

**PREVALENCE AND FACTORS ASSOCIATED WITH
METHICILLIN RESISTANT *STAPHYLOCOCCUS*
AUREUS AMONG SURGICAL PATIENTS IN
KATHMANDU VALLEY, NEPAL**

SHREEJEET SHRESTHA

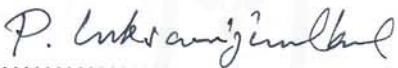
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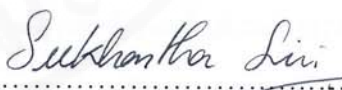
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Thematic Paper
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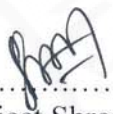

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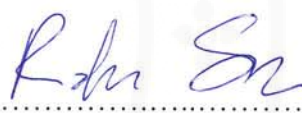

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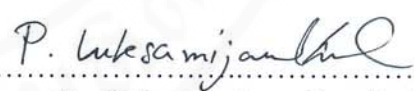
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Shreejeet Shrestha

PREVALENCE AND FACTORS ASSOCIATED WITH METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG PATIENTS WITH SURGERY VISITING HOSPITALS OF KATHMANDU VALLEY, NEPAL

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ABSTRACT

A hospital based cross-sectional analytic study of 797 surgical patients profile was conducted in the hospitals of Kathmandu Valley, Nepal to assess MRSA prevalence and risk factors. Data were analysed by descriptive and inferential statistics. Results revealed that prevalence of MRSA was found to be 9.9%. In this study prevalence was found to be higher in older age group patients (16.7%), males (18.1%), in late autumn season (16.0%), in wound swab (64.7%) and more than 5 days of hospitalization cases (15.5%).

On association of age, odds ratio showed similar risk among the children age group and middle aged people with $p=0.056$. Males were found to be significantly riskier than females with OR 2.98 with $p < 0.001$. In case of seasonal variation, Monsoon and late autumn season had a higher chance of MRSA infection with $p=0.204$. Wound swab and tissue were of significantly higher risk with OR 36.25 and 27.19, respectively, in comparison to other samples with $p < 0.001$. OPD patients were at slightly higher risk in comparison to IPD patients with OR 1.05 ($p=0.832$). The patients with greater than 5 days of hospitalization were at higher risk than patients with less than 5 days of hospitalization or no hospitalization with $p=0.337$. The history of hospitalization before surgery showed similar chance of getting infection of MRSA among both hospitalized and non hospitalized patients with $p=0.97$. The diagnostic diseases of the patient showed similar risk among all types of disease with slightly less risk in hemorrhoid patients in comparison to bone fracture patients with $p=1.0$.

The resistance to antibiotics showed higher resistance to oxacillin, methicillin, penicillin, ampicillin and cloxacillin. Vancomycin was found to be sensitive. The MDR- MRSA with pattern of resistance to Penicillin+ Quinolones+ Aminoglycoside+ Sulfonamides+ Cephalosporin+ Carbapenems was found as a common pattern of MDR-MRSA.

KEY WORDS: MRSA/ANTIBIOTIC RESISTANCE/ PREVALENCE/ RISK FACTORS

62 pages

CONTENTS

	Page
ACKNOWLEDGEMENTS	iii
ABSTRACT	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	x
CHAPTER I INTRODUCTION	1
1.1 Background and justification	1
1.2 Research question	3
1.3 Objectives	3
1.4 Hypotheses	3
1.5 Conceptual Framework	4
1.6 Operational definitions	4
CHAPTER II LITERATURE REVIEW	6
2.1 Methicillin resistant <i>S. aureus</i> and history	6
2.2 Epidemiology of MRSA	8
2.3 Community acquired MRSA (CA-MRSA)	8
2.3.1 Risk factors associated with CA-MRSA	10
2.3.2 Prevention and control of CA-MRSA	10
2.4 Hospital acquired MRSA (HA-MRSA)	10
2.5 Variables	14
2.5.1 Sources	14
2.5.2 Predisposing factors	14
2.5.3 Modes of transmission	15
2.5.4 Prevention and control	16
2.5.4.1 Strategies	16
2.5.4.2 Cleaning, sterilization and disinfection	17

CONTENTS (cont.)

	Page
2.5.4.3 Hand washing	17
2.6 Management of hospital acquired infection	18
2.6.1 Strategies	18
2.6.2 Waste management system	18
2.6.2.1 Documentation, employee health and training	18
2.7 Epidemiology of MRSA carriage	19
CHAPTER III MATERIALS AND METHODS	20
3.1 Study design	20
3.2 Study site	20
3.3 Population sample, sample size and sampling	20
3.4 Inclusion and Exclusion criteria	21
3.5 Data collection	21
3.5.1 Method of collection (Quality control)	22
3.5.2 Research instrument	22
3.5.3 Quality of instrument	22
3.6 Data analysis	22
3.6.1 Data entry	22
3.6.2 Classification criteria	22
3.6.3 Statistics	23
3.7 Ethical consideration	23
CHAPTER IV RESULTS	24
4.1 General characteristics of surgical patients	25
4.2 Prevalence of MRSA among surgical patients	28
4.2.1 Age	28
4.2.2 Gender	28
4.2.3 Seasonal variation	29

CONTENTS (cont.)

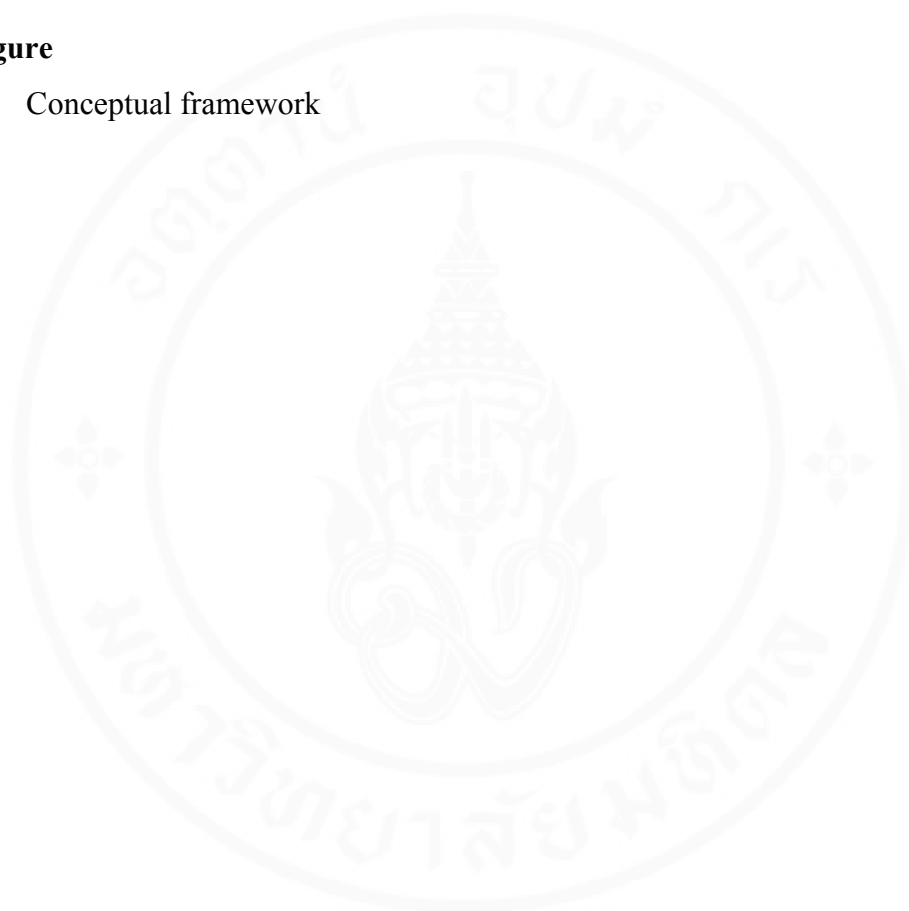
	Page
4.2.4 Types of samples	29
4.2.5 Types of patients	29
4.2.6 Days of hospitalization	30
4.2.7 History of hospitalization	30
4.2.8 Patients diagnostic diseases	31
4.3 Factors associated with MRSA among surgical patients	31
4.4 Resistant percentage of antibiotics	35
4.5 Multiple drug resistant pattern of antibiotics among MRSA patients	36
CHAPTER V DISCUSSION	37
5.1 General characteristic of surgical patients	37
5.2 Prevalence of MRSA among surgical patients	38
5.3 Factors associated with MRSA among surgical patients	41
5.4 Resistant percentage of antibiotics	42
5.5 Multiple drug resistant pattern of antibiotics of MRSA of surgical patient	43
CHAPTER VI CONCLUSION AND RECOMMENDATION	46
6.1 Conclusion	46
6.2 Recommendation	47
6.2.1 Recommendation for implementation	47
6.2.2 Recommendation for further study	48
REFERENCES	49
APPENDICES	57
Appendix A Letter from Hospitals	58
Appendix B Proof of ethical clearance	61
BIBLIOGRAPHY	62

LIST OF TABLES

Table	Page
2.1 Significant events in history of <i>S. aureus</i> and resistant development	7
2.2 Risk factors for hospital associated Methicillin resistant <i>S. aureus</i> (HA- MRSA) infections	12
4.1 Frequency and Percentage of General Characteristics of Surgical Patients	26
4.2 Prevalence of MRSA among surgical patients on basis of Age	28
4.3 Prevalence of MRSA among surgical patients on basis of Gender	28
4.4 Prevalence of MRSA among surgical patients on basis of Season	29
4.5 Prevalence of MRSA among Surgical Patients on basis of types of sample	29
4.6 Prevalence of MRSA among Surgical Patients on basis of types of department	30
4.7 Prevalence of MRSA among Surgical Patients on basis of days of hospitalization	30
4.8 Infection rate of MRSA among Surgical Patients on basis of history of hospitalization	31
4.9 Infection rate of MRSA Surgical Patients on basis of Patients diagnostic diseases	31
4.10 Association of Risk factors of MRSA with different variables with OR, 95% CI and p-value.	32
4.11 Antibiotics Resistant and Percentage of Resistant of MRSA	35
4.12 MDR pattern of antibiotics of MRSA patients	37

LIST OF FIGURES

Figure	Page
1.1 Conceptual framework	4



LIST OF ABBREVIATIONS

MRSA-	Methicillin Resistant Staphylococcus aureus
CDC-	Center for Disease control and Prevention
TUTH-	Tribhuvan University Teaching Hospital
OPD-	Outpatient Department
WHO-	World Health Organization
ICU-	Intensive care unit
ER-	Emergency room
MSSA-	Methicillin Sensitive Staphylococcus aureus
EARSS-	European Antimicrobial Resistance Surveillance System
HAIs-	Hospital acquired infections
SPSS-	Statistical Package for the Social Sciences
MDR-	Multiple Drug Resistant
UTI-	Urinary Tract Infection
C+S	Cefoxidime+ Sulbactam

CHAPTER I

INTRODUCTION

1.1 Background and justification

Methicillin resistant *S. aureus* (MRSA) has become a major problem in many countries, resulting in significant morbidity, mortality and healthcare costs. The origin of antibiotic resistance of *S. aureus* trace back to 1941 when Penicillin was first introduced. Initially, all *S. aureus* were sensitive to Penicillin[1]. Two years after this landmark event, strains of Penicillin resistant *S. aureus* had already emerged. In the ensuing decade, these Penicillin resistant *S. aureus* strains become highly prevalent in hospitals. By the 1960's, the strains had spread outside healthcare setting into the community. Methicillin which was used to combat these Penicillin resistant soon developed resistance within a year of its implementation [2].

As the use of Methicillin and other similar Penicillins increased, so did the incidence and prevalence of MRSA. The first documented United States outbreak of MRSA occurred in 1968 [3]. In the ensuing decade, after a brief decline, MRSA continued to spread throughout healthcare facilities but remained largely confined to healthcare facilities until 1981. However, MRSA outbreak became the first significant exception to this rule, making emergence of Community-Associated MRSA (CA-MRSA) by the outbreak affecting large population in the community settings [4].

According to the Centers for Disease Control and prevention (CDC), Number of both community associated and hospital-acquired infections has increased in the past 20 years. MRSA currently causes 1% of all Staphylococcal infections and more than 50% of healthcare associated infections. Each year in United States, more than 290, 000 hospitalized patients are infected with *S. aureus*. Of these, approximately, 126,000 are related to MRSA [5]. In distributing trend, antibiotic resistance among the isolates has increased because of antibiotic pressure. Currently, less than 5% of clinical isolates remain sensitive to Penicillin. A CDC survey revealed

that the proportion of Methicillin resistant isolates with sensitivity only to Vancomycin increased from 22.8% in 1987 to 56.2% in 1997 [6].

Now, MRSA has become a major clinical and epidemiological problem. Strains of MRSA may be epidemic in character (epidemic MRSA or EMRSA). The appearance of outbreaks of nosocomial infections due to EMRSA is a current problem in most of the countries. Previously limited to the large institutions, outbreaks of MRSA are now quite common in many hospital settings. Thus the notorious MRSA had evolved which 30 years later, is causing immense problems in effective treatment and is associated with considerable morbidity and mortality. The drug of choice for treating MRSA infections is Vancomycin. However, the emergence of Vancomycin-intermediate or Vancomycin-resistant *S. aureus* has become a very great challenge for clinicians[7].

Only scanty number of reports regarding MRSA is available in Nepal. The prevalence rate of MRSA was found to be 26.1% [8]. A bacteriological study at Tribhuvan University Teaching Hospital (TUTH) revealed that the most prevalent bacteria in the studied samples were *S. aureus* (60%) and about 23.5% were resistant to Methicillin[9]. MRSA was found in 31.4% of total *S. aureus* isolates from Kanti Children's Hospital and 11.7% from TUTH [10]. Sanjana et al.[11] also reported 39.6% Methicillin resistant *S. aureus* isolates at College of Medical Sciences-Teaching Hospital, Chitwan.

In Nepal, there is a limited drug regulation regarding the use of antibiotics. Due to the indiscriminate use of antibiotics without proven susceptibility testing, it has further led to the development of resistant strains. Lack of MRSA carriage screening among healthcare workers and healthy carrier contributed to profound transmission not only in healthcare facilities but also among community settings. In HAMS Hospital, Sumeru Hospital, Banepa Hospital the prevalence of nosocomial infection has been found which also includes the cases of resistance pattern of *Staphylococcus aureus* including MRSA in patients having surgical history.

The present study aimed primarily to assess the Methicillin resistant *S. aureus* (MRSA) among patients with surgery visiting at Hospitals of Kathmandu, Nepal. This study also analyzed the associated factors of MRSA. Moreover, the antibiotic susceptibility test of isolated MRSA was studied. Furthermore, the

investigation may also throw lights on screening of MRSA and measures to be taken for the prevention and control of MRSA in broad perspective.

1.2 Research Question

- What is prevalence of MRSA in patients with surgery visiting Hospitals of Kathmandu Valley, Nepal?
- What factors are associated with MRSA among patients with surgery at Hospitals of Kathmandu Valley, Nepal?

1.3 Objectives:

- To determine the prevalence of MRSA in surgical patients at Hospitals of Kathmandu Valley.
- To identify factors associated with MRSA in patients with surgery among patients visiting Hospitals of Kathmandu Valley.

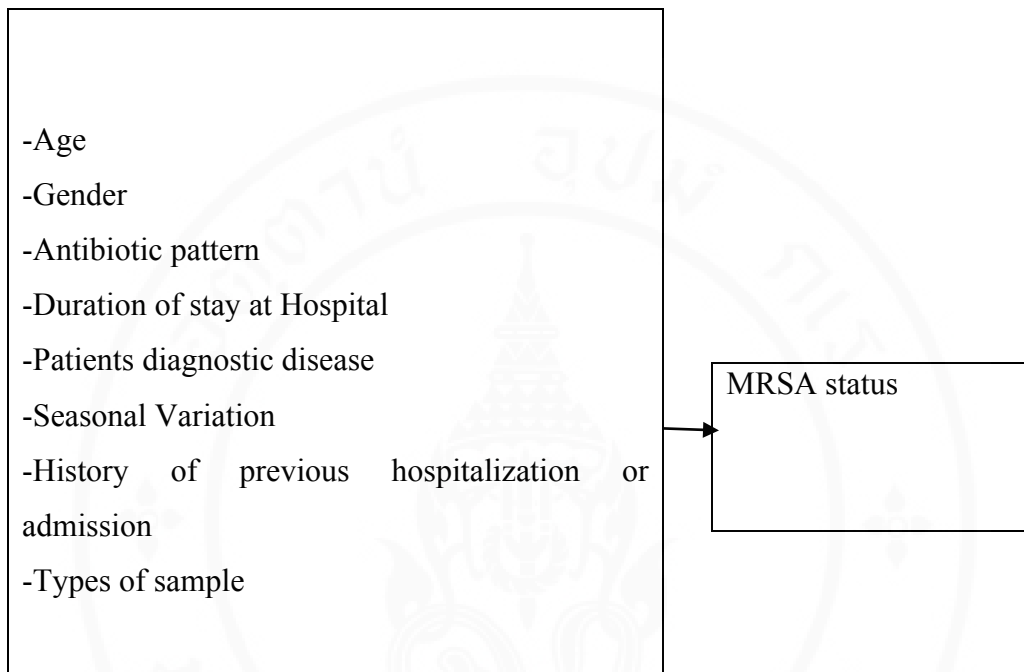
1.4.Hypotheses

There are association between age, gender, antibiotics pattern, duration of stay at hospital, patients diagnostic disease, seasonal variation ,history of previous hospitalization, types of samplewith MRSA status among surgical patients of Hospitals of Kathmandu Valley.

1.5. Conceptual Framework:

Independent Variables

Dependent Variables



1.6. Operational Definitions:

MRSA status: The presence or absence of Methicillin resistance *Staphylococcal aureus* isolated from surgical patients at hospitals of Kathmandu valley, Nepal.

Age: The age of the surgical patients who visit the hospitals of Kathmandu valley, Nepal.

Gender: The gender of the surgical patients who visit the hospitals of Kathmandu valley, Nepal

Antibiotic pattern: It refers to the antibiotics pattern used for diagnosis of MRSA sample from surgical patients.

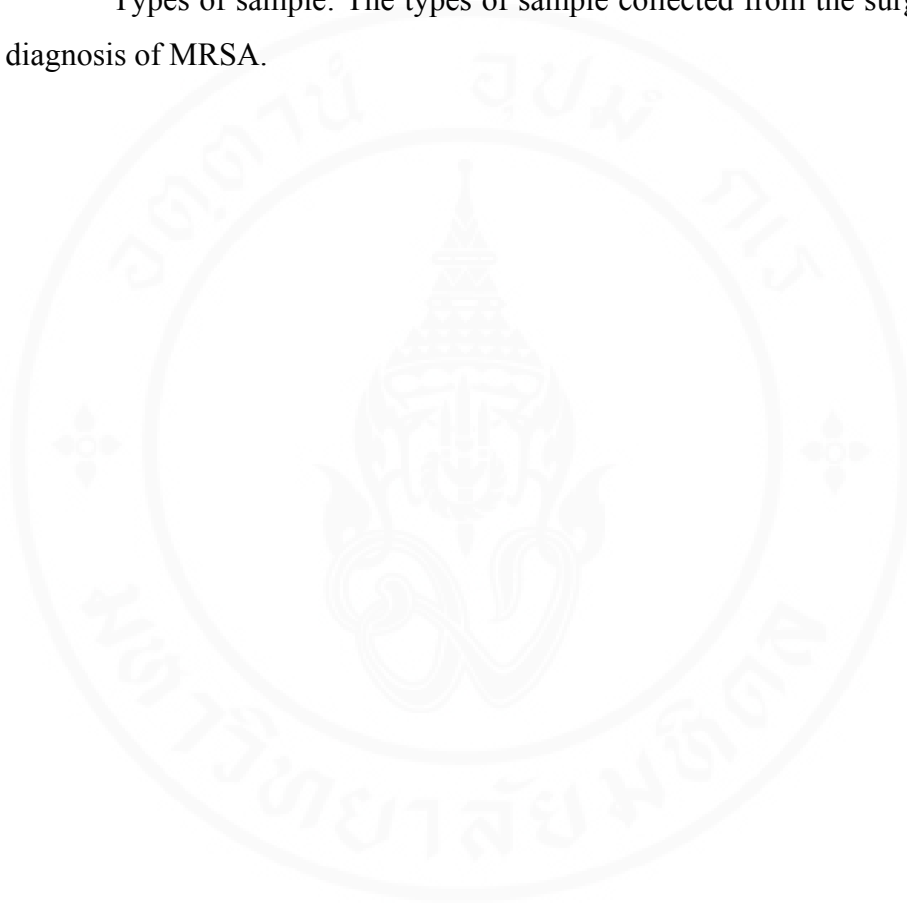
Duration of Stay at hospital: The time duration for the stay of patients at hospital for the recovery after surgery.

Patients' diagnostic disease: The type of diseases which causes patients to visit for surgery at Hospitals of Kathmandu valley, Nepal.

Seasonal Variation: The season of Nepal during one year duration when patients visit for surgery at Hospitals of Kathmandu valley, Nepal.

History of previous hospitalization: The history of patients previous visit at hospital or not who came for surgery at Hospitals of Kathmandu valley, Nepal.

Types of sample: The types of sample collected from the surgical patients for diagnosis of MRSA.



CHAPTER II

LITERATURE REVIEW

2.1. Methicillin resistant *S. aureus* and history

In spite of the fact that Staphylococci were discovered and described more than a century ago and that effective antibiotics have been used for more than 40 years, Staphylococci continue to be a major cause of human disease not only in hospitalized individuals, but also to be a common cause of bacterial infections in the community. All over the world in poor and developed countries alike, *S. aureus* is the leading cause of bacterial infection involving the bloodstream, lower respiratory tract, skin and soft tissues.

Methicillin is semi synthetic Penicillin commonly used to treat infections caused by Penicillin-resistant Staphylococci. Although this class of antibiotic is very effective in treating most Staphylococcal infections, Some Staphylococcus species (particularly *S. aureus*) have developed resistance to Methicillin and can no longer be killed by this antibiotic.

Resistance has been observed to every class of antibiotic, regardless of whether it was derived from natural or synthetic origins. The first example of this was observed with Penicillin resistant *S. aureus* in the 1940s. The second line of defense against bacterial infection was β -lactam where resistance developed within 10 years. The development of Methicillin was thought to deliver the 'magic bullet' but in 1968, Methicillin resistant *S. aureus* (MRSA) was observed. This was followed with Vancomycin resistance in 1996[12].

Table:2.1 Significant events in history of *S. aureus* and resistant development [13]

Date	Event
1881	Ogeston identifies grape like clustering bacteria in human pus
1884	Rosenbach differentiates staphylococcal species based on pigment
Pre-1940s	Surgeons fear staphylococcal wound infections; Significant mortality from invasive infections observed
1950s	Multi-drug resistant strains of <i>S. aureus</i> emerge, resistance spread by phase 80a
1959	Development of Methicillin to treat Penicillin resistant <i>S. aureus</i>
1961	Barber induces Methicillin resistance in <i>S. aureus</i> laboratory strains
1963	Jevons describes the first naturally occurring Methicillin -resistant <i>S. aureus</i> (MRSA)
1960s to 2000s	Genetic basis for Methicillin resistance described; PBP2a characterize
2002	Glycopeptides-intermediate <i>S. aureus</i> strains isolated
2000 to Present	Increased occurrence of community acquired (CA-MRSA) and hospital acquired (HA-MRSA)

Since the emergency of Penicillinase producing *S. aureus* in the middle of the forties, the prevalence of such strains have increased dramatically. Several “epidemic waves” of antibiotic resistant *S. aureus* have occurred both in hospitals and community associations since then.

In USA approximately 60 % of all Staphylococcal infections in the intensive care unit are caused by MRSA [14]. The mortality rate associated with invasive MRSA is approximately 20 %. It is the leading cause of death by a single infectious agent [15]. The rapid emergence of community associated MRSA (CA-MRSA) infections is one of the most alarming events in the history of infectious diseases. It was first reported in Western Australia in the 1990s and spread worldwide in recent years. *S. aureus* infections can occur in healthy individuals outside the risk

groups. The prevalence of MRSA is steadily increasing, particularly in children and young adults.

2.2. Epidemiology of MRSA

Recently, strains of multiple drug resistant *S. aureus* have appeared and proven very difficult to treat [16]. During the late 1950s and early 1960s, *S. aureus* caused considerable morbidity and mortality as a nosocomial or hospital acquired pathogen and has become the head leading cause of nosocomial infection during the last two decades[17]. Since then Penicillinase resistant semi synthetic Penicillin as proved to be successful antimicrobial agents in the treatment of staphylococcus infections. Unfortunately, MRSA (Methicillin resistant *S. aureus*) strains isolated are on increasing resistant to multiple non- β -lactam containing antimicrobial drugs. Recent report of Vancomycin resistant *S. aureus* fore shows further threat by this “super bug”.

MRSA is a problematic pathogen in human medicine and appears to be emerging in the world. Historically, hospital associated MRSA infections have predominated in humans and contributed to significant illness and death. Recently a shift in the epidemiology of MRSA infections have been documented, where by community associated Methicillin resistant *S. aureus* (CA-MRSA) infections have become more common.

The center for Disease Control [5]estimates that 25% to 30 % of the American population is colonized with *S. aureus*, and approximately 1% is colonized with MRSA.

2.3. Community acquired MRSA (CA-MRSA)

The scope of Staphylococcal antibiotic resistance is currently extending from clinical setting to new patient populations. While antibiotic-reistatant *S. aureus* was once thought to be confined to large hospitals, community outbreaks of MRSA are now occurring in individuals without traditional risk-factors for carriage of MRSA. Although outpatients with a history of intravenous drug use, nursing home residency

or recent hospitalization appear to have a heightened risk for MRSA carriage, a significant minority of patient admitted with MRSA carriage do not have such identifiable risk-factor [18].

MRSA infections that are acquired in the community are classified into two categories, with and without healthcare-associated risk factors, the latter category representing the true CA-MRSA infections. Recognized healthcare-associated risk factors are admission to hospital in the previous year, residence in a long-term care facility, dialysis, surgery, permanent indwelling catheters or the use of medical devices inserted through the skin [19].

There is distinction between community-acquired MRSA infections with and without healthcare-associated risk factors. As the healthcare-associated CA-MRSA infections are caused by the strains tends to be relevant with HA-MRSA. The strains show different epidemiological, clinical, microbiological, genetic and therapeutic behaviors. Current evidence suggests that CA-MRSA strains that are acquired in the absence of healthcare-associated risk factors evolved from Methicillin-susceptible *S. aureus* in the community that acquired specific genotypic features, including the Staphylococcal cassette chromosome *mec* (*SCCmec*)IV element [20].

Community associated MRSA infections are commonly reported in miscellaneous groups such as patients with cystic fibrosis, day-care centers, wrestling teams, and prisons [21]. The antimicrobial patterns of these strains are unique and differ from HA-MRSA, because they are resistant to Methicillin but are not multidrug resistance. CA-MRSA isolates were more likely to be susceptible to non β -lactam antibiotics than HCA-MRSA isolates[22]. Many are sensitive to trimethoprim-sulfamethazole, clindamycin, aminoglycosides and quinolones [23].

Initial reports of CA-MRSA initially appeared in the late 1990's, but since 2002 a more acute rise in CA-MRSA cases has occurred in the US [24]. Community epidemiological studies estimate that 1-9% of the United States population is now colonized with CA-MRSA [25]. In a person colonized with CA-MRSA, the risk if invasive infection is greater than with either HA-MRSA or MSSA. In a study of military recruits, 38% of those colonized with CA-MRSA developed infection within 8 weeks[26]. However, studies of this aspect are not carried out, and authentic records on community- associated MRSA in Nepal is not available.

2.3.1. Risk factors associated with CA-MRSA

The following risk factors may be associated with CA-MRSA infections:
[27]

- History of MRSA infection or colonization in patient or close contact.
- High prevalence of CA-MRSA in local community or patient population.
- Recurrent skin disease.
- Crowded living conditions (e.g. homeless shelters, military barracks).
- History of incarceration.
- Participation in contact sports.
- Skin or soft tissue infection with poor response to β -lactam antibiotics.
- Recent and/or frequent antibiotic use.
- Injection drug use.
- Child under two years of age.
- Male with history of having sex with men.
- Shaving of body hair.

2.3.2. Prevention and control of CA-MRSA

Since CA-MRSA is an emerging pathogen, more studies are needed to determine the best methods for controlling and preventing the spread of MRSA in the community. Current strategies that appear to be successful include increased awareness, early detection and appropriate treatment, and maintaining a clean environment.

2.4. Hospital acquired MRSA (HA-MRSA)

Nosocomial or HA-MRSA is defined as the MRSA acquired after 72-hours of hospitalization or related to an intervention during the hospital stay[28]. HA-MRSA strains tend to be multidrug-resistant (MDR) and to colonize and infect patients during hospitalization or in long-term healthcare facilities, after surgery or following contact with a person who has an MRSA infection or is a carrier of MRSA.

Periodic outbreaks of infection were first reported in hospitals where high levels of oxacillin or Methicillin were used and intensive care environments. However, from the 1980s onwards, HA-MRSA has been a significant regional health threat, causing infections initially in large hospitals and later in smaller community hospitals [29].

Hospital acquired MRSA are usually associated with increased expression of multiple genes, including those of aminoglycoside resistance. In HA-MRSA strains, genetic elements vary by isolate. Hospital associated isolates have been found to most commonly carry *SCCmec* types I, II, and III. *SCCmec* types II and III are both larger elements that have the ability to provide resistance to additional antibiotics types, so HA-MRSA has commonly been associated with multi-drug resistance [30]. Contaminated environment surfaces may serve as a reservoir of MRSA in hospitals. Recently, the association between the dispersion of MRSA strains into the environment by colonized patients and MRSA epidemics has been reported via use of molecular typing methods[31]. Many results demonstrated that MRSA from patients could contaminate the environment, whereas MRSA from the environment might be potentially transmitted to patients via health care workers under unsatisfactory infection control.

Table 2.2: Risk factors for hospital associated Methicillin resistant *S. aureus* (HA-MRSA) infections[32].

Exposure to a patient known to be colonized or infected with MRSA
<p>Host factors</p> <ul style="list-style-type: none"> • History of stay in an ICU during the last 5 years • History of surgery during the last 5 years • Prolonged hospital stay (21 days or longer) • Intravenous drug use • Resistance in a skilled nursing facility • Presence of open skin lesions <p>Chronic medical illness</p> <ul style="list-style-type: none"> • Diabetes mellitus Type I • Patients undergoing hemodialysis <p>Impaired immune function</p> <ul style="list-style-type: none"> • AIDS • Quantitative defect in leukocyte function • Quantitative defect in leukocyte function (chronic granulomatous disease, Job's syndrome)

According to the data from US National Nosocomial Infections Surveillance System [31], in the last four decades, MRSA has been one of the most widely known multidrug-resistant organisms in healthcare institutions, about 61 % of all *S. aureus* isolates in intensive care units harbored Methicillin resistance.

The prevalence of HA-MRSA has been varied greatly by geography in Europe. The European Antimicrobial Resistance Surveillance System (EARSS), which collected 50,759 nosocomial isolates from 495 hospitals in 26 countries, reveals that the prevalence of MRSA was found to be largely variations in countries and sometimes between hospitals within a country. In some northern countries, such as Sweden and Denmark, the prevalence was less than 1%; in southern countries, such as Greece and Italy, the prevalence was upwards of 40%. The UK saw the greatest

percentage increase, from 30.5% in 1999 to 44.5% in 2002. Slovenia was the only country to show a significant decrease, declining from 22.3% to 14.7%[32].

In the US and some Asian countries, an increase in HA-MRSA has been observed. One study based in a large Taiwan university hospital found the prevalence of MRSA isolates increased from 26.7% of nosocomial infections in 1990 to 77% in 2001[33].

Hospital-acquired infections (HAIs) (also called “nosocomial infections” or “health care associated infections”) are infections that patients acquires while in hospital being treated for some other condition. Most infections that become clinically evident after 48 hours of hospitalization are considered hospital-acquired. Infections that occur after the patient is discharged from the hospital can be considered healthcare-associated if the organism were acquired during the hospital stay [34].

Hospital acquired infection classified as:[35]

- a) Infection contracted and developing outside the hospitals and require admission to the hospital.
- b) Infections contracted outside, but clinically apparent when the patient is in the hospital.
- c) Infections contracted, and developing when the patient is in hospital.
- d) Infections contracted within the hospital, but not becoming clinically apparent until after the patient has been discharged.
- e) Infections contracted by hospital staff as a consequence of their work, whether or not this involves direct contact with patients.

Nosocomial infections typically affect patients, who are immunocompromised because of age, underlying diseases, or medical or surgical treatment. The patients hospitalized in ICUs are 5 to 10 times more likely to acquire nosocomial infections than other hospital patients.

The bacteria *S. aureus* is found on the skin or in the nose in around 30% of the population with no ill effects. The antibiotic resistance form, MRSA, whose proportion of infection has been increased by nearly 50-fold since 1991 [36].

2.5 Variables

2.5.1. Sources

Sources of the hospital acquired infection are infecting microorganisms from fellow patients, which may be multidrug resistant-infecting organisms from hospital staff, infecting organisms from instrument, blood products, intravenous fluid, from patient's normal flora, insects are also source of multidrug infection, organism may be present in air, dust, water, antiseptic solution, food, surfaces contaminated by patient's secretions, blood fluids, etc[37].

2.5.2. Predisposing factors

Predisposing factors for hospital acquired infection are hospital environment heavily laden with a variety of pathogens, organisms present in air, dust, antiseptic location, water and food or may be spread from sheddings from the patients, and hospital microbial flora is usually multi-drug resistant. Patients have impaired defense mechanism due to disease therapy and investigations in the hospital, instrumentation hospitals may introduce infection, blood, blood products may transmit infections and accidental inoculation of infectious material [38].

Following are some factors responsible for MRSA hospital infection [39-41]

I. Susceptibility to infection

Natural resistance to infection is lower in infants and the elderly patients, who often constituted the majority of hospital patients, because of long their stay and decreased immunity and hence have high risk of getting hospital infections. Pre-existing diseases, such as diabetes, or other conditions for which the patient was admitted to hospital and the medical of surgical treatment including immunosuppressive drugs, radiotherapy or splenectomy, may also reduce the patient's natural resistance to disease. Moreover, the natural defence mechanism of the body surfaces may be bypassed either by injury or by procedures such as surgery, insertion of indwelling catheter, tracheostomy or ventilator support.

II. Contact with other patients and staff

Patients with comparable susceptibility to infection tend to be concentrated in the same area, e.g. in neonate ward, post-operative ward, burn ward etc., where infected and non-infected patients may be cared for by the staff, thus creating numerous opportunities for the spread of *S. aureus* by direct contact.

III. Inanimate reservoirs of infection

S. aureus derived from the body flora of the hospital population, are found in the air, dust, and on equipments and materials in use in hospital where they may survive for a long period and infect the susceptible patient.

IV. Role of antibiotic treatment

Treatment of hospital patients with antibiotics exerts strong selective pressures on the normal microbial flora. Sensitive species or strains which normally do not maintain a protective function are eliminated where as those that are more resistant survive and become endemic in the hospital population.

2.5.3. Modes of transmission

Hospital acquired infections spreads by various routes as follows: [42]

Contact: Main route of transmission, Transmission by hands or clothing of hospital personnel and even patient himself or transmitted by contact with inanimate objects.

Air-borne route: Transmitted by inhalation of droplet, dust from bedding floors, exudates dispersed from wounds, skin, etc., and aerosols produced by nebulizers, humidifiers and air conditioning apparatus.

Oral route: Transmitted by ingestion of contaminated food or water.

Parenteral route: Transmitted by the use of contaminated syringes, needles and other instruments, by administration of contaminated blood, blood products, infusion fluids or tissue.

Inoculation route: infection by inoculation occurs when infected material is inoculated directly into tissue or transfusion of contaminated blood or inoculation of material containing the organisms.

Iatrogenic transfer: Infections may also occur during diagnostic or therapeutic procedures, if proper care is not taken.

2.5.4. Prevention and control

2.5.4.1. Strategies

According to Nayak M.[43], the strategies for the prevention of hospital acquired infection are of following ways

a) Exclusion the source of infection

- Health care providers should avoid direct contact with patients, fomites especially body fluids.
- Should wear barriers such as gloves when contact is necessary.
- Should avoid puncturing oneself with any fluid-contaminated instruments.
- Frequent hand-washing especially between patients.
- Careful handling, cleaning and disinfection of fomites.
- Should do possible use of single-use disposable items
- Should do patient isolation for seriously infected patient.

Air flow system plays an important role in the dissemination of organisms by airborne route. This can be reduced by isolating patients.

b) Enhancing host ability to resist infection:

Host resistance can be enhanced by boosting immunity and reducing risk factors.

- Boosting specific immunity- passive immunization provides short term protection.
- Appropriate use of prophylactic antibiotics prevents infection to an extent. But there is a tendency to misuse antibiotics- by using them too often or for long, thereby increasing the selection pressure for the emergence of resistance organism.
- Care of invasive devices is essential to reduce the risk of endogenous infection from skin and form other instrumental devices.

2.5.4.2. Cleaning, sterilization and disinfection:

Proper infection control procedures need to be followed for both patient safety and health care personnel. Cleaning, Sterilization and disinfection are important procedures need to be carried out for hospital infection control. WHO guidelines recommended routine cleaning of hospital environment to ensure that environment is visibly clean, and free from dust and soil. There must be policies specifying the frequency of cleaning agents used for walls, floors, windows, beds, curtains, screens, fixtures, furnitures, bath and toilets, all reused medical devices [37].

2.5.4.3. Handwashing

Hygiene has been considered one of the cornerstones of infection control. Nosocomial infection, many of which are transmitted from patient to patient by poorly sanitized hands of health care workers; exert a significant toll in human and economic terms every year. So, health care personnel need to follow proper hand washing technique for prevention of hospital infection [44].

The need and importance of hand washing under the following conditions have been adopted by CDC:

- Before all invasive procedures

- Before caring of susceptible patients
- Before and after touching wounds and invasive devices
- After caring for infected patients
- Between contact with patients in high risk areas

2.6. Management of hospital acquired infection

2.6.1. Strategies

Management of hospital acquired infection is not an easy task; it requires education of health care personnel regarding infection control procedures and strict adherence to rules and policies of infection control. There are supportive elements to be considered for the infection control programme[45], which are:

1. The development of an effective surveillance system
2. The development of policies to reduce risk of hospital acquired infection
3. The maintenance of a continuing education programme for hospital personnel

2.6.2. Waste management system:

Segregation at the source and safe storage is the key to whole waste management process. It should be carried out at the point of generation to keep general waste from infectious waste. Treatment of waste is required to disinfect, or decontaminate by chemical disinfectants of waste at right source, so that there is no longer the source of pathogenic microorganisms. After treatment residue can be handled safely, transported, stored or disposed. Infectious waste needs to be destroyed of infected by recommended methods of disinfecting or destruction of biologically infected waste such as autoclaving and microwaving. Incineration is the better option for the large scale infectious waste management.

2.6.2.1. Documentation, employee health and training:

Personal health service can contribute to infection control activities by establishing such policies and procedures as placement evaluations, health and safety education, immunization programmes, monitoring potentially

harmful infectious exposures and instituting appropriating preventive measures. These measures should be implemented among healthcare workers providing information regarding infection risk related to employment and developing guidelines for restricting work because of infectious disease and maintaining health records of all health care workers.

Health care providers have the duty to protect the health personnel as well as patients. Health care workers are exposed to a wide array of health and safety hazards including exposure to biologic agents, stress, injury and chemical agents. Immunization of the personnel is an important component of hospital control programs [46].

2.7. Epidemiology of MRSA carriage

S. aureus has long been known to colonize the human body, and remains as carriage. Colonization has been defined as the presence of a microorganism not part of the normal flora of the host that is multiplying. The most consistent site of *S. aureus* colonization is nares. However cutaneous surfaces, conjunctiva, the intestinal tract, and the throat have been also common sites of colonization [46].

Carriage of MRSA is an important risk-factor for subsequent infection, and facilitates cross-transmission. Colonization is an important step in the pathogenesis of *S. aureus* infection and is instrumental in the epidemiology of these bacteria. Approximately 20 % of the general population is persistently colonized with *S. aureus*, most frequently in the anterior nares, although other body sites, such as the perineum and throat, may also be colonized[22].

The carriage rate of MRSA in the population is starting. A 2004 study showed a carriage rate of about 20 percent. Four years on, that rate is now close to 50 percent, [44]. The figure are very similar in Europe, According to a 2006 review from the EARSS [45] the prevalence of MRSA in Western Europe and the US is equivalent, with the carriage rate in the UK, Ireland, France, Spain and Italy at 25 to 50 percent, with slightly lower rates in Germany and Eastern Europe.

CHAPTER III

MATERIALS AND METHODS

3.1 Study Design:

A hospital based cross-sectional analytic study of surgical patients profiles or records was conducted. During the study period 797 surgical patients diagnosed bacterial infection with bacterial cultures were processed from January 2013 to December 2013.

3.2. Study Site:

The Hospitals of Kathmandu valley, Nepal were study sites.

3.3. Population sample, sample size and sampling:

The surgical patients visiting 3 Hospitals of Kathmandu Valley, Nepal from January 2013 to December 2013 were population sample. In HAMS Hospital which is 150 bedded, Sumeru Hospital which is 100 bedded Hospital and Banepa Hospital which is 50 bedded, there was problem of nosocomial infection and about 1800 patients visit for surgery throughout the year.

Sample Size: The sample size was calculated as follows

$$n = \frac{z^2 \alpha / 2 P (1-P)}{d^2}$$

n = estimated sample size

P = proportion of MRSA in surgical patients = 45% = 0.45

z = level of statistical significance, set on $\alpha = 0.05$, $z^2 \alpha / 2 = 1.96$

d = allowable error = 0.05

Total sample size = 380 (at least)

$$= 380$$

$$nf = n / (1 + n/N)$$

$$= 380 / (1 + 380/1800) = 314$$

3.4. Inclusion and Exclusion criteria:

a) Inclusion:

Inclusion criteria include patients' profiles or records as follows:

- Surgical Patients with all infections with result of culture of bacteria.
- Only the growth of bacteria within 48 hrs in the study.
- Both sex and every age group of surgical patients

b) Exclusion:

• The insufficient information of the patient history including antibiotic usage was excluded.

• MRSA samples that was not labelled (age and gender) properly and with visible signs of contamination was excluded.

3.5 Data collection:

The secondary data were collected from the hospital record which includes patients history and laboratory diagnosis and reporting of antibiotics used.

3.5.1. Method of collection (Quality control)

Records including clinical history, type of infection, ward, gender, age, consumption of antibiotics, were obtained from surgical patients. Their duration of stay, development of infection 48 - 72 hours after admission to the hospital, use of prosthetic devices, use of invasive procedures such as catheterization, and use of hospital instruments were also recorded.

The collected data from the hospital were recorded and entered in Excel. Then the entered data were transferred to SPSS programming for data analysis.

3.5.2. Research instrument:

The chart (records) of the patients history used in the hospital were research instrument for the study.

3.5.3. Quality of instrument:

The verification of the hospital record was done for removing the error of the data entry. All three hospitals followed same protocol for diagnosis of MRSA with 0.5 Mcfarland tube and ATCC culture of *Staphylococcus aureus* 25923.

3.6. Data analysis:

The data analysis was done by SPSS version 18 (Mahidol University) and Microsoft Excel.

3.6.1. Data entry(Quality control):

The data entry was done on Microsoft Excel and SPSS version 18(Mahidol University) and cross checked from the original file of the hospital.

3.6.2. Classification criteria:

The samples of MRSA were classified as positive and negative. Similarly the antibiotics used for the diagnosis of MRSA were categorised as Resistant and sensitive samples. The coding of resistance and sensitivity pattern, types of antibiotics

used during culture, Presence and absence of MRSA were done for analysis of the data.

3.6.3. Statistics:

a) Descriptive statistics: The prevalence of the MRSA was presented by using descriptive statistics such as percentage.

b) Inferential statistics: The association between studied factors and MRSA status (positive/negative) was analysed by using chi-square, 95% CI and odds ratio at critical level of $\alpha=0.05$.

3.7. Ethical consideration:

The ethical consideration proposal was sent to the hospital administration and similarly the ethical approval was obtained from the ethical committee of Faculty of Public Health, Mahidol University with protocol no 185/2556.

SS

CHAPTER IV

RESULTS

The purpose of the study was to find prevalence and risk factors of Methicillin Resistant *Staphylococcus aureus* (MRSA) among surgical patients from the Hospitals of Nepal. The study consisted of 797 surgical patients. Results of the study were presented by descriptive statistics using frequencies and percentage and analyzed by calculating infection rate and 95% CI , OR and p-value using chi-square test for association between dependent and independent variables. The level of significance for all association was set up at p- value of less than 0.05. Similarly the antibiotic resistant pattern was also found for drug sensitivity test. Results of the study are presented as follows:

- 4.1. General characteristics of surgical patients
- 4.2. Prevalence of MRSA among surgical patients
 - 4.2.1. Age
 - 4.2.2. Gender
 - 4.2.3. Seasonal variation
 - 4.2.4. Types of samples
 - 4.2.5. Types of patients
 - 4.2.6. Days of hospitalization
 - 4.2.7. History of hospitalization
 - 4.2.8. Patients' diagnostic diseases
- 4.3. Factors associated with MRSA among surgical patients
- 4.4. Resistant percentage of antibiotics
- 4.5. Multiple drug resistant pattern of antibiotics among MRSA patients

4.1 General characteristics of surgical patients

The general characteristics of studied patients as age, gender, seasonal variation, types of samples, types of patients, days of hospitalization, history of hospitalization, patients diagnostic diseases and types of organisms isolated are described in Table 4.1

Among 797 surgical patients, the maximum age was 88 years and the minimum age was under 1 year (including infants) with mean age of patients as 30.83 years. Approximately 40.9% of surgical patients aged between 16 and 30 years and the least were above 76 years with only 1.5%. Most patients were female with 72.9%. On seasonal variation, the higher number of patients got surgery on summer with 28.4% and least patients in early autumn with 6.8%. The higher number of urine samples was processed for diagnosis with 78.7% and only 2.4% of tissues were cultured as specimens of surgical patients. During the study time, 55.8% of patients were from inpatient department, whereas, 44.2% were from outpatient department. Among the studied participants, 87.0% were hospitalized for less than 5 days and 13.0% were hospitalized for more than 5 days with mean days of hospitalization with 3.45 days. Among the studied participants, 40% of patients have diagnostic disease as Trauma whereas 6.3% have Bone fracture.

During the study 47.1% found to be infection of *E.coli* followed by 34.1% (272 patients) of *Staphylococcus aureus* were found and out of 272 diagnosed Staphylococcus infection, 79 were found to be Methicillin resistance as MRSA. The prevalence of MRSA was found 9.9% among total studied surgical patients.

Table 4.1 Frequency and Percentage of General Characteristics of Surgical Patients(N=797)

Age group	Frequency	Percent
1-15	123	15.4
16-30	326	40.9
31-45	197	24.7
46-60	101	12.7
61-75	38	4.8
76-90	12	1.5
Mean± SD	30.83±17.25	
Minimum, Maximum	0,88	
Gender		
Male	216	27.1
Female	581	72.9
Seasonal variation		
Spring	161	20.2
Summer	226	28.4
Monsoon	151	18.9
Early Autumn	54	6.8
Late Autumn	94	11.8
Winter	111	13.9
Types of Sample		
Urine	627	78.7
Blood	100	12.5
Wound Swab	51	6.4
Tissue	19	2.4

Table 4.1 Frequency and Percentage of General Characteristics of Surgical Patients(N=797) (Cont.)

General characteristics	Frequency	Percent
Outpatient and Inpatient		
IPD	445	55.8
OPD	352	44.2
Days of Hospitalization		
No Hospitalization	352	44.2
< 5 days	387	48.6
> 5 days	58	7.3
Total	797	100.0
Minimum, Maximum	0,9	
Patients diagnostic disease		
Bacterimia	119	14.9
Bone fracture	50	6.3
Hemorrhoid	55	6.9
Skin infection	108	13.6
Tracheal infection	69	8.7
Trauma	319	40.0
UTI	77	9.7
Types of Organisms		
E.coli	375	47.1
Staphylococcus sps		
a) MSSA	193	24.2
b) MRSA	79	9.9
Salmonella sps	90	11.3
Klebsiellasps	28	3.5
Streptococcus sps	20	2.5
Proteus sps	11	1.4
Citrobactersps	1	0.1
Prevalance of MRSA among total surgical patients=79/797=9.9%		

4.2 Prevalence of MRSA among Surgical Patients

The infection rate or prevalence of MRSA was calculated among 797 surgical patients as follows.

4.2.1 Age

The prevalence of MRSA was found to be relatively higher among patients of above 75 years of age and least among patients of age group 16-30 years. Details are presented in Table 4.2

Table 4.2 Prevalence of MRSA among surgical patients on basis of Age

Age Group(years)	Total Cases	MRSA cases	Prevalence
Below 15 years	123	16	13.0
16-30	326	21	6.4
31-45	197	22	11.2
46-60	101	14	13.9
Above 60 years	50	6	27.2
Total	797	79	9.9

4.2.2 Gender

The male surgical patients were found to be relatively higher prevalence than female surgical patients, 18.1% Vs 6.9%. Details were shown in Table 4.3

Table 4.3 Prevalence of MRSA among surgical patients on basis of Gender

Gender Group	Total Cases	MRSA cases	Prevalence
Male	216	39	18.1
Female	581	40	6.9
Total	797	79	9.9

4.2.3 Seasonal Variation

The prevalence in late autumn season (16.0%) was found to be relatively higher than that in other seasons. Least infection was found in early autumn season (1.9%).

Table 4.4 Prevalence of MRSA among Surgical Patients on basis of Season.

Seasonal Variation	Total cases	MRSA cases	Prevalence
Spring	161	14	8.7
Summer	226	21	9.3
Monsoon	151	17	11.3
Early Autumn	54	1	1.9
Late Autumn	94	15	16.0
Winter	111	11	9.9
Total	797	79	9.9

4.2.4 Types of Samples

The high prevalence of MRSA was found in wound swab samples with 64.7% followed by tissue with 57.9% collected from the surgical patients.

Table 4.5 Prevalence of MRSA among Surgical Patients on basis of types of samples

Types of Sample	Total Cases	MRSA cases	Prevalence
Urine	627	20	3.2
Blood	100	15	15.0
Wound swab	51	33	64.7
Tissue	19	11	57.9
Total	797	79	9.9

4.2.5 Types of Department

The prevalence was found similar in both inpatient and outpatient (10.1% and 9.7%, respectively)

Table 4.6 Prevalence of MRSA among Surgical Patients on basis of types of department

Department	Total cases	MRSA cases	Prevalence
IPD	445	45	10.1
OPD	352	34	9.7
Total	797	79	9.9

4.2.6 Days of Hospitalization

The prevalence was found high in case of more than 5 days of hospitalized patient with 15.5%.

Table 4.7 Prevalence of MRSA among Surgical Patients on basis of days of hospitalization

Days of Hospitalization	Total cases	MRSA cases	Prevalence
No Hospitalization	352	34	9.7
< 5 days	387	36	9.3
> 5 days	58	9	15.5
Total	797	79	9.9

4.2.7 History of Hospitalization

In case of MRSA infection the history of hospitalization and not hospitalization both had similar prevalence with nearly 10%.

Table 4.8 Infection rate of MRSA among Surgical Patients on basis of history of hospitalization

History of Hospitalization	Total cases	MRSA cases	Prevalence
No	422	42	10.0
Yes	375	37	9.9
Total	797	79	9.9

4.2.8 Patients Diagnostic Disease

The patients diagnostic diseases shows similar infection rate in all samples with nearly 10%.

Table 4.9 Infection rate of MRSA Surgical Patients on basis of Patients diagnostic diseases

Patients diagnostic Disease	Total cases	MRSA cases	Prevalence
Trauma	319	31	9.7
Bacterimia	119	12	10.1
Skin Infection	108	11	10.2
UTI	77	8	10.4
Hemorrhoid	55	6	10.9
Tracheal infection	69	6	8.7
Bone Fracture	50	5	10.0
Total	797	79	9.9

4.3 Factors associated with MRSA among Surgical patients

The association between MRSA presence and absence with associated risk factors shows results as Table 4.18.

On association of age OR showed less risk among young adult, adult and senior citizen comparative to children age group and similar risk among middle aged people with $p = 0.056$.

In case of Gender male were risky than female with OR 2.98 and $p < 0.001$.

In case of seasonal variation Monsoon and late autumn season were of chance of MRSA infection comparative to winter season and spring, summer, and late autumn season bears less risk of getting infection with $p\text{-value} = 0.204$.

In case of types of samples as wound swab and tissue were of higher risk with OR 36.25 and 27.19 respectively in comparison to other samples with $p < 0.001$. In case of type of department OPD patients were slightly at risk in comparison to OPD patient with OR 1.05 and $p = 0.832$.

In case of days of hospitalization during surgery, patients with greater than 5 days of hospitalization were at higher risk than patients with less than 5 days of hospitalization and no hospitalization with $p = 0.337$.

The history of hospitalization before surgery shows similar chance of getting infection of MRSA among both hospitalized and non hospitalized patients with $p = 0.97$.

The diagnostic diseases of patient shows similar risk among all types of disease with slightly less risk in hemorrhoid patients in comparison to bone fracture patients with $p = 1.0$.

Table 4.10 Association of Risk factors of MRSA with different variables with OR, 95% CI and p-value.

		Non-		Lower	Upper	OR	p
		MRSA	MRSA				
Age	Young						
	Adult	21	305			1	0.056
	Children	16	107	1.23	4.81	2.43	0.011
	Adult	22	175	1.03	3.65	1.93	0.041
	Middle						
	Aged	14	87	1.19	5.08	2.46	0.015
Senior							
	Citizen	6	44	0.79	5.48	2.08	0.135

Table 4.10 Association of Risk factors of MRSA with different variables with OR, 95% CI and p-value (Cont.)

		MRSA	Non-MRSA	Lower	Upper	OR	p
Gender	Male	39	177	1.86	4.78	2.98	<0.001
	Female	40	541				
Seasonal Variation							
	Spring	14	147	0.378	1.985	0.866	0.734
	Summer	21	205	0.432	2.007	0.931	0.856
	Monsoon	17	134	0.517	2.57	1.153	0.727
	Early Autumn	1	53	0.022	1.365	0.172	0.096
	Late Autumn	15	79	0.751	3.967	1.726	0.199
	Winter	11	100				0.204
Types of Sample							
	Urine	20	607	0.01	0.066	0.24	<.001
	Blood	15	85	0.04	0.372	0.128	
	Wound swab	33	18	0.45	3.913	1.333	
	Tissue	11	8				
Outpatient and Inpatient							
	IPD	45	400	0.66	1.682	1.052	0.832
	OPD	34	318			1.0	
Days of Hospitalization							
	No Hospitalization						
	on	34	318	0.26	1.288	0.582	0.337
	< 5 days	36	351	0.25	1.229	0.558	
	> 5 days	9	49				

Table 4.10 Association of Risk factors of MRSA with different variables with OR, 95% CI and p-value (Cont.)

	MRSA	Non-MRSA	Lower	Upper	OR	p
History of Hospitalization						
Hospitalization						
No	42	380	0.63	1.608	1.01	0.968
Yes	37	338				
Patients underlying Disease						
Trauma	31	288	0.38	2.487	0.967	1
Bacterimia	12	107	0.30	3.115	0.958	
Skin						
Infection	11	97	0.35	3.237	1.056	
UTI	8	69	0.37	2.559	0.978	
Hemorrhoid	6	49	0.27	2.498	0.821	
Tracheal						
infection	6	63	0.41	2.109	0.928	
Bone						
Fracture	5	45			1.0	

4.4 Resistant Pattern of Antibiotics

The resistant pattern of antibiotics showed high rate of resistant pattern in Oxacillin and methicillin with 100% resistant in MRSA followed by penicillin 98.7%. Vancomycin was found to be sensitive to MRSA patients.

Table 4.11 Antibiotic Resistance and Percentage of Resistance of MRSA

Antibiotics	Resistant	Sensitive	Total used	Antibiotics % Resistant of
Oxacillin	79	0	79	100.00
Methicillin	4	0	4	100.00
Penicillin	76	1	77	98.70
Ampicillin	61	1	62	98.39
Cloxacillin	16	1	17	94.12
Imipenem	26	3	29	89.66
Erythromycin	63	12	75	84.00
Cotrimoxazole	43	18	61	70.49
Ciprofloxacin	54	24	78	69.23
Amoxicillin	42	22	64	65.63
Ceftriazone	44	24	68	64.71
Meropenem	18	11	29	62.07
Ofloxacin	48	31	79	60.76
Gentamycin	44	33	77	57.14
Chloramphenicol	27	36	63	42.86
Amikacin	20	44	64	31.25
C+S	3	11	14	21.43
Vancomycin	0	67	67	0.00

4.5 Multiple Drug resistant Pattern of Antibiotics among MRSA patients

The antibiotics used for diagnosis of MRSA were categorized in 7 main groups.

The highest number of antibiotics resistance found in pattern of Penicillin+ Quinolanes+ Aminoglycoside+ Sulfonamides+ Cephalosporin+ Carbapenems with 10 cases followed by Penicillin+ Quinolanes+ Aminoglycoside+ Sulfonamides+ Cephalosporin with 9 cases.

Table 4.12 MDR pattern of antibiotics of MRSA patients

Antibiotics Resistance pattern	Frequency
Penicillin	5
Penicillin+ Quinolanes	5
Penicillin+ Aminoglycosides	1
Penicillin+Cephalosporin	2
Penicillin+Carbapenams	1
Penicillin+Quinolanes+Sulfonamides	2
Penicillin+Quinolanes+Cephalosporins	3
Penicillin+Aminoglycoside+Carbapenems	1
Penicillin+Sulfonamides+Cephalosporins	3
Penicillin+Sulfonamides+Carbapenems	2
Penicillin+ Cephalosporin+Carbapenems	2
Penicillin+ Quinolanes+Aminoglycoside+Sulfonamides	5
Penicillin+ Quinolanes+Aminoglycoside+Cephalosporin	6
Penicillin+ Quinolanes+Aminoglycoside+Carbapenems	1
Penicillin+ Quinolanes+Aminoglycoside+CS	2
Penicillin+ Quinolanes+Sulfonamide+Cephalosporin	3
Penicillin+ Quinolanes+Sulfonamides+ Carbapenems	1
Penicillin+ Aminoglycosides+Sulfonamide+Cephalosporin	4
Penicillin+ Aminoglycosides+ Sulfonamide+ Carbapenems	1
Penicillin+ Quinolanes+Aminoglycoside+Sulfonamides+Cephalosporin	9
Penicillin+ Quinolanes+Aminoglycoside+Sulfonamides+ Carbapenems	2
Penicillin+ Quinolanes+Aminoglycoside+Cephalosporin+ Carbapenems	6
Penicillin+Aminoglycosides+Sulfonamide+Cephalosporin+ Carbapenems	1
Penicillin+Quinolanes+Aminoglycoside+Sulfonamides+Cephalosporin+Carbapenems	10
Penicillin+ Quinolanes+Aminoglycoside+Cephalosporin+ Carbapenems+CS	1

CHAPTER V

DISCUSSION

The discussion part of this study is presented with following topics:

- 5.1 General characteristics of surgical patients
- 5.2 Prevalence of MRSA among surgical patients
- 5.3 Factors associated with MRSA among surgical patients
- 5.4 Resistant percentage of antibiotics
- 5.5 Multiple drug resistant pattern of antibiotics of MRSA of surgical patients

5.1 General characteristics of Surgical Patients

The general characteristics of surgical patients showed high frequency among patients of age group 16-30 years followed by 31-45 years with mean age 31 years and SD 17.25. The high frequency might be due to working age group and exposure to risk groups. Regarding the gender, female patients found to come for surgery with 72.9% followed by male only 27.1%. On seasonal variation summer season was found to have more surgical cases followed by spring season with least cases in early autumn season. The higher frequency in summer season may be due to hot climate and exposure to risk factors.

The urine samples were found to be at high percent with 78.7% for check up at laboratory followed by blood with 12.5%. The urine sample was found high as for diagnosis of diseases, urine was easier sample for confirmatory of infection. Surgical patients were found to be at inpatient wards with 55.8%. The surgical patients were found be hospitalizaed with mean days of hospitalization 3.45 days and maximum days upto 9 days. Nearly half of those surgical patients were found to be previously hospitalized. The higher rate of hospitalization and inpatients were due to complexity of surgical cases.

The surgical patients were found to come for diagnosis of trauma with 40% followed by bacteraemia and skin infections with 14.9% and 13.6% respectively in this study. *S.aureus* is responsible for a wide range of diseases, such as bacteraemia, pneumonia, endocarditis, osteomyelitis, septicarthritis and skin and soft tissue infections. An important distinctive feature of *S.aureus* strains is the susceptibility to methicillin as methicillin-resistant *S.aureus*(MRSA) which was first reported in 1960s in the hospital setting.[47].

5.2 Prevalence of MRSA among surgical patients

CDC (2006) in US estimated about 1% colonization with MRSA in US population [48]. But in this study prevalence of MRSA were detected 9.9 % which is in accordance with the findings disseminated by other studies[49-53] and various region in Nepal as well [11, 54-57].The earlier study from Nepal has reported the prevalence of 29% MRSA in 1990[55] during the period of where there were fewer health care institution and less access to antibiotics. A study in eastern Nepal by Kumari *et al.*, (2008) has reported the prevalence of MRSA to be 26.14%, in Western Nepal; by Subedi and Brahmadathan, (2005) reported low prevalence of 15.4% of MRSA where antibiotics were not easily available. In a study from tertiary care hospital has revealed 44.9% of MRSA among the nosocomial *S.aureus* isolates [56].Sanjana *et al.*, (2010) reported 39.60% of MRSA in Nepal. In a study at a tertiary care hospital of Nepal, Mishra has reported 42.42% MRSA in 2008. In accordance with this study, the prevalence of MRSA was 29.47%, has depicted the alarming condition due to MRSA isolates which is still in increasing trends. In India, prevalence of MRSA has been reported in the range of 20-39.5%, [58-62]. Instead of abundance of MRSA isolates across the world are not the same, the most MRSA isolates have been reported from Taiwan, Canada and Australia as much as 66-77% [49, 63-65]. There are higher prevalence rate in the well developed countries like USA in which it ranged from 50%-60% by mutated strains of *S.aureus*[53]. But in the developing countries like Nepal, the higher prevalence of MRSA may have contended the fact that the inappropriate use of antibiotics for community as well as hospital

acquired infections has resulted in the increment of the pressure to select MRSA and other resistant bacteria [11, 54, 55, 57].

Age:

The prevalence of MRSA was found to be high at age group above 76 years with 16.7% followed by between 46-60 years age group with 13.9% and below 15 years age group with 13%. In similar study carried out at Gangtok East Skkim, it was contrast reported that MRSA were high in age group 20-40 years of age and less in age groups below 20 years and above 40 years [66].

Gender:

Male were found to higher prevalence to MRSA than female in this study. Vaez *et al.*,(2011) also showed male were more prevalent with 38.5% males vs 33.7% females, with significant relationship whereas similar result to this study was disseminated by the study done by Osmon *et al.*, 2004 (54.1% females vs 49.1% males). In both outpatients and inpatients groups, male carried maximum carrier rate. The factors responsible may be as their active life, chances of more contact with other carrier, maximum exposure to outer environment, smoking habit or may be some other immunological and physiological phenomenon.[53]

Types of Samples:

In this study, the highest percentage MRSA was isolated from wound swab (64.7%). This result was very similar with the study by Tiwari *et al.* 2009 in which wound pus, swabs isolates of MRSA contributed to be 71.2%. It seemed that the MRSA should be responsible for the skin infection most commonly, however the percentage of MRSA isolates from urine may be due to contamination during specimen collection by the patients and the highest percentage of MRSA from Tissue may be due to the low sample size of the specimen [67]. Blood stream infection by MRSA was very low in this study with respect to the study conducted by Rahabarand Hajia, (2007) and Osmon *et al.*, (2004) in which they resulted 81% and 84.39% of blood stream infection caused by *S.aureus* respectively. The present study supported the study done by Sanjana *et al.*,(2010) who also reported low percentage (5%) of

blood stream infection caused by MRSA. Rahabarand Hajia, (2007) and Osmon *et al.*, (2004) reported higher detection rate of MRSA in blood stream infection when blood culture was requested for secondary sepsis while detection of MRSA in blood was low when it was requested to culture for the diagnosis of enteric fever [10].

Inpatient and Outpatient:

Kumari *et al.*, (2008) and Tiwari *et al.*, (2009) have reported 70% and 66.9% of the MRSA isolates from inpatient department respectively and suggested for the more sophisticated condition in hospital. In accordance with the study done by Kumari *et al.*, (2008) and Tiwari *et al.*, (2009), this study showed high hospital associated MRSA infection than community associated [68]. In spite of the low rate of MRSA strains isolated from outpatient department (23.85%), it can't leave without controls of the rate from outpatient also triggered us for the surveillance program for MRSA to be conducted in the community as well. but in this study prevalence among IPD and OPD was found similar result.

The carriage of MRSA among outpatients and inpatients has indicated the chances of transmission of the organisms to healthcare settings. Moreover, the inpatients are also susceptible to the staphylococcal infection of wounds and other sites from endogenous source during hospital stay. [54]

MRSA strains are usually resistant to several antibiotics that are used on a large scale in the hospital. This mechanism of increased spreading under antibiotic misuse might have contributed to the worldwide increase in the prevalence of MRSA [69].

In comparisons to international reports, the rate of isolation of MRSA from our study was found slightly high. It may be due to certain limitation to our study such as- lack of proper acknowledgement of different risk factors, selection of sample randomly without uniform distribution of different age groups and due to limited time and constrain resources.

5.3 Factors associated with MRSA among surgical patients

Although, MRSA infection was not significant relation with age group (p value >0.05), the highest risk of MRSA was represented by age group of middle aged group with OR 1.01 revealing high age group is more vulnerable to MRSA infection. Instead of many similar and independent studies that is not showing any relation between age, site of infection and rate of MRSA, only a few report has been showing increase rate of MRSA in elder people age 64 years and more significantly studied by Arbique *et al.*, (2001); Fatholahzadeh *et al.*,(2008); Hsueh *et al.*,(2004); Merlino *et al.*,(2002); Waness, (2010) are the supportive findings to this study. It has been clear that age is a risk factor because of its role in long term hospitalization; loss of immunity and longer antibiotic therapy [70].

The findings of this study revealed that, most of the MRSA carrier from middle age-class people was in agreement with Ankur *et al.* (2008). However, on contrary to the findings of Arch *et al.* (2006); Rajbhandari *et al.* (2002) and Thapa *et al.* (2004) [71-73]concluded that older adults were less likely to be colonized with MRSA than the younger population.

Regarding the genderwise distribution of MRSA isolates, high risk was recovered from the male patients with OR 2.98 than female patients, however there was significant association between infection and gender ($p<0.001$)which showed the similarity in the study conducted by Boucher and Corey,(2008). and Vaez *et al.*,(2011) [53, 74] showing males were more prevalent 64.4% males vs 35.6% females, and 38.5% vs 33.7% respectively with significant relationship whereas similar result to this study was disseminated by the study done by Osmon *et al.*, 2004[75](54.1% females vs 49.1 % males).

Risk factors for acquisition of MRSA included the administration of multiple antibiotics by patients[76].The influencing factors for higher carrier rate in male may be due to more out-door activities of male and hence frequent exposure to the overcrowded environment and with other infected person. But these assumptions were not significant; they may have been happened by chance factor only. There was no such correlation between the gender and the carriage of *S.aureus*. The difference in the carriage pattern could be due to difference in study population of different age group.

Risk factors associated with MRSA acquisition included older age, prolonged hospitalization, prior antibiotic therapy, more underlying disease and degree of disability, surgical procedures, wound infections, use of devices and exposure to other infected or colonized individuals etc. Antibiotic therapies predispose an individual to colonization by MRSA and increase the risk of infection. The high infection to colonization ratio has been observed as significant correlation[50].

5.4 Resistance percentage of antibiotics

Almost all isolates of *S.aureus* were resistant to Oxacillin, Methicillin, Penicillin and Ampicillin, but fortunately vancomycin was sensitive to all isolates of *S.aureus*. This result is similar to the result disseminated by Zhanel *et al.*,(2000); Zhanel *et al.*,(2008); Fatholahzadeh,(2008); Khorvash,(2008) and Shrestha *et al.*,(2009) indicating vancomycin as a therapeutic alternative for MRSA infection.[77-79]

Although most of the MRSA was found resistant to β -lactam antibiotics (ampicillin 98.39% and penicillin 98.70%), fortunately resistance towards vancomycin was not observed. Amikacin, and chloramphenicol were other effective antibiotics to MRSA isolates, whereas cefotaxime, ceftazidime, amoxicillin, gentamycin, ciprofloxacin, ofloxacin and cotrimoxazole were found to be weak in action against MRSA with reference to the resistant pattern. These results are basically in agreement with other studies done by Fatholahzadeh *et al.*, (2008); Khorvash *et al.*,(2008); Mishra,(2008); Tiwari *et al.*, 2009 b; Zhanel *et al.*,(2000); Zhanel *et al.*,(2008). It is noteworthy to depict the high resistivity of erythromycin to MRSA isolates which is in accordance with the finding disseminated by Tiwari *et al.*,(2009b) (68.7%) whereas greater than Mishra, (2008) (14.29%). The higher percentage of erythromycin resistance was obtained in this study shows the use of erythromycin also became critical.

A study carried out in India showed that Vancomycin and Ciprofloxacin were the most effective drug for *S. aureus*[80]. A similar study in Shiraz- Iran was showed also that Vancomycin (100%) was the most effective drug followed by Ciprofloxacin (87%) for treating MRSA infections [81].

Risk factors for acquisition of MRSA include the administration of multiple antibiotics by patients [76]. The carriage of MRSA among outpatients and inpatients has indicated the chances of transmission of the organisms to healthcare settings. Moreover, the inpatients are also susceptible to the staphylococcal infection of wounds and other sites from endogenous source during hospital stay.

MRSA strains are usually resistant to several antibiotics that are used on a large scale in the hospital. This mechanism of increased spreading under antibiotic misuse might have contributed to the worldwide increase in the prevalence of MRSA [50].

MDR *S. aureus* is the biggest offender for the hospital infection. Already resistance to most of the β -lactams class, MRSA is now often resistant to antibiotics outside the β -lactams. As a result of which, MDR MRSA has now developed resistance towards the drug of choice i.e. Glycopeptides (Vancomycin).

Vancomycin has been the mainstay of therapy for treatment of MRSA infections in hospitalized patients. With the increased prevalence of MRSA infections both in the hospital and community settings, the use of Vancomycin has been increasing. Recent emergence of Vancomycin-intermediate resistant (VISA) and Vancomycin resistant (VRSA) *S. aureus*, has compromised the use of Vancomycin as therapeutic failure. The isolation of such strains from several parts of the world suggests that VRSA will continue to emerge worldwide, portending the emergence of MRSA strains for which there will be no effective therapy, a situation similar to that in the pre-antibiotic era [50].

5.5 Multiple drug resistant pattern of antibiotics of MRSA of surgical patients

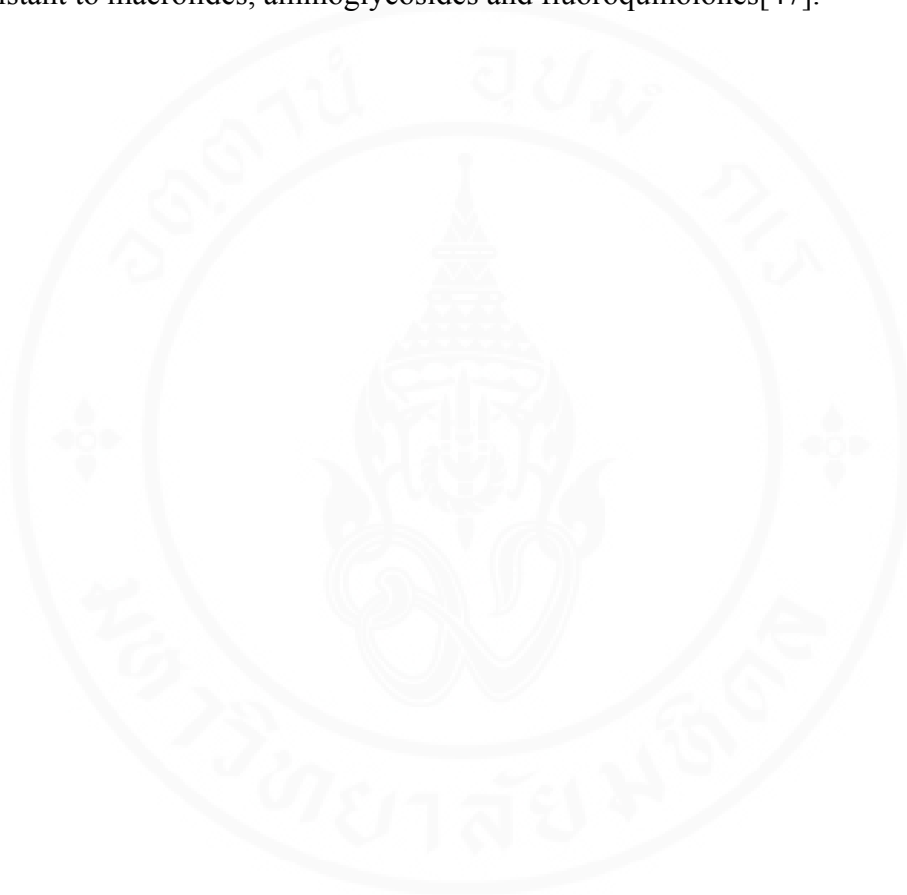
Multi-drug resistant (MDR) MRSA were identified by their antibiotics sensitivity pattern. *S. aureus* resistant to two or more drugs from different antimicrobial classes were called MDR MRSA. Although this study is in accordance with the previous studies from Nepal and other countries showing high percentage of MDR among MRSA: 78% by Subedi and Brahmadathan, (2005); 41% by Tiwari *et al.*, (2009b) and similarly 63% by Salab *et al.*, (2012) from Sudan, it is contrast to some

of report such high prevalence 100% MDR MRSA in this study[82]. The risk factors for MDR MRSA are self medication with antibiotics haphazardly by the patients, patient perception, patient compliance, physician prescribing practices and antibiotic use in hospitals [57, 82, 83]. This study depicts the paramount need for MRSA surveillance and strict drug policy to be conducted nationwide or else the threat will increase.

The resistance of MRSA to a multitude of antibiotics is one of the greatest challenges for modern antimicrobial therapy, particularly since the emergence of *S.aureus* with intermediate susceptibility to glycopeptides[84]. Vancomycin has been used extensively to treat MRSA although there have been several reports of failure at in treating MRSA lower respiratory tract infections [8 5] due to vancomycin molecule due and poor penetration of vancomycin into lung tissues[8 6] and although there has been supposed nephrotoxicity and ototoxicity of vancomycin[8 7] initially which were further purified and vancomycin nephrotoxicity reduced as low as 1.4%(similar to that seen for some β -lactams) and the absence of any ototoxicity [88]. Increasing frequency of MRSA infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of macrolide lincosamide streptogramin B(MLS_B) antibiotics to treat such infections. However, their widespread use has led to an increase in the number of MRSA strains resistant to MLS_B antibiotics[89].

It is also of crucial importance to depict antimicrobial susceptibility pattern of *S.aureus* in a country like Nepal where empirical therapy is unavoidable. The antimicrobial resistance crisis can be stepped down through efforts by clinicians, microbiologist, patients and public health officials by following the recommend at ion given by summit on antimicrobial resistance, like not indulging patients demand for unneeded antibiotics, educating patients on appropriate antibiotic use, identifying pathogens, choosing short course narrow spectrum antibiotics, encouraging patients to get vaccinated, improving resistance surveillance system will help controlling this situation. Although some resistance is inevitable with the use of antibiotics, steps can be taken to curtail practices that cause and propagate resistance [5 7 , 6 8 , 8 2] .In this way, it will be able to maintain or prolong the efficacy of existing drug thus in the reduction of morbidity and mortality of patients. Most of the MDR MRSA

pattern were found as Penicillin+ Quinolanes+ Aminoglycoside+ Sulfonamides+ Cephalosporin+ Carbapenems with 10 cases followed by Penicillin+ Quinolanes+ Aminoglycoside+ Sulfonamides+ Cephalosporin which is similar with study of Pantosi et al., 2007 as most MRSA strains are multi drug-resistant, being commonly resistant to macrolides, aminoglycosides and fluoroquinolones[47].



CHAPTER VI

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

From this study, it could be concluded that the prevalence of MRSA was 9.9%. The prevalence tended higher in higher age group patients (16.7%), male (18.1%), in late autumn season (16.0%), in wound swab (64.7%) and more than 5 days of hospitalization cases (15.5%). Similar type of prevalence was found in IPD and OPD department (10.1% vs 9.7%), previous hospitalization and no hospitalization (9.9% vs 10.0%) and patients diagnostic diseases with nearly 10 % in each. All isolated MRSA were multi-drug resistance(MDR) which is the significant public health problem in our context.

On association of age OR showed less risk among young adult, adult and senior citizen comparative to children age group and similar risk among middle aged people with $p = 0.056$. In case of Gender male were risky than female with OR 2.98 and $p < 0.001$. In case of seasonal variation Monsoon and late autumn season were of chance of MRSA infection comparative to winter season and spring, summer, and late autumn season bears less risk of getting infection with $p = 0.204$.

In case of types of samples as wound swab and tissue were of higher risk with OR 36.25 and 27.19 respectively in comparison to other samples with $p < 0.001$. In case of type of department OPD patients were slightly at risk in comparison to IPD patient with OR 1.05 and $p = 0.832$. In case of days of hospitalization during surgery, patients with greater than 5 days of hospitalization were at higher risk than patients with less than 5 days of hospitalization and no hospitalization with $p = 0.337$.

The history of hospitalization before surgery shows similar chance of getting infection of MRSA among both hospitalized and non hospitalized patients with $p = 0.97$. The diagnostic diseases of patient shows similar risk among all types of disease with slightly less risk in hemorrhoid patients in comparison to bone fracture patients with $p = 1.0$.

The resistance to antibiotics showed higher resistance to oxacillin, methicillin, penicillin, ampicillin, cloxacillin. Vancomycin was found to be sensitive. Similarly amikacin, chloramphenicol, gentamycin were also sensitive.

The MDR-MRSA with pattern of resistance to Penicillin+ Quinolones+ Aminoglycoside+ Sulfonamides+ Cephalosporin+ Carbapenems and Penicillin+ Quinolones+ Aminoglycoside+ Sulfonamides+ Cephalosporin found as common pattern of MDR.

6.2 Recommendations

6.2.1 Recommendation for implementation

I. Medical doctors should take more concern for preventing MRSA infection among elderly patients, child patients, male and patients with wound or tissue injury.

II. During treatment of MDR-MRSA patients doctor should select Vancomycin for drug of choice.

III. Screening of methicillin resistance among *S.aureus* should be performed in all hospital laboratory as well as surveillance program for MRSA should be conducted in the community as well.

IV. It is also of crucial importance to depict antimicrobial susceptibility pattern of MRSA in a country like Nepal where empirical therapy is unavoidable. Inappropriate or excessive antibiotic therapy and prophylaxis should be avoided in all healthcare settings.

V. Early detection of MRSA through surveillance is fundamental to preventing spread. Patients as well as healthcare workers should be screened for MRSA in routine screening procedures and decolonization of nasal or body sites can be carried out in order to reduce the bacterial load, which in turn reduces the risk of spread and infection.

VI. Programs to educate health care personnel about infection control precaution against MRSA with intermediate glycopeptide resistance should be developed and infection control specialist should monitor compliance.

6.2.2 Recommendation for further study

1. MRSA , being a major threat should be investigated epidemiologically with various risk factors, including following informations:

- Body site infection or colonization
- Racial categories
- Admission date and duration
- Immunocompetence or severity of illness
- Treatment given, especially antibiotics

2. Along with biotyping, phagotyping, serotyping, plasmid profiling of the isolates should be carried out simultaneously for complete characterization and to establish relationship with the epidemic strains.

3. Infection-control and laboratory personel should implement active surveillance for *S. aureus* with intermediate glycopeptide resistance particularly in population at high risk such as patients on dialysis and patients in whom Vancomycin threapy is unsuccessful.

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APPENDIX A



HOSPITAL FOR ADVANCED MEDICINE AND SURGERY

GPO Box: 3832, Buddhanagar, Kathmandu, Nepal, Helpline.: 4786111, Fax: 077-1-4785165
E-mail: info@hamshospital.org, website: www.hamshospital.org



29 November 2013

To Whom It May Concern

This is letter of ethical clearance for Mr. Shreejeet Shrestha, who is currently enrolled in MPH course 2013-14, Mahidol University, Thailand, to use the data from the reports of Hospital on prevalence and factors associated with MRSA among patients with surgery, for the purpose of his thematic paper. The data which contain information about the lab report over a period of years for survey of associated factors in year 2013 will be used by him.

It gives us immense pleasure to help Mr. Shrestha for this purpose.

Thank you .

Janardan Man Shrestha

Director

Health Ministry Ref. No.: 6081/063/12/14

Reg No.: 44627/063-064



बनेपा हस्पिटल एण्ड एजुकेशन फाउण्डेशन प्रा. लि.
Banepa Hospital and Education Foundation Pvt. Ltd.

E-mail: drgehendra@hotmail.com
Tel No.: 011-661180, 011-662418, 011-664299
Banepa-5, Kavre, Nepal

Ref. No.: 90-2070

Date :

Date: 11/11/2013



TO WHOM IT MAY CONCERN

This is letter of no objection for Mr. Shreejeet Shrestha who is currently enrolled in MPH course 2013, Mahidol University, Thailand, to use the data from our laboratory department of Banepa Hospital And Education Foundation Pvt.Ltd. for his educational purpose.

It gives us immense pleasure to help Mr. Shreejeet Shrestha for this purpose.

Thank You.

Sincerely yours,

Dr.Gehendra Raj Kayastha

Medical Director

Banepa Hospital and Education Foundation Pvt. Ltd.



Reg: 56762/065/066



सुमेरु हस्पिटल प्रा.लि
Sumeru Hospital Pvt. Ltd
Dhapakhel, Lalitpur, Ph.No:- 01-5003377, 5003388, 5003399

Ref:- 161/070/071


Date : Nov. 28, 13

To whom it may concern

This is letter of no objection for Mr. Shreejeet Shrestha, who is currently enrolled in MPH course 2013-14, Mahidol University, Thailand, to use the data from the reports of Hospital on **Prevalence and factors associated with MRSA among Patients with surgery, for the purpose of his thematic paper. The data which contains information about the patients clinical history and lab report over a period of years for survey of associated factors in year 2013** will be used by him.

It gives us immense pleasure to help Mr. Shrestha for this purpose.

Thank you.


Dinesh Acharya
Administrator
Sumeru Hospital

Fax:- 977-01-5554555, E-mail:- sumeru@wlink.com.np, P.O.Box:-15137, Websit:- www.sumeru.com.np

APPENDIX B



Documentary Proof of Exemption
Ethical Review Committee for Human Research
Faculty of Public Health, Mahidol University

Protocol Title : PREVALENCE AND FACTORS ASSOCIATED WITH METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AMONG SURGICAL PATIENTS IN KATHMANDU VALLEY, NEPAL

Protocol No. : 185/2556

Principal Investigator : Mr. Shreejeet Shrestha

Affiliation : Master of Public Health (International Program)
Faculty of Public Health, Mahidol University

This protocol complies with a "Research with Exemption" category

Date of Issue : 11 December 2013

The aforementioned project have been reviewed and approved according to the Standard Operating Procedures of Ethical Review Committee for Human Research, Faculty of Public Health, Mahidol University:

A handwritten signature in blue ink, appearing to read 'S. Nantham'.

(Assoc. Prof. Dr. Sutham Nanthamongkolchai)

Chairman of Ethical Review Committee for Human Research

BIOGRAPHY

NAME	Shreejeet Shrestha
NATIONALITY	Nepalese
DATE OF BIRTH	28 th December, 1984
PLACE OF BIRTH	Lalitpur, Nepal
EDUCATION	November 2009 M.Sc. Medical Microbiology Tribhuvan University, Nepal
WORKING EXPERIENCE	January 2010-March 2013 Lecturer, Kantipur college of Medical Sciences January 2007-December 2012 Lecturer, Pinnacle College
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