid Cases	227		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.29.

Test is not statistically significant

3. Gender and prevalence of *Staphylococcus aureus*

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2.882[a]	1	.090		
Continuity Correction[b]	2.435	1	.119		
Likelihood Ratio	2.886	1	.089		
Fisher's Exact Test				.101	.059
Linear-by-Linear Association	2.870	1	.090		
N of Valid Cases	227				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 40.81.
b. Computed only for a 2x2 table

Test is not statistically significant

4. Age and Gender and prevalence of MRSA.

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	27.199[a]	5	.000
Likelihood Ratio	33.214	5	.000
Linear-by-Linear Association	17.221	1	.000
N of Valid Cases	109		

a. 2 cells (16.7%) have expected count less than 5. The minimum expected count is 2.59.

Test is statistically significant**.**

5. Ward and prevalence of MRSA and MSSA

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.074[a]	1	.785		
Continuity Correction[b]	.019	1	.890		
Likelihood Ratio	.074	1	.785		
Fisher's Exact Test				.791	.445
Linear-by-Linear Association	.074	1	.786		
N of Valid Cases	227				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 48.02.
b. Computed only for a 2x2 table

Test is not statistically significant.

6. *Staphylococcus aureus* with amikacin

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	10.588[a]	1	.001		
Continuity Correction[b]	9.662	1	.002		
Likelihood Ratio	10.804	1	.001		
Fisher's Exact Test				.001	.001
Linear-by-Linear Association	10.542	1	.001		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 32.17.
b. Computed only for a 2x2 table

Test is statistically significant.

7. *Staphylococcus aureus* with Chloramphenicol

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.029[a]	1	.864		
Continuity Correction[b]	.000	1	.993		
Likelihood Ratio	.029	1	.864		
Fisher's Exact Test				.873	.497
Linear-by-Linear Association	.029	1	.865		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 23.53.
b. Computed only for a 2x2 table

Test is not significant.

8. *Staphylococcus aureus* with Ciprofloxacilin

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	19.461[a]	1	.000		
Continuity Correction[b]	18.299	1	.000		
Likelihood Ratio	19.729	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	19.375	1	.000		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 48.50.
b. Computed only for a 2x2 table

Test is statistically significant.

9. *Staphylococcus aureus* with cefoxitin

Chi-Square Tests

	Value	Df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	227.000[a]	1	.000		
Continuity Correction[b]	223.011	1	.000		
Likelihood Ratio	314.332	1	.000		
Fisher's Exact Test				.000	.000
Linear-by-Linear Association	226.000	1	.000		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 52.34.
b. Computed only for a 2x2 table

Test is statistically significant.

10. *Staphylococcus aureus* with Clindamycin

Chi-Square Tests

	Value	Df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.579[a]	1	.003		
Continuity Correction[b]	7.715	1	.005		
Likelihood Ratio	8.652	1	.003		
Fisher's Exact Test				.004	.003
Linear-by-Linear Association	8.541	1	.003		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 28.33.
b. Computed only for a 2x2 table

Test is statistically significant.

11. *Staphylococcus aureus* with Cotrimoxazole

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.229[a]	1	.040		
Continuity Correction[b]	3.701	1	.054		
Likelihood Ratio	4.243	1	.039		
Fisher's Exact Test				.047	.027
Linear-by-Linear Association	4.211	1	.040		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 54.26.
b. Computed only for a 2x2 table

Test is statistically significant.

12. *Staphylococcus aureus* with Erythromycin

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.258[a]	1	.007		
Continuity Correction[b]	6.538	1	.011		
Likelihood Ratio	7.327	1	.007		
Fisher's Exact Test				.009	.005
Linear-by-Linear Association	7.226	1	.007		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 40.81.
b. Computed only for a 2x2 table

Test is statistically significant.

13. *Staphylococcus aureus* with Gentamicin

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.482[a]	1	.034		
Continuity Correction[b]	3.928	1	.048		
Likelihood Ratio	4.493	1	.034		
Fisher's Exact Test				.042	.024
Linear-by-Linear Association	4.462	1	.035		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 44.18.
b. Computed only for a 2x2 table

Test is statistically significant.

14. *Staphylococcus aureus* with Meropenem

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.009[a]	1	.005		
Continuity Correction[b]	7.151	1	.007		
Likelihood Ratio	8.081	1	.004		
Fisher's Exact Test				.005	.004
Linear-by-Linear Association	7.974	1	.005		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 25.93.
b. Computed only for a 2x2 table

Test is statistically significant.

15. *Staphylococcus aureus* with Nitrofuraton

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.042[a]	1	.837		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.042	1	.839		
Fisher's Exact Test				1.000	.670
Linear-by-Linear Association	.039	1	.843		
N of Valid Cases	14				

a. 3 cells (75.0%) have expected count less than 5. The minimum expected count is .86.
b. Computed only for a 2x2 table

Test is not statistically significant.

16. *Staphylococcus aureus* with Tetracycline

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.048[a]	1	.025		
Continuity Correction[b]	4.450	1	.035		
Likelihood Ratio	5.061	1	.024		
Fisher's Exact Test				.028	.017
Linear-by-Linear Association	5.026	1	.025		
N of Valid Cases	227				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 40.81.
b. Computed only for a 2x2 table

Test is statistically significant.

17. *Staphylococcus aureus* and D test

Chi-Square Tests			
	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	11.214[a]	2	.004
Likelihood Ratio	11.297	2	.004
Linear-by-Linear Association	8.813	1	.003
N of Valid Cases	142		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 14.87.

APPENDIX: X

Formulas: The sensitivity, specificity, PPV and NPV values were calculated by using the following formula:

Sensitivity = $\frac{TP}{TP+FN}$X 100% Specificity = $\frac{TN}{TN+FP}$X 100%

PPV = $\frac{TP}{TP+FP}$X 100% NPV = $\frac{TN}{TN+FN}$X 100%

PPV = positive predictive value

NPV = negative predictive value

TP = true positive

TN = true negative

FP = false positive

FN = false negative