

**PREVALENCE OF NOSOCOMIAL INFECTION IN SURGICAL  
WOUNDS AMONG POSTOPERATIVE PATIENTS AND THEIR  
ANTIMICROBIAL SUSCEPTIBILITY PATTERN**

*Dissertation submitted to*  
**THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY**

*In partial fulfillment of the regulations  
for the award of the degree of*

**M.D.(MICROBIOLOGY)**  
**BRANCH – IV**



**MADRAS MEDICAL COLLEGE**  
**THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY**  
**CHENNAI – TAMILNADU.**

**APRIL 2015**

## **CERTIFICATE**

This is to certify that this dissertation titled **“PREVALENCE OF NOSOCOMIAL INFECTION IN SURGICAL WOUNDS AMONG POSTOPERATIVE PATIENTS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN”** is a bonafide record work done by Dr. C.ABBA RUBA SUNANTHINI, during the period of her Post Graduate study from MAY 2012 to APRIL 2015 under guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai- 600003, in partial fulfilment of the requirement of **M.D MICROBIOLOGY** degree Examination of The TamilnaduDr. M.G.R Medical University to be held in April 2015.

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## **DECLARATION**

I declare that the dissertation entitled “**PREVALENCE OF NOSOCOMIAL INFECTION IN SURGICAL WOUNDS AMONG POSTOPERATIVE PATIENTS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN**” submitted by me for the degree of M.D. is the record work carried out by me during the period of September **2013** – August **2014** under the guidance of **Dr.G.Jayalakshmi, M.D.,D.T.C.D.**, Director & Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfilment of the University regulations for the award of degree of M.D., Branch IV (Microbiology) examination to be held in April 2015.

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#### INTRODUCTION

Amassing sick people together under one roof has many advantages, but some disadvantages, notably easier transmission of infection from one patient to another. In the past, the major environment for this interaction had been the hospital, which led to the term **nosocomial infection**. The term nosocomial is derived from the Greek word *nosokomeion*, meaning hospital. Later it was termed as **health care associated infection(HAI)** because of the increase in modes of healthcare in home care settings by skilled nurses. Even then, hospitals remains the major environment associated to Health care associated infection (HAI)<sup>(6)</sup>.

Nosocomial infections or HAI according to World Health Organization (WHO) is **an infection contracted by a patient while in a hospital or health care facility (and not present or incubating on admission)**. Such infections may become evident during their stay in hospital or only after their discharge<sup>(7)</sup>. HAI is a serious health hazard as their prevalence are about 9% worldwide. Approximately 5-10% of patients in developed countries and 25 % of patients in developing countries have been found to acquire nosocomial infection. According to Rita Dutta<sup>(9)</sup>, a member of **Hospital Infection Society (HIS) - Mumbai** ten to thirty per cent of patients admitted to the hospitals in

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# **PREVALENCE OF NOSOCOMIAL INFECTION IN SURGICAL WOUNDS AMONG POSTOPERATIVE PATIENTS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN**

## **Abstract:**

**Background:** Surgical site infections accounts for about 24% of all nosocomial infections among 16 million patients who undergo surgery every year. SSIs have a major impact on the patient's quality of life since they are associated with considerable morbidity, occasional mortality, extended hospital stay and financial burden on the patient and the health care provider. The present cross sectional study was done to isolate and identify the aerobic and anaerobic microorganisms causing SSI and to evaluate the antibiotic susceptibility pattern of these pathogens, to assess the risk factors for SSI, to assess the microbial contamination levels in operating theatres and postoperative wards using both active and passive sampling methods and to assess any linkage between the environmental isolates and the isolates causing SSI.

**Materials and method:** The present study was done in the Institute of Microbiology, Madras Medical College, Chennai. The samples were collected from 200 postoperative inpatients with clinically diagnosed SSI in the department of General surgery, Cardio Thoracic surgery and Vascular Surgery, Rajiv Gandhi Government General Hospital, Chennai. Infected wounds were studied bacteriologically. Samples such as wound swabs from the infected wound site, blood from peripheral vein were collected as indicated and processed as per standard operating procedure( SOP). Environmental sampling from operation theatres and postoperative wards, nasal swabs from anterior nares and hand imprint culture of hospital personnel were collected and processed as per SOP. The results were analyzed. Antibiotic resistance pattern of 2 *MRSA* strains from general surgical SSI patient were found to be similar to 1 *MRSA* strain from General surgery Operation theatre. These strains were subjected to 16S rRNA gene amplification and Amplified Ribosomal DNA Restriction Analysis (ARDRA) to identify the strain relatedness.

**Results:** The overall postoperative surgical site infection rate was 15.56% .

Males (64.29%) had a higher SSI rate compared to females(35.71 %). The rate of SSI was higher(10.22% ) in clean contaminated surgeries ( class II) compared to 5.3% in clean surgeries (class I). There was a significant increase in the rate of infection with the an increase in duration of the pre-operative hospitalization, ASA score > 2 and duration of surgery. In general surgery, the infection rate was highest in post appendectomy wounds and lowest in wounds following thyroidectomy, adrenalectomy and hysterectomy. In Cardiothoracic surgery SSI rate was higher in patients who underwent lobectomy and least in valve replacement surgery. In Vascular surgery the SSI rate was higher in bypass graft wounds. The commonest aerobic isolate from the surgical wound infections was *Escherichia coli*, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and the least common was *Proteus mirabilis*. In general surgical patients anaerobes namely, *Peptostreptococcus spp.* and *Bacteroides fragilis* were isolated. Among all the wounds, isolation of monomicrobials predominated over polymicrobials. Majority of the Gram negative isolates were sensitive to Amikacin and Piperacillin Tazobactam but were resistant to commonly prescribed antimicrobial agents namely Cephalosporins, Gentamycin and Ciprofloxacin. Cefotaxime and Ceftriaxone, the commonly used third generation cephalosporins as surgical prophylaxis to prevent SSIs were found to be less effective against most of the gram negative organisms. The present study also observed an increase in SSIs caused by ESBL producing enteric Gram negative bacilli.

Air sampler system was found to measure the microbial burden more accurately compared to settle plate method. Environmental sampling done in the operation theatres and postoperative wards revealed that the Index of microbial contamination of air were within acceptable limits. 16S rRNA gene amplification and Amplified Ribosomal DNA Restriction Analysis (ARDRA) performed on the

*MRSA* isolates with similar antibiotic resistant pattern ,revealed that the isolates fr om SSI were distinct from the one isolated from the OT.

### **Conclusion:**

The conclusion drawn from the present study indicates the necessity for implementing routine wound culture and sensitivity and the test reports to guide the choice of antibiotics, periodic review and adherence to Hospital infection control policy and guidelines. The study also recommends consideration of anaerobic bacteria as a cause in all SSIs, identification of relevant gene responsible for antibiotic resistance of the pathogens by molecular methods as an epidemiologic measure, limiting the use of third generation cephalosporin in surgical prophylaxis to prevent development of further resistance to these antibiotics, performance of routine surveillance of all Operation Theatres once in every two months instead of once in four months done presently and strict adherence to the OT protocol on asepsis and to prefer newer less toxic disinfecting agents.

## INTRODUCTION

Amassing sick people together under one roof has many advantages, but some disadvantages, notably easier transmission of infection from one patient to another. In the past, the major environment for this interaction had been the hospital, which led to the term **nosocomial infection**. The term nosocomial is derived from the Greek word *nosokomeian*, meaning hospital. Later it was termed as **healthcare associated infection(HAI)** because of the increase in modes of healthcare in home care settings by skilled nurses. Even then, hospitals remains the major environment associated to Health care associated infection (HAI)<sup>(8)</sup>.

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WHO described Nosocomial infection as one of the major infectious diseases causing a huge economic impact. In spite of many advanced measures in

the control and prevention of Nosocomial infection, they continue to be a major side effect of hospital treatment contributing to a significant rate of morbidity, mortality and cost of care.

Infections commonly acquired in the hospitals are respiratory tract infection(13.8%), surgical site infection (13.80%), urinary tract infection(19.70%) and Bacteremia(6.8%). Among 16 million patients undergoing surgical procedures every year, it has been estimated that 2%-5% develop surgical site infections which accounts for about 24% of all nosocomial infections. The SSI rate is said to be higher in developing countries due to scarcity of resources and inadequacy of staff

Surgical site infection(SSI) is defined as the invasion of microorganisms through tissues following a breakdown of local and systemic defenses. SSI, one of the HAI s occurs after an invasive (surgical) procedure. It ranges from a spontaneously limited wound discharge within 7–10 days of an operation to a life-threatening postoperative complication, such as a sternal infection after open heart surgery.

There are four classes of surgical wound<sup>(5,6)</sup> with an increasing risk of SSIs namely, Class I- clean wound, Class II – clean-contaminated wound, Class III - contaminated wound and Class IV- dirty wounds. This study includes only two surgical wound classes namely, clean and clean contaminated wounds as the degree of contamination is higher in Class III & Class IV<sup>(114)</sup>.

There are many risk factors that contribute to the susceptibility of surgical wound to infection. These risk factors include pre-existing illness, longer duration of surgery and surgical wound class(III & IV). Other factors include extremes of ages, long duration of preoperative hospitalization, malnutrition, immunosuppression, remote site infection, emergency procedures and co morbidities like malignancy and metabolic diseases.

Surgical site infections can have a significant effect on patients quality of life since they are associated with considerable morbidity and extended hospital stay. In addition, surgical site infections result in a considerable financial burden to healthcare providers also.

Sources of pathogen in a surgical site infection may be 1.endogenous from the flora on the patients skin, mucous membrane or hollow viscera, 2.Exogenous sources namely cross infection from other patients , surgical personnel or environment, instruments and other material brought into the sterile field during operation.

Focussing on Hospital acquired infections, sources are invariably the hospital environment, medical and paramedical staff who are in close contact with the patient in various stages of treatment. Potential sources of hospital cross infections are doctors' white coats, nurses' uniforms, hospital garments, privacy drapes, stethoscopes, curtains, AC vent, bed rails and common hospital surfaces. Identification of nosocomial cluster isolates from postoperative wound infection warrants an epidemiological study to trace the source of infection. A nosocomial

cluster is defined as the isolation of closely related isolates ( $\geq 90\%$ ) from  $\geq 2$  patients in the same ward or at the same hospital within a period of 90 days. One important aspect which has to be borne in mind at this juncture is the ability of microorganisms that get transferred to survive for a considerable length of time on these surfaces.

The knowledge about the chain of transmission of HAI is important for both epidemiological investigation and infection control. **This chain is comprised of Pathogen source, Susceptible person, Colonisation and transmission to others.** A break in every link by implementing comprehensive policies and procedures like minimizing the preoperative stays in the hospital, following pre operative procedures like shaving the operative site using clippers(not razors) just before the procedure, using antibiotic prophylaxis only when indicated and according to established protocols, providing sterile instruments in individually wrapped sterile packages., using effective antiseptic, such as 10 % povidone iodine, to prepare the incision site, including perioperative scrub with antiseptic for hand and forearm antiseptics for surgical teams will reduce Health care associated Surgical Site Infection to a considerable extent.

Another important source or reservoir of microorganisms could be air, therefore in controlled environments such as operating theatres regular microbial monitoring by air sampling method is useful to measure air quality and identify microbial contamination if any. In hospital wards with outbreak of cross infections it is required to examine the air for its content of particular pathogen. Various methods

have been devised for measuring the bacterial content of air. The settle plate method is used chiefly for larger particles which settle by gravity from air on to exposed surfaces and the slit sampler method is used to count the number of bacteria contained particles in a given sample of air.

It is obvious through various studies conducted by World Bank that HAI increases the cost of health care, as two-thirds of the developing countries have spent more than 50% of their health care budgets on hospitals. This could be reduced by effective Infection Control(IC) programs as they decrease the spread of nosocomial infections and hence the morbidity, mortality, and health care costs.

As a clinical microbiologist and a part of infection control committee, it is our responsibility to develop and implement specific policies and procedures to prevent the spread of infections among patients during their stay in a hospital or health care facility.



**AIMS AND OBJECTIVES**  
**OF THE STUDY**

## **AIMS AND OBJECTIVES**

1. To isolate and identify the aerobic and anaerobic microorganisms causing Surgical Site Infection among post-operative patients and to evaluate the antibiotic susceptibility pattern of these pathogens .
2. To assess the risk factors influencing the rate of Surgical Site Infection.
3. To evaluate the proportion of infections caused by clustered isolates .
4. To assess the microbial contamination levels in operating theatres and postoperative wards using both active and passive sampling methods.
5. To assess any linkage between the environmental isolates and the isolates causing SSI<sup>(9)</sup>.

REVIEW OF  
LITERATURE

# REVIEW OF LITERATURE

## HISTORY

In 1850, the Austrian obstetrician Ignac Semmelweis demonstrated that many hospital infections are preventable when he made the unpopular suggestion that puerperal fever was carried on the hands of physician who came directly from attending an autopsy to the delivery ward without washing . A death of 8.3 % was reduced to 2.3% by introducing the simple measure of hand washing before and after any clinical examination. Thus nosocomial wound infection came into vogue at the start of Microbiology.

The introduction of “antisepsis” by Joseph Lister in 1960s changed the science of surgery from an activity of infection and death, to one that relieved suffering and prolonged life. These advances however did not yield the expected impact on the rates of Surgical site infection due to the emergence of drug resistant pathogenic microorganisms, increase in surgical patients with immunocompromised state and various chronic debilitating illness<sup>(9)</sup>.

Infections most commonly acquired in hospitals are

- Urinary Tract Infection
- Surgical Site Infection
- Respiratory Tract Infection
- Bacteremia

Surgical Site Infection has been documented for more than 4000 years. Egyptians prevented putrefaction by mummification due to their notion about infection. Hippocrates introduced the use of antimicrobials namely vinegar and wine to irrigate infected wounds before performing a secondary closure. Galen recognized that suppuration of wounds often led to recovery especially after drainage. This dictum was later misunderstood that production of pus was curative and hence healing would occur only with pus production. They even applied noxious substances like faeces to induce pus production.

Only in 19<sup>th</sup> century, the dictum of microbes as cause of wound infection according to Koch's postulates came into understanding.<sup>( 10)</sup> "Magic Bullet", the concept of killing microorganisms but not the host came into existence in mid twentieth century when sulphonamide chemotherapy was discovered.

Alexander Fleming discovered Penicillin with which he treated a police constable with severe staphylococcal bacteremia with metastatic abscesses. Nowadays most Staphylococci are resistant to Penicillin due to acquisition of Beta lactamases which break the beta lactam ring present in antibiotic molecules. The emergence of drug resistant strains namely, Methicillin resistant Staphylococcus aureus(MRSA), Vancomycin resistant enterococci(VRE), Extended spectrum Beta Lactamase(ESBL) producers and Metallobetalactamase(MBL) producing Gram negative bacilli are of great concern presently.

**Definitions:**

- According to National Nosocomial Infection Surveillance (NNIS) Nosocomial surgical site infections are those infections involving the incision site, with purulent discharge, occurring 30 days within the operative procedure that are acquired during hospital care (48 hours or more after admission) and were not present or incubating on admission.
  - In 1992 US Centre for Disease Control defined that Surgical site infections are the ones which occur within 30 days of surgery and must have at least one of the following features,
    - Purulent discharge from the superficial infection
    - Microorganisms isolated from aseptically obtained wound swab culture.
  - Must also have one of the following signs of infection
    - Pain or tenderness
    - Localised swelling

**Classification of operative wounds by level of Bacterial contamination<sup>(5)</sup>**

The risk of developing a postoperative surgical site infection is effected by the degree of microbial contamination of the operative site. A widely accepted system of classifying operative site by the degree of contamination was developed by National Research council in 1964. Accordingly surgical wounds are classified into 4 classes.

**Class I Clean wounds:**

An operative wound which is not infected, not inflamed and the respiratory , genital , urinary tract or alimentary is not entered. These wounds are closed primarily and if needed drained through closed drain.

For example: Mastectomy

**Class II Clean-contaminated wounds:**

An operative wound in which entry is made with controlled access into the alimentary, respiratory, urinary or genital tract without significant spillage of contents. Minor breach in technique and no signs of infection.

For example: surgical procedures involving biliary tract, appendix, vagina.

**Class III Contaminated wounds:**

These include open, fresh accidental wounds or operations with major lapse in sterile technique or gross spillage from gastrointestinal tract. Acute non-purulent inflammation at operative sites come under this category.

**Class IV Dirty and infected wounds:**

These wounds are defined to harbor microorganisms in the operative field during the surgical procedure. Operative wounds where perforated viscous or infection has been encountered and also old traumatic wounds with devitalized tissue fall under this category.

**According to CDC Surgical Site Infection<sup>(7)</sup> are further classified into**

**Superficial Incisional Surgical Site Infection-**

These are infections that occur within 30 days after the surgery involving only skin and subcutaneous tissue of the incision with purulent drainage with or without culture report.

**Deep Incisional Surgical Site Infection**

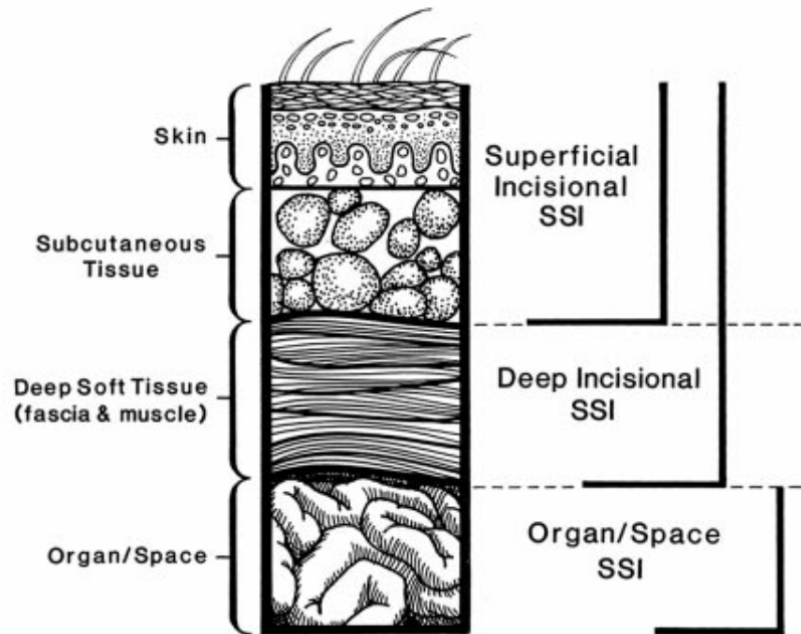
Infection that occurs within 30 days of surgical procedure without implant or in one year with implant in place and involves deep soft tissue, associated with purulent discharge. It also includes dehiscence of incision wound either spontaneously or by surgeon with signs of infection/inflammation.

**Organ/Space Surgical Site Infection**

Infection that occurs in 30 days after the surgery without implant or in one year with implant in place and the infection involving any part (e.g., organs and spaces) other than the incision with Purulent drainage from a drain placed into the organ/space.

Organ/Space Surgical Site Infection is one in which fluid/tissue culture from the organ/space is positive for micro organisms, or any evidence of infection is identified on direct examination, or by histopathologic or radiologic examination, or during reoperation or diagnosis is made by a surgeon or attending physician.





## **MICROBIOLOGY OF SURGICAL SITE INFECTIONS:**

### **Normal Human microbial flora<sup>(13)</sup>**

The term “Normal Microbial Flora” denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons.

The skin and mucous membranes always harbor a variety of microorganisms that can be arranged into two groups.

1. The resident flora consists of relatively fixed types of microorganisms regularly found in a given age, if disturbed it promptly re establishes itself.
2. The transient flora derived from the environment, consists of non pathogenic microorganisms that inhabit the skin and mucous membranes for hours, days

or weeks; it neither produces disease, and does not establish itself permanently on the surface.

However, if the resident flora is disturbed, transient microorganisms may colonize, proliferate and produce disease.

The normal microbial flora found in the various sites are as follows<sup>(13)</sup>

1. **SKIN:** Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyogenes, Diphtheroids, Enterococci, Propionobacterium, Fungi and yeast in skin folds.
2. **UPPER RESPIRATORY TRACT:** Staphylococcus aureus, Staphylococcus epidermidis), Streptococcus pyogenes, Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Diphtheroids, Mycoplasma, Prevotella and Fusobacterium
3. **Oral cavity:** Streptococcus salivarius, Streptococcus mutans, Streptococcus sanguis, Bacteroides
4. **GASTROINTESTINAL TRACT:**
  - Aerobes:** Various enterobacteriaceae namely Escherichia coli, Klebsiella sp. Enterobacter sp., Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus and Lactobacillus.
  - Anaerobes:** Gram positive cocci namely Peptococci and Peptostreptococci.

Gram negative bacilli namely Bacteroides fragilis, Bacteroides melaninogenicus, Bifidobacterium bifidum, Clostridium septicum, and Prevotella spp.

## **5. UROGENITAL TRACT:**

**Urinary tract:** Staphylococcus epidermidis, Enterococcus faecalis and occasionally alpha haemolytic Streptococcus.

**Genital tract:** Staphylococcus, Escherichia coli, Diphtheroids and Lactobacillus acidophilus(Doderlein's bacillus)

### **Pathophysiology and determinants of Surgical Site Infection:**

Intact epithelial surfaces normally prevent infection of tissues. Surgery breaks down this protective barrier and also other natural mechanisms namely humoral and cell mediated immunity<sup>(10)</sup>.

Postoperative wound infection depends on the complex interaction between<sup>(3)</sup>,

1. Patient related factors namely, nutritional status, host immunity, co morbid conditions like Diabetes mellitus
2. Wound related factors namely, Magnitude of tissue trauma, devitalization that accompany the surgery
3. Microbial factors namely, the production of enzymes that mediate tissue invasion or that enable the bacterium to survive the host defenses and also the pharmacologic defense that is administered prophylactically.

The milieu of the surgical wound is reviewed as a balance of opposing forces and the determinants of surgical site infection are<sup>(3)</sup>.

Microbial Concentration & virulence of bacteria, Injured wound or tissues,  
Resistance to Antibiotics,

Foreign Material

$$\text{SSI} = \frac{\text{Foreign Material}}{\text{General \& local immunity of host, Perioperative Antibiotics}}$$

The risk of SSI is also determined as follows;

$$\text{SSI risk} = \frac{\text{Bacterial contamination dose} \times \text{Virulence of the bacteria}}{\text{Resistance of the host.}}$$

SSI risk is said to be markedly increased if the microbial contamination is more than 1,00,000 per gram of tissue.

As the number and virulence of contaminating bacteria increases, so does the chance for development of postoperative infection. Use of foreign materials and surgical trauma to the tissues act as adjuvants that potentiate the risk of infection, opposed by the systemic and local host immune response which prevent infection.

The local response ranges from mild inflammatory oedema, erythema to frank suppuration. The systemic response ranges from mild fever, leucocytosis to severe septicemia and shock<sup>(16)</sup>.

If the inoculum is less, The infection limits with local edema, erythema and subsides with antibiotics. A heavy inoculum leads to pus formation and systemic response<sup>(3)</sup>. Any defect in phagocytosis or migration of phagocytes will lead to prolonged intracellular survival and multiplication of the organisms leading to severe sepsis. Hence elective operation are avoided if possible in case of immunodeficiency diseases.

Local factors namely tissue hypoxia, a change in ionic environment , local fibrin deposition, alteration in levels of opsonic proteins, accumulation of bacterial products like lipopolysaccharides , proteolytic enzymes such as fibrinolysins and heparinase alter the local host defence mechanisms and contribute to persistent infection.

Toxins and other substances produced by Microorganisms increase their ability to invade and survive in a host, producing damage to the host. Endotoxins produced by Gram negative bacteria stimulates cytokine production and hence triggers systemic inflammation syndrome which sometimes leads to Multiple System Organ Failure.

**According to the National Health Safety Network the Most common pathogenic microorganisms isolated from surgical site infections in 2006-2007**

<b>Pathogens isolated from SSI</b>	<b>Percentage of pathogenic isolates reported</b>
<b>Staphylococcus aureus</b>	<b>30%</b>
<b>Coagulase-negative staphylococci</b>	<b>13.7%</b>
<b>Enterococcus spp.</b>	<b>11.2%</b>
<b>Escherichia coli</b>	<b>9.6%</b>
<b>Pseudomonas aeruginosa</b>	<b>5.6%</b>
<b>Enterobacter</b>	<b>4.2%</b>
<b>Klebsiella pneumoniae</b>	<b>3.0%</b>
<b>Candida spp.</b>	<b>2.0%</b>
<b>Klebsiella oxytoca</b>	<b>0.7%</b>
<b>Acinetobacter baumannii</b>	<b>0.6%</b>

In addition to the above, unusual micro organisms are also recognized for example, Rhizopus/rhizopodiformis in contaminated adhesive tapes, Rhodococcus bronchialis after Coronary Artery Bypass Graft surgery and rapidly growing Mycobacterium species have been reported<sup>(65)</sup>.

**Clinical features of SSI:****Local manifestation:**

Surgical site infection(SSI) presents as Superficial Incisional Surgical Site Infection, Deep Incisional Surgical Site Infection and Organ/Space Surgical Site Infection.

**Wound abscess:**

Presents with swelling, pain, redness and warmth

**Cellulitis and lymphangitis:**

Cellulitis is a non suppurative invasive infection with signs of inflammation and lymphangitis presenting as red painful streaks at the site.

**Systemic manifestation:****Bacteremia and septicemia:**

It usually presents with fever associated with chills and rigor. It is important in patients with prosthesis and may be associated with Multi System Organ Failure.

**SOURCES OF PATHOGENS CAUSING SURGICAL SITE INFECTION:**

The sources can be classified as

**A) Primary:** Acquired from community or endogenous wound

**B) Secondary:** Acquired from operating theatres or ward (nosocomial) or from contamination at surgery<sup>(10)</sup>.

**Sources can also be classified under the following heads:**

**A)Direct inoculation**

1. **At the time of surgery:** Resident flora of patient's skin, Hands of surgical team members (via torn gloves), Contaminated surgical material, Contaminated host tissue.
2. **During postoperative period:** Drains & irrigating catheters, transient/resident flora of patient, infected tissues.

**B)Airborne contamination**

1. **At the time of surgery:** Skin and clothing of patient and operating room staff, inanimate theatre environment, malfunctioning air filtration equipment.
2. **During postoperative period:** Only in case of open wounds and burns.

**C)Haematogeneous – lymphatic seeding:**

1. **At the time of surgery:** Pre-existing infection of non wound sites (pneumonia, UTI), Intravascular / Intravenous lines.
2. **During postoperative period:** Postoperative infection of non wound sites (pneumonia , UTI), Intravascular / Intravenous lines.<sup>(3)</sup>

In short, the source of pathogens causing SSI could be listed as

1. The endogenous flora of patient's skin, mucous membrane or hollow viscera.
2. Seeding of pathogen into the postoperative wound from a distant site.
3. Exogenous sources like the surgical environment, surgical personnel, instruments, materials brought into the sterile surgical field during the procedure<sup>(9)</sup>.



## **RISK FACTORS THAT MAY INFLUENCE SURGICAL SITE INFECTIONS**

**ARE:**

### **A) Endogenous sources**

#### **1.Age**

Extremes of age have been found to have influence on the likelihood of wound infection. In the bivariate analysis, by Jeanne Lee et al<sup>(23)</sup> surgical site infection was associated with an age of seventy-five years or older ( $p = 0.03$ ),

In 2003, Keith S. Kaye et al<sup>(24)</sup> by their study proposed that older patients showed increased risk of SSI because they were less likely to mount a strong immune response, such as fever or leukocytosis, which in turn led to a more subtle presentation and hence a delay in the diagnosis.

#### **2.Pre- existing illness:**

The American Society of Anaesthesiologists(ASA)<sup>(5)</sup> preoperative assessment physical status is considered to be highly predictive of subsequent wound infection.

Code	Patient's Preoperative Physical Status
1	Normal healthy patient
2	Patient with mild systemic disease
3	Patient with severe systemic disease
4	Patient with an incapacitating systemic disease which is a constant threat to life
5	Moribund patient who is expected to survive for 24 hours with or without operation.

In a study , by Jeanne Lee et al<sup>(23)</sup> an ASA score of  $\geq 3$  was estimated to be a significant predictor of SSI with odds ratio = 9.92 and  $p < 0.001$ .

In 2010, Alavi K et al<sup>(25)</sup> by their study on SSI in inflammatory bowel disease , found that an ASA score  $> 2$  is a strong predictors of surgical site infections.

### **3.Diabetes Mellitus**

Several studies indicate that Diabetes Mellitus has a significant impact in wound infection . In a study by Sam Chen, Matt V Anderson et al on association of Diabetes mellitus(DM) with Surgical Site Infections in Spinal Arthrodesis, DM was the highest of all the independent risk factors causing SSI and in another study by Ata A, Vallerian Bt et al, the patients with DM were 1.32 ( $P < 0.05$ ) times more likely than nondiabetics to develop SSI after colorectal and non-colorectal surgeries<sup>(20,29)</sup>.

According to a study by Robert Latham et al<sup>(40)</sup>, Postoperative hyperglycemia and undiagnosed diabetes have been found to have strong association with development of SSIs among patients undergoing cardiothoracic surgery.

### **4.Obesity:**

In a study by Ardeshriri M et al<sup>(19)</sup>, effect of obesity on mortality and morbidity after coronary artery bypass grafting surgery in Iranian patients, the incidence of surgical site infection was found to be increased in patients with Body Mass Index  $\geq 30$ .

According to Debra et al in a study analyzing the risk factors for superficial and deep chest surgical-site infections after a coronary artery bypass graft surgery , Obese diabetic patients were found to have 7.7 fold increased risk of developing deep chest infections following surgery.

### **5.Nutritional status**

In 2002, Debra L. Malone et al, in their study on reanalysis of risk factors in surgical site infection on 5031 noncardiac surgical patients, found that malnutrition (defined as significant loss of weight in 6 months prior to the surgery) was significantly associated with increased SSI<sup>(21)</sup>.

### **6.Coexisting infection**

Coexisting infections namely, indolent dental, skin soft tissue or urinary infections at the time of surgery may serve as a source for 1) haematogenous spread, causing late infections of prostheses or cardiac valves, or 2) cause bacterial transfer to contiguous sites<sup>(77,78)</sup> . These infections have been found to cause 3-5 fold increase in SSI rates<sup>(79)</sup> and hence such infections should be identified and treated before the surgery.

### **7.Length of preoperative stay in hospital**

In a study regarding the risk factors in SSI in abdominal surgeries by S. M. Patel et al, in patients who stayed preoperatively for 2-6 days and 7-13 days **the SSI** rate were high(12.8% and 33.3% respectively) compared to 5.550% in those who stayed for 0-1 day<sup>(81)</sup>.

**Stephane Leung Wai Sang** et al in their study regarding modifiable risk factor for mediastinitis after cardiac surgery, inferred that, preoperative hospital stay increased the risk of mediastinitis by 15% per week of stay<sup>(22)</sup>.

### **8.Remote site infection:**

In a prospective 5 year study of Surgical wound infections by M Olson et al at the Minneapolis VA Medical Center, epidemiologic correlation was found between remote site infection and subsequent SSI<sup>(58)</sup>.

### **9.Malignancy**

In a study conducted by Claesson and Holmlund in 190 patients who underwent colorectal surgery, the wound infection rate was 17 % in patients with malignancy compared to 4.5% in those without malignancy.

### **10.Cigarette smoking**

In 2013, Durand F et al assessed the risk factors of organ/space SSI in a prospective cohort including 3,908 patients who underwent orthopaedic surgery with implants and found that a significant association existed between smoking and organ/space SSI in these patients. According to David E Reichman et al, Cigarette smoking is considered to interfere with primary wound healing, possibly due to constriction of peripheral blood vessels which in turn leads to tissue hypovolemia and hypoxia<sup>(59)</sup>.

## Exogenous sources

### 1. Length of Surgical procedure

Risk of Surgical site infection has been shown to be proportional to the length of the surgical procedure by various studies<sup>(43, 63)</sup>. G. Peersman<sup>(62)</sup> in a study on patients who underwent Total knee replacement correlated that prolonged Operative Time of duration  $127 \pm 45$  min ( $p < 0.001$ ) resulted in an increased SSI rate compared to those patients who underwent surgery for  $94 \pm 28$  min .

According to S.M. Patel et al<sup>(81)</sup>, in their study regarding risk factors of SSI in abdominal surgeries, inferred that the rate of SSI increased when the length of operation exceeded the T hrs (where T is the 75<sup>th</sup> percentile of duration of a specific operation performed).

### The T Point for Common Surgical Procedures

Operation	T Point (hrs)
Coronary artery bypass graft	5
Bile duct, liver or pancreatic surgery	4
Craniotomy	4
Head and neck surgery	4
Colonic surgery	3
Vascular surgery	3
Joint prosthesis surgery	3
Abdominal or vaginal hysterectomy	2
Herniorrhaphy	2
Ventricular shunt	2
Limb amputation	1
Appendectomy	1
Caesarean section	1

## **2. Emergency Procedures**

Several studies have shown that emergency operations have a greater likelihood of SSI.<sup>(41,81)</sup> In a study conducted by Hulam Asghar Channa et al, in 1120 post surgical patients 82 (7.3%) patients developed SSI, among them infection rate was higher in emergency procedures (13.1%) compared to elective procedures (2.9%).

## **3. Airborne contamination:**

Air in operation theatre environment and Critical care units, also plays a major role as a reservoir for microorganisms in causation of SSI<sup>(82)</sup>. These pathogens may cause deep surgical infection which may even require reoperation or may endanger life.<sup>(82)</sup>

### **Wound level factors:**

The SSI rate has been found to increase with the Wound class. In a study regarding risk factors for SSI in abdominal surgeries by S.M.Patel et al, infection rate was found to increase in the order by 3%, 11.5%, 20% and 40% in wounds of Classe I, II, III and IV respectively<sup>(81)</sup>.

**According to the guidelines for prevention of infections in operating room by Hospital Infection society, precautions to be taken include**

**A. Preoperative precautions related to patient**

1. **Preoperative antiseptic bath** with chlorhexidine is said to reduce the microbial load reduction by 9 fold compared to Povidone iodine which reduces the load by 1 or 2 fold<sup>(9)</sup>.
2. **Preoperative shaving** done immediately before surgery reduces the risk of SSI compared to the same being done on the prior night, as any cuts in the skin due to prior night shaving serves as a foci for bacterial multiplication.
3. **Patient skin preparation** with povidone iodine, 4% chlorhexidine gluconate, alcohol containing products are recommended to reduce SSI.
4. **Antimicrobial prophylaxis** given empirically before surgical procedure reduces the microbial burden due to intraoperative contamination of the surgical site and hence the incidence of SSI. They act empirically even before the host defence get activated. Hence maximum tissue and blood levels of antibiotic should be present before first incision is made and before contamination occurs. According to M.E.E. Van Kasteren et al, in their study on Antibiotic prophylaxis and the risk for SSI following total hip arthroplasty, the most important prophylaxis related factor for increased risk of SSI was timely administration of the first dose of antibiotic. The importance of administering the antibiotics shortly before the incision is that,

at the end of the procedure, the concentration of the antibiotic will still be high enough to prevent infection and reduces the necessity of repeating doses during prolonged surgery.<sup>(61)</sup>

**B.Preoperative precautions related to the surgical team:**

1. Surgical scrub, hand and forearm antiseptics is mandatory for the surgical team members who come into contact with the sterile instruments and operating field.
2. Infected surgical personnel have been linked with outbreaks of SSI. In a study<sup>(27)</sup> by Boyce JM et al, a single strain of *S. epidermidis* infection caused outbreak of SSI among postoperative patients who underwent cardiothoracic surgery. One out of the 8 surgical personnel carried the epidemic strain in his hands which had been identified by identical susceptibility pattern, Restriction Endonuclease digestion pattern and plasmid profile. Hence the hospital should implement policies to prevent such transmission to the patients by restricting these healthcare workers from exposure prone environment /procedures or even exclude them from work.

The policies should be designed in such a way that the infected personnel should report their illnesses readily instead of masking the reality.



### **C. Precautions related to Operation theatre environment:**

Sources of micro organisms in the theatre environment are epithelial cells, aerosol, dust, respiratory droplets. The microbial level in the theatre environment is directly proportional to the total number of people moving about in the room. Hence minimizing traffic during surgery and avoiding turbulence in the theatre environments reduces the risk of SSI.

#### **1) Heating Ventilation Air conditioning system(HVAC)**

Maintains indoor humidity, temperature, controls odors, removes contaminated air and minimizes airborne transmission risk<sup>(9)</sup>.

Modern operating theatres have latest air filtration systems namely, **HEPA** (High efficiency particulate filters), Laminar Airflow air ventilation system and UV Germicidal Irradiation for upper room air irradiation.

#### **2) DISINFECTION OF SURFACES:**

Broad spectrum disinfectants grouped under classes of glutaraldehyde, Phenolics, alcohols, iodophors and chlorine-based agents which are safe , economical and approved by Hospital Infection Control Committee should be used.<sup>(60)</sup> Common antiseptics used in general surgical practices are Chlorhexidine, povidone iodine, cetrimide, alcohols hypochlorites and hexachlorophane.

### **3)FUMIGATION/ FOGGING:**

Fogging an ancient routine though no longer recommended in the west, is still being used in our country(9). Formalin is being potentially carcinogenic is replaced with safer agents such as hydrogen peroxide, peracetic acid and other compounds of formaldehyde<sup>(57)</sup>.

### **4)STERILIZATION OF INSTRUMENTS:**

Surgical instruments should be disinfected by soaking in germicidal detergents, washed thoroughly and sterilized by steam under pressure or other approved methods. Barriers and Strict adherence to aseptic techniques must be adhered to.

### **5) MONITORING:**

The sophisticated systems namely HEPA and HVAC need monitoring regarding maintenance of filter systems, determination and quantification of pressure differentials , particle counts and cleanliness of duct .

For microbial monitoring of closed workplace at biorisk, Perugia university devised a system called Microbial environmental monitoring ( MAM-monitoraggio ambientale microbiologico). Air monitoring formed the fundamental part of MAM system and the same was assessed as the Index of the microbial air contamination (IMA) by many methods under the following heads;

- i. Count of colony forming units per cubic meter of air (CFU/m<sup>3</sup>);
- ii. Count of CFU on settle plates;
- iii. Measurement of a chemical component(ATP, DNA)of the microbial cells/m<sup>3</sup> of air
- iv. Counting the microbes using microscope , flow cytometry or fluorescent in situ hybridization.

The former two methods of counting the CFU are considered to be effective means of quantification of airborne microbes as the latter 2 methods are not practical presently and are still under study. The CFU count is the most important parameter, as it measures the live microorganism which are not only harmful by themselves but also can lead to more harm by their multiplication.

Air sampling is done in two ways, either active or passive methods . <sup>(35-39)</sup>

### **Active air sampling**

The microbial air contamination is measured by counting the number of CFU per cubic metre (CFU/m<sup>3</sup>) of air by using **active air samplers**. These air samplers collect a known volume of air from the environment which is blown on to a nutrient medium through different techniques.

There are many different types of active samplers namely Impingers, Centrifugal samplers, Impactors (slit-type), Impactors (sieve type), Surface Air System sampler (SAS), Filtration samplers, Electrostatic precipitation samplers and Thermal precipitation samplers.

In this study Centrifugal samplers is used. It operates under the principle of air being drawn into the drum and subjected to centrifugal acceleration which leads the air borne pathogens to impact on the blood agar borne on plastic strips. After sampling, the plastic strips are removed and incubated at 37°C for 48 hrs and the colonies grown are counted<sup>(4)</sup>.

### **Advantages of active air sampling**

1. Collects a known volume of air
2. The microbial air contamination is measured by counting the number of Colony Forming Units per cubic metre of air (CFU/m<sup>3</sup>)
3. Sample collection is faster compared to passive air sampling .

### **Disadvantages of active air sampling:**

1. Expensive
2. Noisy
3. Difficulty to sterilize the device
4. Different samples taken by the same sampler give different results
5. Results obtained by different active sampler in the same place show high variability .
6. Does not evaluate the fallout microorganisms.
7. The sampler needs frequent calibration.
8. The impact on the nutrient medium have been said to inactivate certain number of micro organisms<sup>(37)</sup>.

### **Passive air sampling:**

Passive air sampling is done using **settle plates**.

Petri dishes containing an agar medium of known diameter are left open to air for a measured period of time. Microbes carried by dust particles settle on the surface of the medium<sup>(4)</sup>, with an average deposition rate of 0.46 cm/s . Generally the plates are incubated aerobically at 37°C for 24 hours and the number of colonies formed shows the total number of bacteria containing particles settled on the media. Blood agar is suitable for all pathogenic, saprophytic and commensal bacteria. In case of any particular pathogen appropriate selective media can be used.

### **LAB DIAGNOSIS**

#### **INVESTIGATIONS:**

1. Complete haemogram
2. Urine routine examination
3. Blood urea, sugar and serum creatinine
4. X- ray chest and Abdomen
5. Radiodiagnostic procedures namely, ultrasonogram, CT and MRI if necessary
6. Microbiological investigations
  1. Direct Gram staining
  2. Aerobic bacterial culture and Antimicrobial susceptibility test for the isolates.
  3. Anaerobic bacterial culture
  4. Fungal culture
  5. Environmental study

**Investigation of strain relatedness among isolates:**

When an outbreak occurs or when an increased incidence of infection is suspected investigation should be done to isolate the causative organism and the source should be identified by phenotypic and genotypic methods. A good typing technique must be discriminatory, reproducible and should have a high degree of typability.

**Antibiotic susceptibility pattern:**

This test is readily performed and provides preliminary clue to distinguish between two isolates in dispute. However this is not ultimate since different strains may have same pattern. Hence more specialized typing techniques are necessary.

**Specialized typing techniques:****Serotyping:**

Serotyping distinguishes between the strains of same species by the antigenic determinants expressed on their cell surface structures using specific antisera. These antigenic variation is exhibited by cell surface structures such as membrane proteins, lipopolysaccharides, flagella, fimbriae and capsular polysaccharides. Strains thus differentiated are known as 'serotypes' and this typing is used in several gram positive and gram negative bacteria.

**Bacteriophage typing:**

Phage typing compares the pattern of lysis obtained when isolates are exposed to a series of phage suspension. This method can be used to type bacteria namely,

*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi* and *Pseudomonas aeruginosa* .

### **Molecular typing:**

#### **Plasmid profiles- First generation molecular epidemiology:**

In this method, plasmids serve as markers of various bacterial strains which can encode genes for antibiotic resistance and virulence factors. Plasmids are circular deoxyribonucleic acid molecules which exist in bacteria, independent of the chromosome. The study of plasmids is important to medical microbiology because they have been used to map the spread of antibiotic resistance<sup>(44)</sup> among hospital pathogens<sup>(8)</sup>.

#### **Restriction enzymes and probes – second generation molecular epidemiology:**

Restriction enzyme digestion of total cellular DNA from isolates result in a pattern of different sized fragments separated and compared by agarose gel electrophoresis restriction enzyme analysis(REA)<sup>(8)</sup> . In a study by Abadall O A Ahmed et al, assessment of preoperative nasal carriers of *Staphylococcus aureus* and their development of postoperative wound infection was studied using restriction enzyme analysis of the protein A and coagulase genes. Out of 98 nasal carriers, 6 patients developed SSI by the same strain. Another strain (type IV, D4) was found among 7 hospital staff representing a nosocomially prevalent strain. The same strain was also encountered in the isolates recovered from postoperative wounds. Thus REA helps in epidemiologic surveillance to find out cross infection from the hospital settings.<sup>(45)</sup>

Amplified Ribosomal DNA Restriction Analysis(ARDRA) is an extension of Restriction Fragment Length Polymorphism is done for strain typing, where, enzymatic amplification of conserved genes at the ends of the 16s gene is done using specific primers and the pattern obtained is analysed . In this technique, a minimum of 3 restriction enzymes are used to differentiate unrelated organisms which may yield similar patterns when a single restriction enzyme is used.

**Pulse Field Gel Electrophoresis(PFGE) and PCR- Third generation molecular epidemiology:**

Very large DNA fragments(>30 kb) get blocked in the upper part of the gel and are not separated by conventional electrophoresis. This problem is resolved by PFGE, in which the electrophoretic current is 'paused' in different directions for different length of time.

In a study conducted in the department of cardiac surgery in Netherlands by Jan Kluytmans et al during a period of 1-year, 6 SSI were observed to be caused by *Staphylococcus schleiferi* . Extensive environmental surveillance and case control study were performed by molecular typing of the causative microorganism using PFGE to identify potential sources of infection<sup>(46)</sup>.

P Y Liu et al used Pulsed-field gel electrophoresis and ERIC-based( enterobacterial repetitive intergenic consensus ) PCR were used to generate and study DNA fingerprints for 14F. Oryzihabitans isolated from 8 episodes of nosocomial infections in a period of 2 years. The isolates were identified to be eight



distinct genotypes, hence all these episodes of infections were inferred to be independent<sup>(47)</sup>.

### **DNA sequence analysis- Third generation molecular epidemiology:**

Genotype information can be determined precisely as DNA (or RNA) nucleotide-base sequences. While comparing total chromosomal sequences is not practical, a subset of nucleotide sequence could be analysed by amplifying a known DNA segment using Polymerase Chain Reaction (PCR). The amplified product can be sequenced by automated techniques and multiple isolates can be compared by sequence based approach using microarrays, multilocus sequence typing etc<sup>(8)</sup>.

Thus a typing method which is discriminatory, reproducible, easy to perform and interpret and also has a high degree of typability is used in epidemiological studies This in turn helps in implementing infection control policies in the hospital preventing further spread of infection from the source.

### **COMPLICATIONS OF SSI:**

#### **Local Complications:**

1. Wound gapping and dehiscence
2. Abscess and sinuses
3. Antibiooma and seroma
4. Burst abdomen
5. Incisional hernia

**Systemic complications:**

If the SSI is not treated appropriately, local wound infection may become generalized. Bacteremia leading to septicemia causing multiple metastatic abscesses in distant organs which in turn leads to Multi System Organ Dysfunction and death.

**TREATMENT:**

1. In patients with superficial abscess, suture removal with incision and adequate drainage of pus should be done.
2. In patients with sepsis of open wounds, thorough debridement with appropriate antibiotics should be given according to the AST report.
3. In patients with significant systemic symptoms, adjunctive systemic antimicrobial therapy can be given in conjunction with incision and drainage even without the AST report.
4. Once the diagnosis of wound infection is confirmed, the pathogen and antimicrobial sensitivity are identified, patient is treated with the appropriate antibiotics . The other two main wound management products which reduce the bacterial burden in the wound are Iodine and silver compounds.

**MATERIALS AND  
METHODS**

## **MATERIALS METHODS**

The present study was done in the Institute of Microbiology, Madras Medical College, Chennai. The samples were collected from 200 postoperative inpatients of General surgery, Cardio Thoracic surgery and Vascular Surgery departments of Rajiv Gandhi Government General Hospital, Chennai .

### **Study design & period:**

Cross sectional study. One year (from September 2013- August 2014)

#### **■ Study population:**

Postoperative patients with clinically infected wounds in the General surgical, Vascular and Cardiothoracic surgical wards.

### **Ethical clearance:**

Before the commencement of the study, approval was obtained from Institutional Ethics Committee. Informed consent was obtained from all the inpatients who satisfied the inclusion criteria. Patients belonging to study population were interviewed with structured questionnaire.

### **Inclusion criteria:**

- Postoperative patients of age more than 18 years of either sex who underwent surgery with Class I and Class II surgical wound.
- Patients with superficial, deep incisional site and organ/ space surgical site infections.
- Infections occurring within 30 days of surgery.

## **Exclusion Criteria**

- Patients with foci of infection prior to surgery.
- Patients with signs of infection on admission.
- Patients already receiving antibiotics for more than 1 week.
- Patients with redressed wounds.
- Patients undergoing re-operation.

## **Collection of data:**

Data were collected from patients who satisfied the inclusion criteria, using preformed structured questionnaire. Demographic details like name, age, sex, address, date of admission, diagnosis, name of surgery, date of surgery, type of surgery, wound class, duration of surgery, duration of pre and postoperative stay in hospital, clinical history namely, presenting complaints including history of fever on admission, past medical history, history of any malignancy or associated immunocompromised state, physical examination including temperature, local examination finding of the surgical site date of infection, and details regarding antimicrobial prophylaxis were collected.

## **Specimens**

1. Wound swabs from Surgical site infection.
2. Blood from peripheral vein.
3. Environmental sampling from operation theatres and postoperative wards
4. Nasal swabs from anterior nares and hand culture of hospital personnel working in postoperative wards.

## **COLLECTION OF SAMPLE**

### **Pus Swabs:**

Pus samples were collected from patients with SSI in 3 sterile swabs, one for direct Grams staining for detecting pus cells and microorganisms, second swab for aerobic culture and third swab for anaerobic culture. The swab were taken from the leading edge of the wound and placed in a sterile test tube and transported to the laboratory.

### **Abscess:**

Pus from an abscess was collected at the time of incision and drainage taking care to avoid contamination from the skin commensal organisms. Under strict aseptic precautions, the area over the abscess was wiped with sterile saline or 70% alcohol. Upto 5 ml of pus was aspirated with a sterile syringe and needle and transferred to aerobic and anaerobic containers(67). Thioglycollate broth and Robertson's cooked meat media were used for collecting samples for anaerobic culture. The samples were properly labelled and transported to the laboratory in appropriate conditions and processed immediately.

### **Blood:**

Under strict aseptic precautions, venepuncture site was cleaned with 70% alcohol and then with 2 % Povidone Iodine. The disinfectant was allowed to act for 1 minute and then 7ml of blood sample was collected with a sterile syringe of which 5ml was added into a sterile screw capped blood culture bottle containing 25 ml of

sterile Brain Heart Infusion broth(BHI broth) and 2ml was added to Thioglycollate broth at bed side.

### **Processing of sample:**

#### **Gram stain :**

Smear of the specimens were prepared by evenly spreading on a new glass slide, air dried, fixed and stained using Gram staining technique. The smear was examined for the presence or absence of bacterial cells, their gram reaction, morphology, arrangement and pus cells.

#### **Aerobic culture:**

Second swab was inoculated onto Blood agar(BA), Nutrient agar(NA) and MacConkey agar plate and were incubated at 37°C for 48 hours. After incubation, bacteria from positive cultures were identified by motility, catalase test, oxidase test, coagulase test and by means of various other biochemical reactions as per standard microbiological techniques. [68]. The antibiotic sensitivity test of all isolates were performed according to CLSI guidelines. If no growth was detected after 48 hours of incubation the culture was declared negative for aerobic bacterial growth.

#### **Antimicrobial susceptibility testing(AST)<sup>69</sup>:**

AST is done to identify the sensitivity and resistant patterns of all isolates according to CLSI guidelines.

#### **Non fastidious organisms:**

Antimicrobial susceptibility testing was done by disc diffusion method using

Kirby Bauer's technique on Mueller Hinton agar (HiMedia, Mumbai), using appropriate antimicrobial drugs as directed by CLSI guidelines<sup>70</sup>.

**Antimicrobial susceptibility testing by Kirby – Bauer Disc Diffusion method:**

1. With a sterile bacteriological wire loop 3- 5 well isolated identical colonies on an agar plate culture were touched and transferred and emulsified in 3-4ml of sterile nutrient broth.
2. Suspension of organism in growth medium is matched to a 0.5 McFarland standards
3. Using a sterile cotton swab, the suspension is inoculated onto a plate of Mueller Hinton Agar and streak the surface evenly over the surface of the medium in three directions rotating the plate approximately 60 °C to ensure even distribution.
4. The surface of the inoculated agar was allowed to dry for 3 to 5 minutes with the lid in place before adding the antibiotic discs.
5. Appropriate antimicrobial discs , five discs per plate of 90mm diameter were placed on the surface of the agar using sterile forceps.
6. After overnight incubation at 37°C, the diameters of zone of inhibition were measured in mm with a ruled template.

Quality control tests were done every week using the following standard ATCC control strains for testing the performance of media & drugs .Interpretation of Zone of inhibition diameters were done according to CLSI guidelines<sup>(71)</sup>.



**ATCC control strains:**

- *Staphylococcus aureus*–ATCC 25923
- *Escherichia coli*-ATCC 25922
- *Pseudomonas aeruginosa*-ATCC 27853
- *Klebsiella pneumoniae* (ESBL)-ATCC 700603

**Panel of antibiotics included for testing antimicrobial sensitivity of Gram negative bacilli.**

Antibiotic	Disc content	Gram negative bacilli	Diameter of Zone of inhibition in mm.Break points		
			Sensitive	Intermediate	Resistant
<b>Amikacin</b>	30µg		≥ 17	15-16	≤ 14
<b>Cefotaxime</b>	30µg	Enterobacteriaceae	≥26	23-25	≤22
		<i>Acinetobacter</i>	≥23	15-22	≤14
<b>Ceftazidime</b>	30µg	Enterobacteriaceae	≥21	18-20	≤17
		<i>P.aeruginosa</i> & <i>Acinetobacter sp.</i>	≥18	15-17	≤14
<b>Cotrimoxazole</b>	1.25/ 23.75µg		≥16	11-15	≤10
<b>Ciprofloxacin</b>	5 µg		≥21	18-20	≤17
<b>Gentamicin</b>	10µg		≥15	13-14	≤12
<b>Imipenem</b>	10µg	Enterobacteriaceae	≥23	20-22	≤19
		<i>P.aeruginosa</i>	≥19	16-18	≤15
		<i>Acinetobacter sp.</i>	≥16	14-15	≤13
<b>Piperacillin-Tazobactam</b>	100µg/10 µg		≥21	18-20	≤17

**The panel of antibiotics included in the antimicrobial sensitivity testing for Gram positive cocci were (Himedia),**

Antibiotics	Disc content	Inhibition zone in mm		
		Resistance	Intermediate	Sensitive
Amikacin	30µg	14	15-16	17
Ciprofloxacin	5µg	15	16-20	21
Cotrimoxazole	1.25/23.75µg	10	11-15	16
Chloramphenicol	30µg	12	13-17	18
Clindamycin	2µg	14	15-20	21
Penicillin	10units	28	-	29
Rifampin	5µg	16	17-19	20
Erythromycin	15µg	13	14-22	23
Cefoxitin	30µg	21	-	22

**METHODS OF DETECTION OF  $\beta$  LACTAMASE ENZYME PRODUCTION IN GRAM NEGATIVE BACILLI:**

**A) EXTENDED SPECTRUM  $\beta$ - LACTAMASES (ESBL) DETECTION**

**METHODS<sup>72</sup>:**

ESBLs are classified under Bush class A  $\beta$ - lactamases. They are capable of hydrolyzing penicillins – Oxyiminocephalosporins and Monobactams (Aztreonam) and inhibited by  $\beta$ -lactamase inhibitors (Clavulanic acid, Sulbactam and

Tazobactam) but have no detectable activity against Cephamycins or Carbapenems (Imipenem, Meropenem).

**1. ESBL Screening method:** <sup>[72]</sup>

Isolates of gram negative bacilli (*Klebsiella pneumoniae*, *Klebsiella oxytoca* and *E.coli*) showing the following resistance pattern were considered to be possible ESBL producing strains.

<b>Antibiotic</b>	<b>Zone diameter for possible ESBL producing strain</b>
Cefpodoxime(10 µg)	≤17 mm
Ceftazidime(30µg)	≤22mm
Cefotaxime(30µg)	≤27mm
Ceftriaxone(30µg)	≤25mm
Aztreonam(30µg)	≤27mm

Isolates of *Proteus mirabilis* showing the following resistance pattern were considered to be possible ESBL producing strains.

<b>Antibiotic</b>	<b>Zone diameter for possible ESBL producing strain</b>
Cefpodoxime(10 µg)	≤22 mm
Ceftazidime(30µg)	≤22mm
Cefotaxime(30µg)	≤27mm

## **2) CLSI phenotypic confirmatory method:**

3-5 colonies of the isolates grown on a non selective culture medium were added to 5 ml of nutrient broth and incubated at  $35\pm 2^{\circ}\text{C}$  for 2-4 hrs. The resulting turbidity was matched with 0.5 McFarlands standard. The inoculum was lawn cultured onto Mueller Hinton Agar plate (HiMedia, Mumbai). Ceftazidime (30 $\mu\text{g}$ ) disc and Ceftazidime/Clavulanic acid disc (30 $\mu\text{g}$ /10 $\mu\text{g}$ ) were placed on the surface of the plate and incubated overnight at  $35\pm 2^{\circ}\text{C}$ . A  $\geq 5\text{mm}$  increase in the zone diameter for Ceftazidime- Clavulanic acid combination compared to the zone diameter of inhibition when tested with Ceftazidime alone confirmed production of ESBL by the organism.

## **3) Double disk diffusion synergy test:**

Standard disc diffusion test was done with the 0.5 McFarlands matched test isolate inoculum, with Ceftazidime (30 $\mu\text{g}$ ) and Ceftazidime/Clavulanic acid (20 $\mu\text{g}$ /10 $\mu\text{g}$ ) (HiMedia, Mumbai) which were kept 30mm apart from centre to centre and incubated at  $35 \pm 2^{\circ}\text{C}$  in ambient air for 16-18 hours. The test organisms with an increased Ceftazidime zone size towards the Ceftazidime/Clavulanic acid disc was interpreted as an ESBL producer.

**Methicillin resistance detection in *Staphylococcus aureus*<sup>(74)</sup>:**

**Disc diffusion method:**

0.5 Mcfarland's suspension of test isolate was lawn cultured on MHA plates.

30 µg Cefoxitin disc is placed on the surface of lawn culture ,incubated at 33–35 °C; in ambient air for 16–18 hours. Isolates showing inhibition zone diameter ≥22 mm ,were considered as Methicillin sensitive strains and those that show inhibition zone diameter ≤21 mm ,were considered as Methicillin resistant isolates.

**Detection of Vancomycin MIC for *Staphylococcus aureus* isolates by macrobrothdilution methods<sup>(74)</sup> :**

Cation adjusted Mueller Hinton broth.(pH 7.2-7.4) was used

**Media Preparation of stock antibiotic solution:**

Formula:

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

W = the Weight of the antibiotic to be dissolved in the volume V

V = the Volume of the stock solution to be prepared (10ml)

C =the final Concentration of the antibiotic solution (1024µg/ml)

P= the Potency of the antibiotic in relation to the base. (For vancomycin, Potency is 950/1000 mg; Himedia)

### **Preparation of working antibiotic solution:**

- Two rows of 10 sterile plugged test tubes were arranged in the racks.
- In a sterile tube, 8ml of broth containing the concentration of antibiotic(128 µg/ml) required for the first tube in each row was prepared from appropriate stock solution(1024 µg/ml) .
- The contents of the above container were mixed thoroughly and using a sterile pipette, 1ml of the stock solution was transferred to first tubes in each row.
- Using a fresh pipette, 4ml of MH broth was added to 4ml of the stock solution , mixed well and from this concentration, 1ml was transferred to the second tube in each row
- The procedure was repeated till the 11th tube
- The first row of tubes were inoculated with test organism
- The second row of tubes were inoculated with ATCC *Staphylococcus aureus* 25923.
- 1 ml of the antibiotic free broth was placed in the last tube in each row as growth control.
- 1 ml of antibiotic solution in each concentration were kept as sterility control.

**Inoculum preparation for the test and ATCC control and incubation:**

- To 9.9 ml of MH broth in a sterile container , 0.1 ml of 0.5 Mcfarland turbidity matched test organism was added and mixed well.
- Using 2 ml sterile syringe, 1 ml of the above inoculum was transferred to each antibiotic containing tubes in the first row and also to the growth control tube.
- Similarly ATCC control strain inoculum was prepared and transferred to the tubes in the second row.
- These tubes were incubated at 37°C overnight.

**Observation & Interpretation:**

- The MIC of ATCC control strain were observed, they were within sensitive range, hence the test was considered to be valid.
- The lowest concentration of the antibiotic in which there was no visible growth was taken as the MIC of the drug for the test organism.

**Interpretation criteria: for vancomycin MIC values.**

MIC <2µ/ml –sensitive.MIC :4-8 µg/ml –Intermediate .MIC >16µg/ml.

**Anaerobic culture:**

The third swab collected from necrotic tissue and exudates from deep wounds were inoculated into thioglycollate broth for anaerobic culture and were transported to the laboratory immediately. Inoculated thioglycollate broth were incubated at

37°C for 48- 72 hours and examined daily for 3 days for the presence of turbidity. If turbidity was observed in the broth, smears were prepared and examined by Grams staining and subcultured onto Selective Anaerobic Blood Agar Plates . The inoculated plates were placed in McIntosh Fildes anaerobic jar with the media facing upwards. Commercially available Gas-Pak (Hi media Laboratories Pvt limited) were cut open at one corner and placed in the jar , the lid was closed immediately and incubated for 48 hours. A plate inoculated with *Pseudomonas aeruginosa* was used as the biological indicator to check the anaerobiosis.

After appropriate period of incubation, plates were examined for evidence of growth. If growth were present Colony morphology was observed, smears were made and Gram staining was done to determine the cell morphology and organisms were identified upto genus level.

Isolates with morphology suggestive of anaerobes were subcultured onto RCM. Aerotolerance of those isolates were checked by inoculating them onto Blood agar plates and incubating them aerobically at 37°C. Those isolates which did not grow on the aerobically incubated subculture plates were taken as obligate anaerobes.

Further, smears were prepared from the RCM subcultures to check the purity of the isolates.

If the plates did not show any growth at 48 hrs, it was incubated for a further period of 72 hrs before discarding.



**Blood Culture:**

The patient's blood sample were inoculated into Brain Heart Infusion (BHI) Broth and Thioglycollate broth and incubated at 37°C aerobically and examined for turbidity at 24 and 48 hours. If turbidity was observed in BHI, subcultures were done onto Blood Agar and MacConkey Agar. These plates were incubated aerobically at 37°C for 24 hrs. Any growth observed was identified up to species level by colony morphology, Gram staining, catalase test, oxidase test, motility and biochemical reactions. Subcultures were done every third day for a period of 10 days and a negative report was given if no growth was observed.

**Environmental study:****Air quality surveillance:**

**Air quality surveillance** is done to examine the content of the air for any particular pathogens. Since it is an expensive, time-consuming process complicated by various protocols, analysis, and interpretation, air sampling should be done only if warranted. As air still remains as a cause of nosocomial postoperative wound infection, this study included air sampling for linking microorganisms from environmental samples with clinical isolates from post operative wound infections<sup>(75,76)</sup>.

Air quality surveillance was performed in the General surgery, Cardio Thoracic Surgery and Vascular Surgery operation theatres and postoperative wards, simultaneously using an air sampler device (LA 002) and the settle plate technique. A total of 11 operation theatres and 3 postoperative wards were subjected to

surveillance .The study was carried out in the OT before the start of the routine surgeries to avoid trafficking while the sampling procedure is done.

#### **Passive air sampling -Settle plate method :-**

Standard petridishes of 9 cm diameter containing sheep blood agar, labelled with date, time and period of exposure, were left open to air according to 1/1/1 scheme<sup>31</sup>, ie, for 1 hour, atleast 1 m away from walls and 1m from the floor adjacent to one end of the operating table(53). The plates were closed after 1 hour and were incubated at 37° C for 48 hours. After incubation, the colonies observed on each plate were counted and noted as the number of bacteria carrying particles which have settled over the area of the given plate in the given period of time.

CFU is calculated by the formula( Polish standard PN89/2-04088/08)<sup>(75)</sup>

$CFU/m^3 = \frac{a \times 1000}{p \times t \times 0.2}$  where a= the number of colonies on the Blood agar plate p= the surface measurement of the Blood agar plate used t= the time of exposure of the Blood agar plate.

The colonies on each plate were identified upto species level by Grams staining, motility, catalase test, Oxidase test and routine biochemical reactions<sup>(51)</sup>. Antimicrobial susceptibility pattern was identified and compared with that of the clinical isolates from postoperative wound infection.

#### **Active air sampling :**

Active air sampling was done by air sampler system (Himedia-LA002) using sterile plastic air sampler strips containing blood agar. The air sampler works under

the principle of centrifugal impaction and samples total volume of 280L/min with a rotational speed of 3970 RPM. The blood agar strips were inserted into the sampler and a volume of 280L/min air was sampled at rest, as a single continuous drawing for 7 min, with no people in the OT upto 6 feet height and 1 feet above the operation table<sup>(51,53)</sup>. The strips were incubated at 37 ° C for 24 hrs. The Microbial Air Contamination were measured as CFU/m<sup>3</sup> by the formula<sup>(52)</sup>

$$\text{CFU /m}^3 = \text{Total No. of colonies on blood agar strip} \times 25 / \text{Time of exposure in minutes.}$$

#### **Surface swabs:-**

Swabs were collected from various surfaces in all OTs (OT table, light, Air conditioner, Boyles' apparatus, washing room, drug rack) using peptone water and Robertson's cooked meat medium (RCM). The peptone water swabs were streaked on nutrient agar, blood agar plates and MacConkey agar. The plates were incubated at 37°C for 24hrs and the colonies observed were identified by biochemical tests.

The swabs collected in RCM were kept in water bath at 80°C for 30min<sup>(67)</sup> and incubated at 37°C for 5 days. Smears were prepared from these tubes, Gram stained and examined for *Clostridium tetani* spores. Tubes showing spores were subjected to anaerobic culture on blood agar using Gaspak system and aero tolerance test.<sup>(10, 84)</sup> Details of the results were recorded.

### **Nasal swabs and hand culture:**

Nasal swabs were taken from anterior nares and hand imprints on 90mm 5 % blood agar plate from 30 hospital personnel which included nursing staff, doctors, interns, postgraduates, sanitary workers and other personnel working in the postoperative wards.

The hand imprint blood agar plates were incubated at 37°C for 48hours. The nasal swabs were streaked onto 5% Blood Agar plates and incubated at 37°C for 48hours. The colonies observed on the plates following incubation were identified by Grams stain , Catalase test, Oxidase test and various biochemical reactions. Antibiotic sensitivity of the isolates were identified by Kirby Bauer Disc Diffusion method according to CLSI<sup>(71)</sup> .

### **MOLECULAR METHOD:**

#### **Polymerase chain reaction:**

Two strains of *Methicillin resistant Staphylococcus aureus* isolated from wound culture in patients who underwent General surgery had an antibiotic resistant pattern similar to that of *Methicillin resistant Staphylococcus aureus* strain isolated by passive air sampling method from General surgery Operation theatre. DNA of these isolates were extracted, 16S rRNA gene amplification and Amplified Ribosomal DNA Restriction Analysis (ARDRA) were performed in order to differentiate the bacterial strains.

### **Protocol for DNA extraction:**

- ❖ Mid log phase cultures were harvested by centrifugation and washed twice in TE buffer (10mM Tris; 1mM EDTA pH-8.0).
- ❖ Pellets were suspended in 400µl of sucrose TE for lysis.
- ❖ Lysozyme (10 mg/ml) was added and the mixture was incubated at 37°C for 30 mins.
- ❖ After 30 mins 100µl of 0.5M EDTA (pH-8), 60µl of 10% SDS and 3µl of proteinase K (20 mg/ml stock) were added and incubated at 55°C for 12 hours.
- ❖ To remove the proteins, purification was done by adding 500µl of equilibrated phenol and chloroform 1:1 ratio.
- ❖ Spun at 10,000 rpm for 10 mins.
- ❖ The above step was repeated once again.
- ❖ Extracted once with chloroform: isoamyl alcohol (24:1) 500µl.
- ❖ Spun at 10,000 rpm for 10 mins.
- ❖ the supernatant was precipitated with 2.5 volume of 100% ethanol.
- ❖ Centrifuged at 10,000 rpm for 10 mins.
- ❖ Discarded the ethanol and to the pellet added 70% ethanol and spun.
- ❖ After air drying, the tubes were resuspended with 20 µl of Milli Q and kept at -20° C.

**Amplification of 16S rRNA gene from *Staphylococcus aureus* isolates of surgical OT and PO ward :**

Bacterial 16S rRNA gene was amplified from the extracted genomic DNA using the following universal eubacterial 16S rRNA gene primers<sup>(16)</sup>,

**Forward primer** 5' AGAGTTTGATCCTGGCTCAG 3' (*E. coli* positions 8-27)

**Reverse primer** 5' ACGGCTACCTTGTTACGACTT 3' (*E. coli* positions 1492-1513).

**Reaction volume = 50 µl**

<b>Components</b>	<b>Stock concentration</b>	<b>Working concentration</b>
Taq buffer with MgCl <sub>2</sub>	10X	1X
dNTPs	10 mM	50µM
Forward primer	10 µM	0.2 µM
Reverse primer	10 µM	0.2 µM
Taq polymerase	1U/µl	1 U
Template		~20 ng

**Thermal-cycling profile**

- Initial denaturation - 95 °C for 5 min
- Denaturation - 94 °C for 1 min
- Annealing - 55 °C for 1 min 40 cycles
- Extension - 72 °C for 2 min
- Final extension - 72 °C for 10 min

## **Amplified Ribosomal DNA Restriction Analysis (ARDRA)**

Amplified Ribosomal DNA Restriction Analysis (ARDRA) was performed in order to differentiate the bacterial strains.

### **Protocol:**

- ❖ The 16S rRNA-PCR products were digested with four selected restriction enzymes (*Hinf* I, *Rsa* I, *Msp*I and *Alu*I) in separate reactions.
- ❖ The digestion was performed for 3 h at 37°C in 20µl reaction volume containing 10µl of PCR product, 2µl of commercially supplied incubation buffer, 7µl of milli Q water and 1µl (10U/µl) of restriction enzyme.
- ❖ Digested products were run on 2% agarose (Amersham, USA) gel in 1X TAE buffer for 4 h at 2V/cm. Gels were stained with Ethidium Bromide and visualized under UV transilluminator and results were analyzed.

# RESULTS



## RESULTS

Total number of patients with clinically suspected SSI: 200

Total number of culture positive patients: 140

Total number of culture negative patients: 60

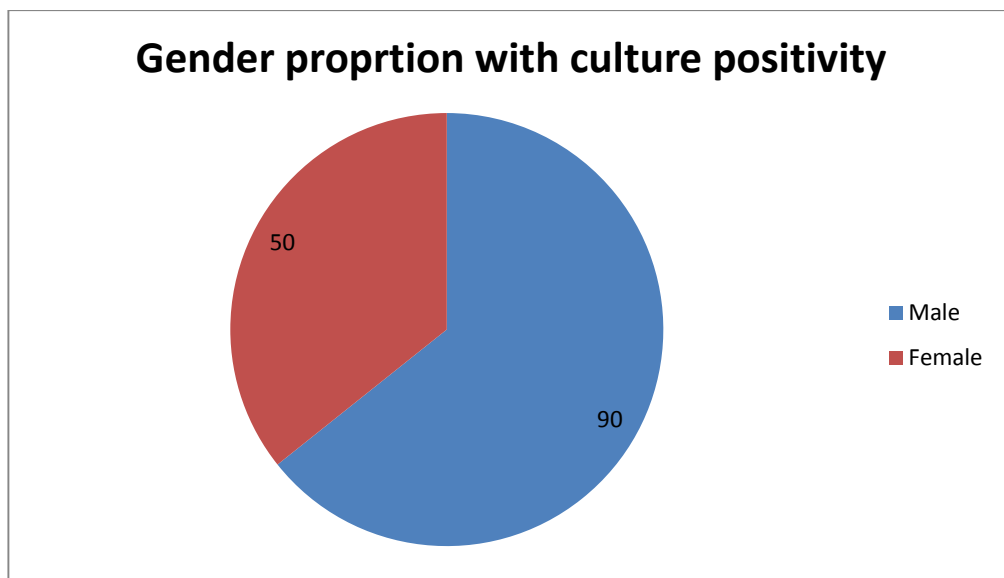
**Table 1: Master table showing the age wise break up of patients with clinically suspected SSI and culture positivity in various departments:**

AGE	General Surgery							Cardiothoracic surgery							Vascular surgery						
	Total No. of clinically suspected SSI	Male	Female	No. of Culture positives	No. of culture negatives	Aerobic isolates	Anaerobic isolates	Total No. of clinically suspected SSI	Male	Female	No. of Culture positives	No. of culture negatives	Aerobic isolates	Anaerobic isolates	Total No. of clinically suspected SSI	Male	Female	No. of Culture positives	No. of culture negatives	Aerobic isolates	Anaerobic isolates
≤ 20yrs	10	5	5	8	2	8	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0
21-30	21	12	9	15	6	15	3	4	2	1	3	1	3	0	1	0	1	1	0	1	0
31-40	31	16	15	21	10	21	0	10	9	1	5	5	5	0	0	0	0	0	0	0	0
41-50	28	14	14	25	3	25	1	6	6	0	5	1	5	0	3	3	0	3	0	3	0
51-60	39	24	15	24	15	24	1	8	8	0	5	3	5	0	2	2	0	2	0	2	0
≥ 60 yrs	29	17	12	20	9	20	2	3	3	0	1	2	1	0	4	3	1	3	1	3	0
<b>Total Perc</b>	<b>158</b>	<b>88 (55%)</b>	<b>70 (44%)</b>	<b>113 (71.5%)</b>	<b>45 (28.5%)</b>	<b>113</b>	<b>7</b>	<b>31</b>	<b>28 (90.3%)</b>	<b>3 (9.6%)</b>	<b>18 (58.1%)</b>	<b>13 (41.9%)</b>	<b>19</b>	<b>0</b>	<b>11</b>	<b>9 (82%)</b>	<b>2 (18%)</b>	<b>10 (90.9%)</b>	<b>1 (9.1%)</b>	<b>11</b>	<b>0</b>

Total number of clinically suspected SSI in General surgery : 158  
 Total number of culture positivity in General surgery : 113(71.5%)  
 Total number of clinically suspected SSI in Cardiothoracic surgery : 31  
 Total number of culture positivity in Cardiothoracic surgery : 18(58.06%)  
 Total number of clinically suspected SSI in Vascular surgery : 11  
 Total number of culture positivity in Vascular surgery : 10(90.09%)

**Table:2 GENDER DISTRIBUTION IN SSI**

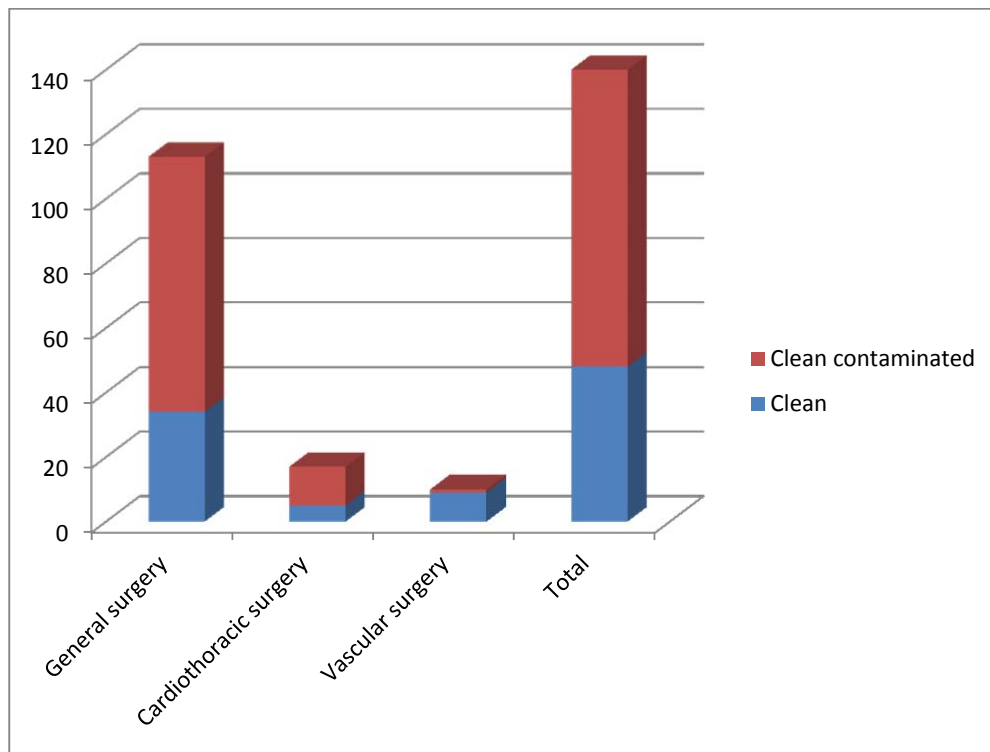
<b>Gender</b>	<b>No. with culture positivity</b>	<b>Percentage n=140</b>
Male	90	64.29%
Female	50	35.71%
<b>Total</b>	<b>140</b>	<b>100%</b>



Males (64.29%) had a higher SSI rate compared to females(35.71 %)

**TABLE: 3 ANALYSIS OF SSI RATE BY WOUND CLASSIFICATION:**

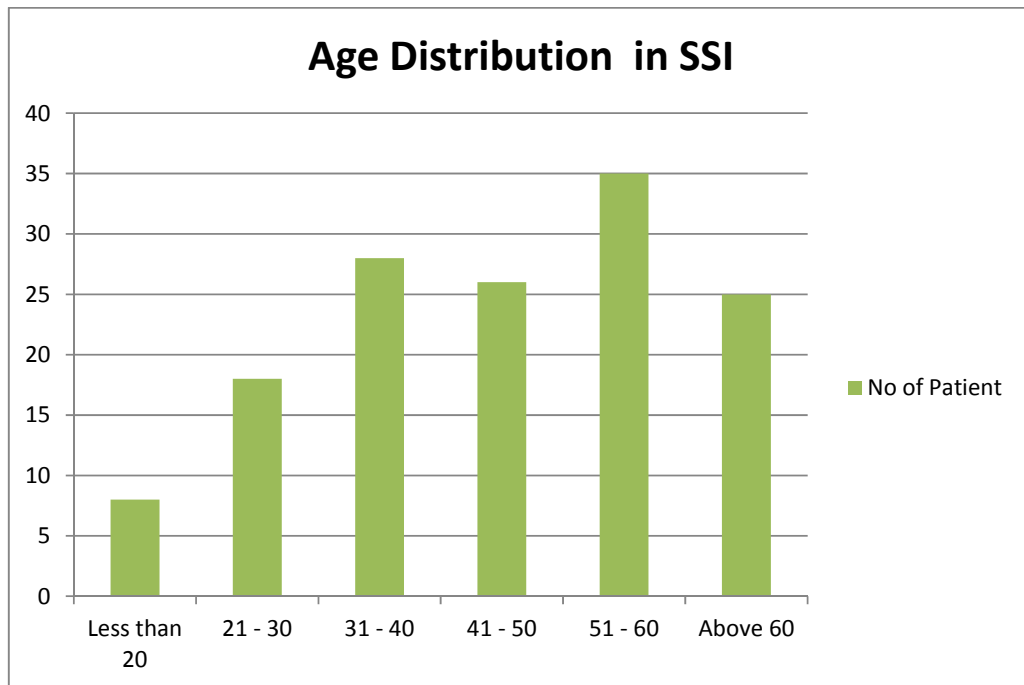
Various surgeries	Clean	Clean contaminated
General surgery	34	79
Cardiothoracic surgery	5	12
Vascular surgery	9	1
Total	48(5.3%)	92(10.22%)



Clean contaminated surgical wounds(10.22%) had higher SSI rate compared to clean wounds(5.3%).

**Table:4 ANALYSIS OF AGE DISTRIBUTION AMONG WOUND CULTURE POSITIVE PATIENTS**

Age	No. of Patient with SSI	Percentage(n=140)
Less than 20	8	5.71%
21 - 30	18	12.86%
31 - 40	28	20%
41 - 50	26	18.57%
51 - 60	35	25%
Above 60	25	17.86%
Total	140	100%

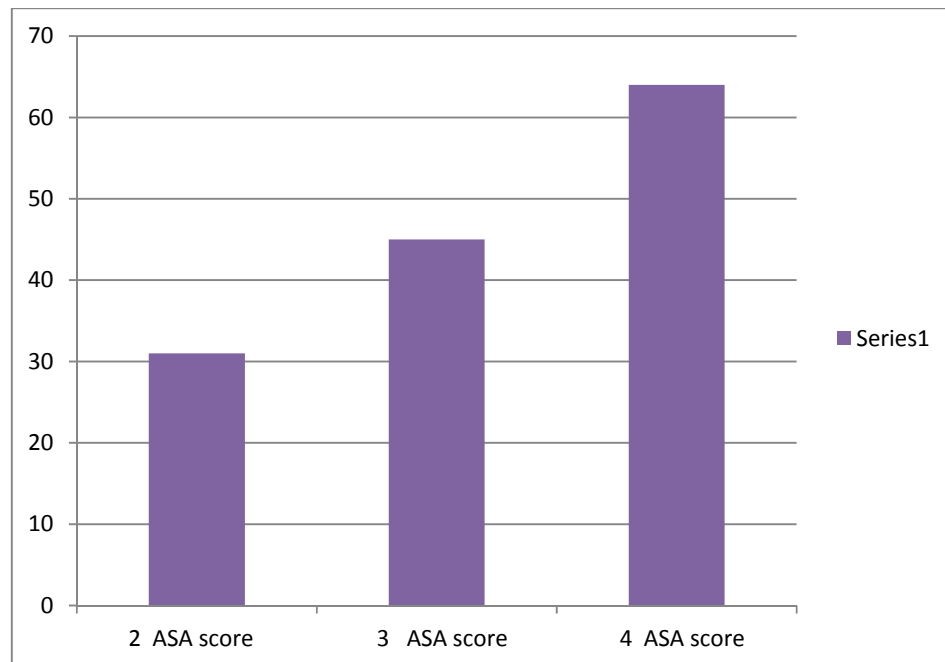


The Surgical site infection was relatively high in the age group of 51-60 years

**Table:5 ANALYSIS OF SSI RATE IN PATIENTS WITH  
VARIOUS ASA SCORE**

<b>ASA*score</b>	<b>No.of pts With SSI</b>	<b>Percentage (n=140)</b>
2 ASA score	31	22.14%
3 ASA score	45	32.14%
4 ASA score	64	45.72%
<b>Total</b>	<b>140</b>	

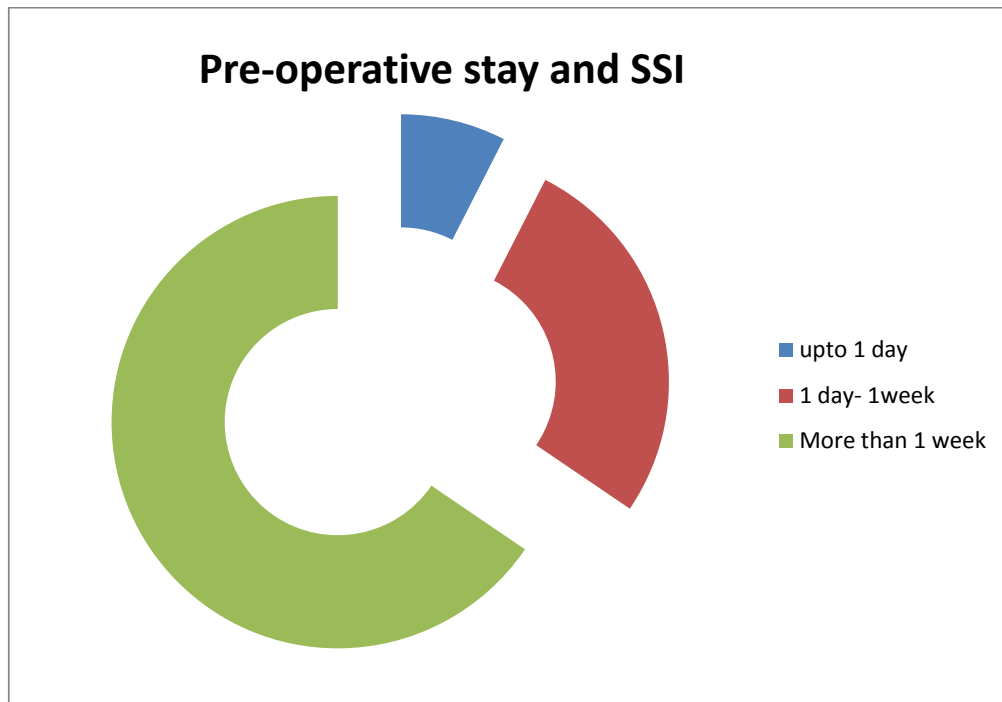
\*ASA - The American Society of Anaesthesiologists



The SSI rate was found to be higher in patients with ASA score >2.

**TABLE:6 CORRELATION OF DURATION OF PRE OPERATIVE HOSPITAL STAY WITH DEVELOPMENT OF SSI POSTOPERATIVELY:**

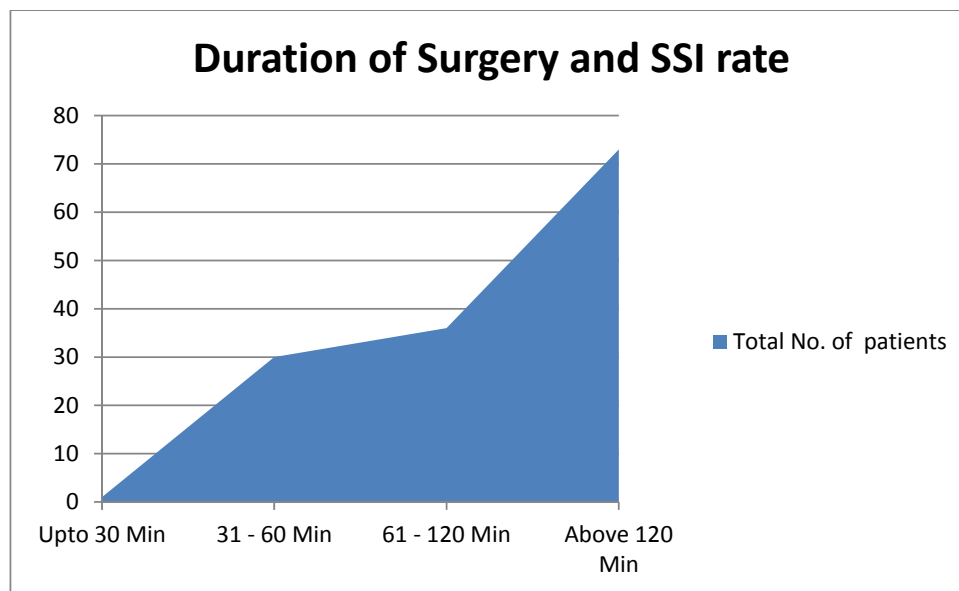
S.No.	Preoperative stay	Total No. of Patients with SSI	Percentage (n=140)
1	0- 1 day	10	7.5%
2	1 day- 7 days	38	27%
3	More than 7 days	92	65.5%
	Total	140	100%



The rate of SSI increased with the duration of preoperative hospital stay.

**TABLE:7 CORRELATION OF DURATION OF SURGERYWITH  
DEVELOPMENT OF SSI POSTOPERATIVELY:**

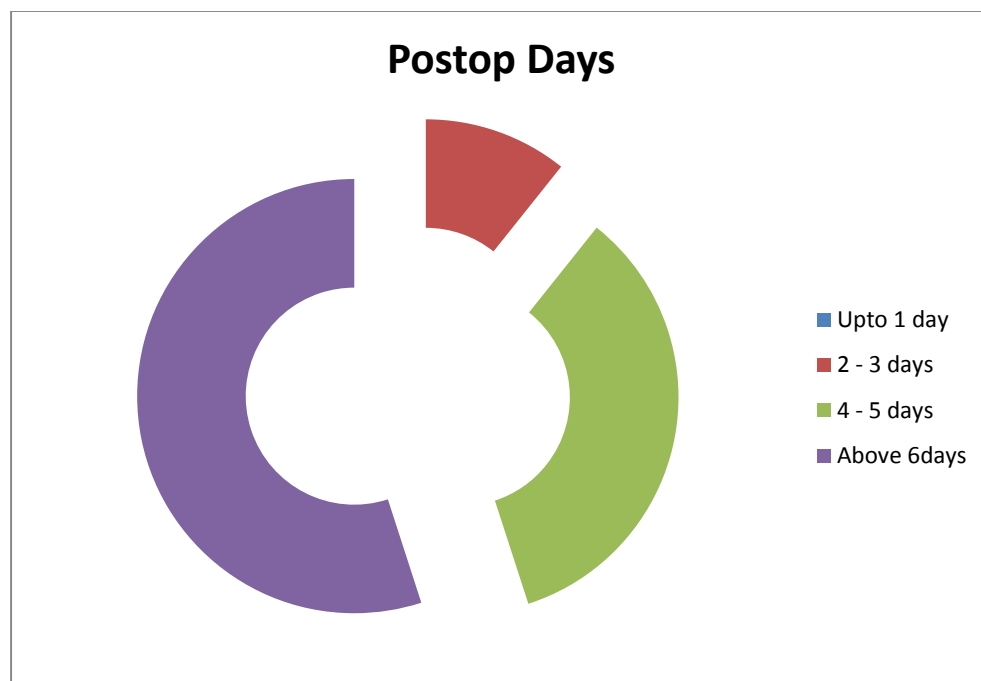
<b>Duration of Surgery</b>	<b>Total No. of patients with SSI</b>	<b>Percentage (n=140)</b>
Upto 30 Min	1	0.70%
31 - 60 Min	30	21.30%
61 - 120 Min	36	26%
Above 120 Min	73	52%
Total	140	



The SSI rate was found to be higher in patients who were operated for more than 2 hours(52%).

**TABLE:8 ANALYSIS OF TIME DURATION OF INFECTION AFTER THE SURGERY:**

<b>Post Operative Stay</b>	<b>Total No of Patients with SSI</b>	<b>Percentage (n=140)</b>
Upto 1 day	0	0%
2 - 3 days	15	10.72%
4 - 5 days	48	34.28%
Above 6days	77	55%
Total	140	100%



The rate of postoperative wound infection was found to increase with the increase in postoperative days. SSI rate was higher among patients with postoperative stay of more than 6 days (55%) compared to a stay of 2-3days(10.71%).

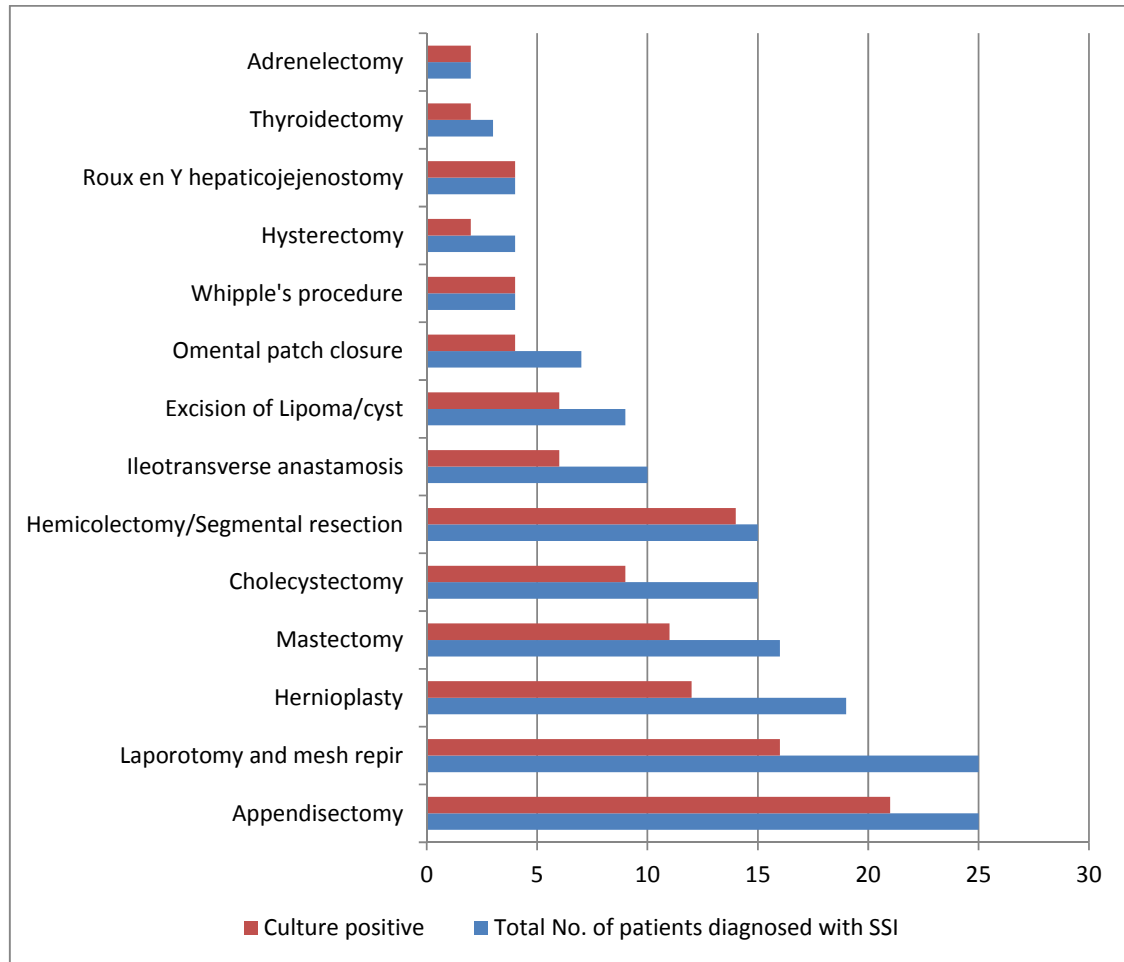


**TABLE:9 INFECTION RATE IN VARIOUS SURGERIES IN GENERAL****SURGERY :**

S.No	Type of the surgery	Total No. of patients diagnosed with SSI	Culture positive			Culture negative	Proportion of infection in various surgeries ( n=158)
			T*	M*	P*		
1	Appendicectomy	25	21	17	4	4	13.29%
2	Laporotomy and mesh repair	25	16	16	0	9	10.13%
3	Hernioplasty	19	12	12	0	7	7.6%
4	Mastectomy	16	11	10	1	5	7%
5	Cholecystectomy	15	9	9	0	6	5.7%
6	Hemicolectomy/Segmental resection	15	14	12	2	1	8.9%
7	Ileotransverse anastomosis	10	6	6	0	4	3.8%
8	Excision of Lipoma/cyst	9	6	6	0	3	3.8%
9	Omental patch closure	7	4	4	0	3	2.5%
10	Whipple's procedure	4	4	4	0	0	2.5%
11	Hysterectomy	4	2	2	0	2	1.3%
12	Roux en Y hepaticojejunostomy	4	4	4	0	0	2.5%
13	Thyroidectomy	3	2	2	0	1	1.3%
14	Adrenelectomy	2	2	2	0	0	1.3%
	Total	158	113	106	7	45	71.6%

T\*-Total, M\*-Monomicrobials, P\*-Polymicrobials

## INFECTION RATE IN VARIOUS GENERAL SURGERIES:

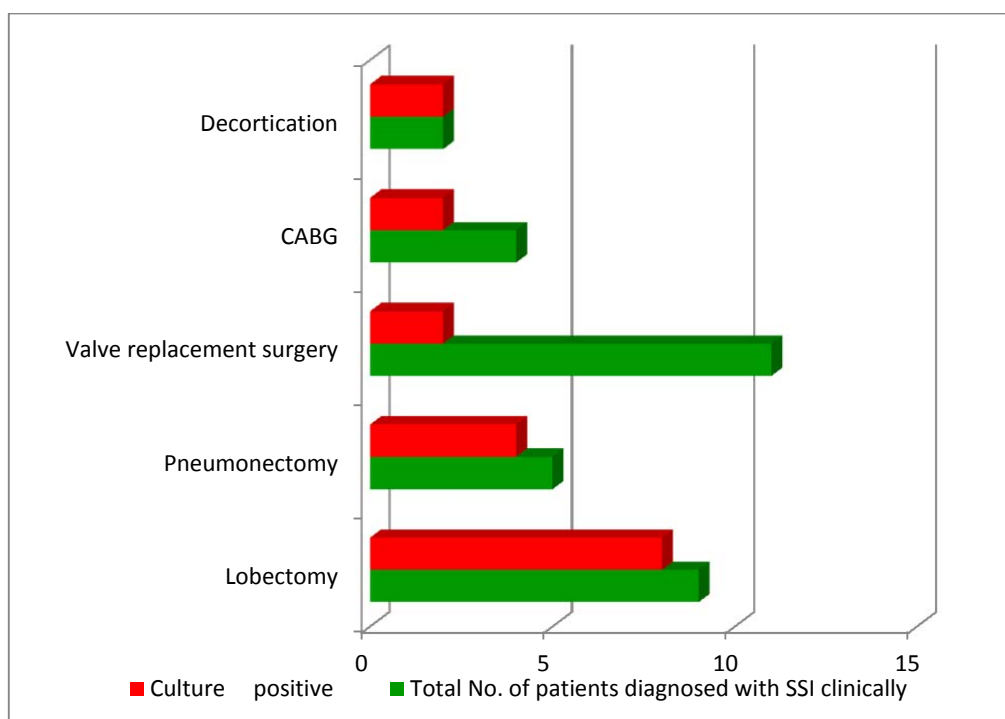


Among 158 General surgery patients who were clinically diagnosed with SSI, 113 were culture positive, of which appendicectomy had the highest rate of infection(13.29%) followed by laparotomy with mesh repair(10.13%) . SSI rate was least(1.3%) in patients who underwent thyroidectomy, adrenelectomy and hysterectomy. Monomicrobial isolates predominated over polymicrobial isolates in General surgical patients.

**TABLE:10 SSI RATE IN VARIOUS CARDIOTHORACIC SURGERIES**

S. No	Type of surgery	Total No. of patients diagnosed with SSI clinically	Culture positive			Culture negative	Proportion of infection in various CTS surgeries n=31
			T*	M*	P*		
1	Lobectomy	9	T*	M*	P*	1	25.8%
			8	7	1		
2	Pneumonectomy	5	4	4	0	1	13%
3	Valve replacement surgery	11	2	2	0	9	6.45%
4	CABG	4	2	2	0	2	6.45%
5	Decortication	2	2	2	0	0	6.45%
	Total	31	18	17	1	13	58.06%

T\*-Total, M\*-Monomicrobials, P\*-Polymicrobials

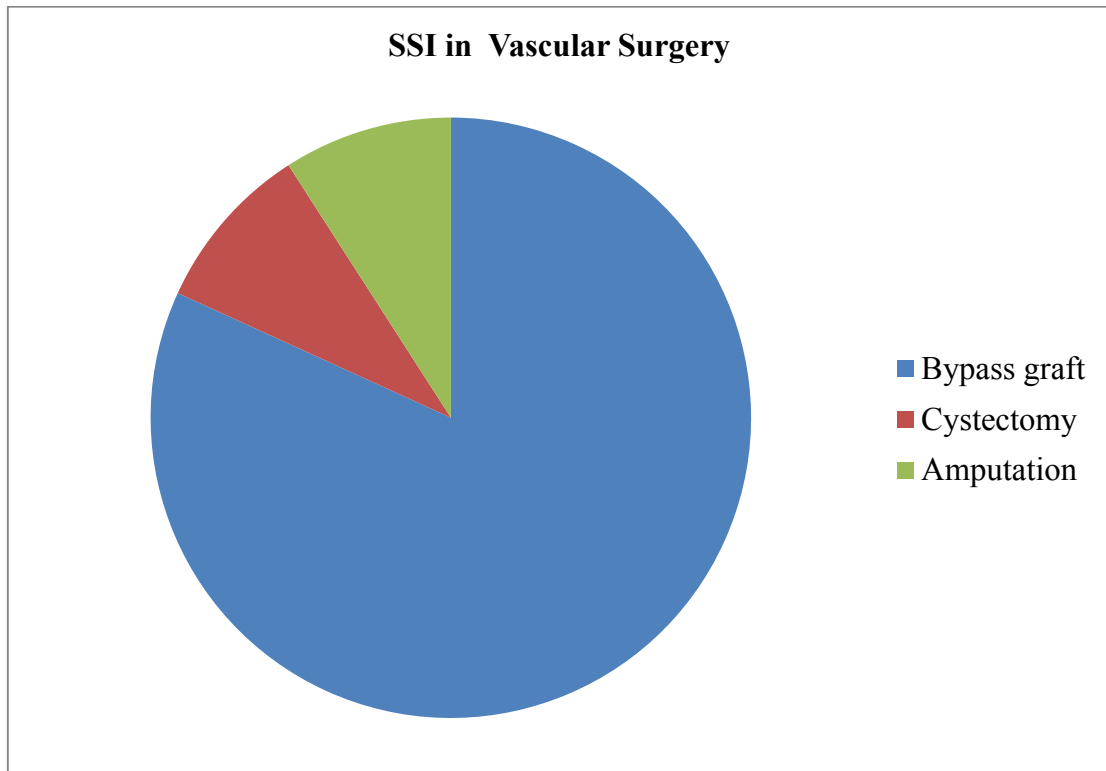


Out of 31 Cardiothoracic surgery patients who were clinically diagnosed to have SSI, 18 were culture positive. Among these culture positive patients, lobectomy patients(25.8%) formed the highest proportion. In patients who underwent Valve replacement surgery, 11 were clinically diagnosed with SSI but only 2 were culture positive and the SSI rate was also low(6.45%).Among the 18 culture positives in Cardiothoracic patients 17 were mono microbials and 1 was polymicrobial.

**TABLE:11 INFECTION RATE IN VARIOUS VASCULAR SURGERIES**

S.No	Procedure	Total pts. With SSI clinically diagnosed SSI	Culture positives			Culture negatives	Proportion of infection in various surgeries n=11
			T*	M*	P*		
1	Bypass graft	9	8	8	0	1	72.72%
2	Arterial Cystectomy	1	1	1	0	0	9.09%
3	Amputation	1	1	0	1	0	9.09%
	Total	11	10	9	1	1	90.09%

T\*-Total, M\*-Monomicrobials, P\*-Polymicrobials

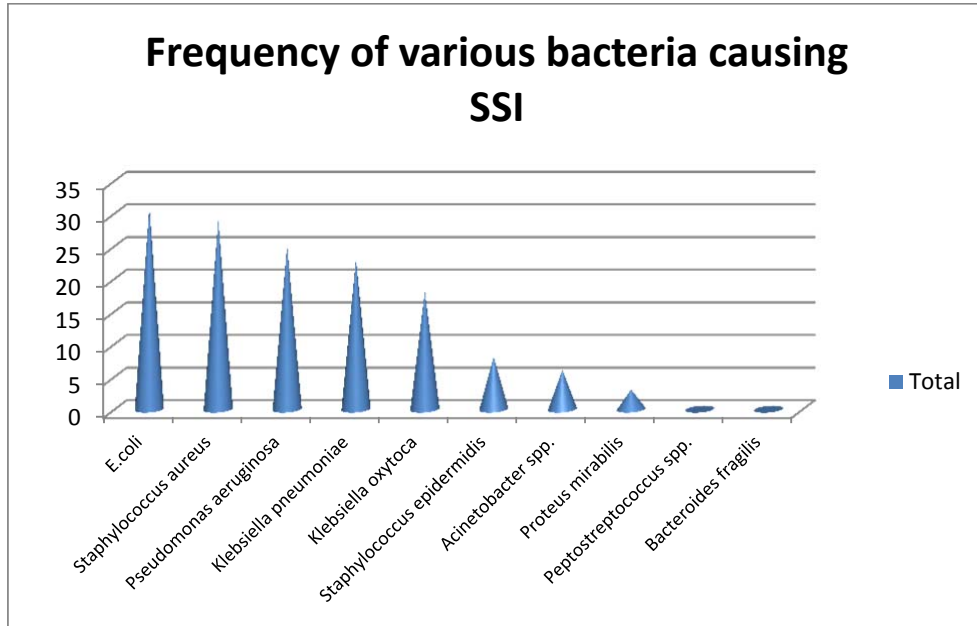


Among 11 patients with clinically diagnosed SSI, 10 were culture positive and patients who underwent Bypass graft(72.72%) had higher rate of SSI. Of these 10 culture positive wounds, 9 were due to monomicrobials and 1 due to polymicrobial.

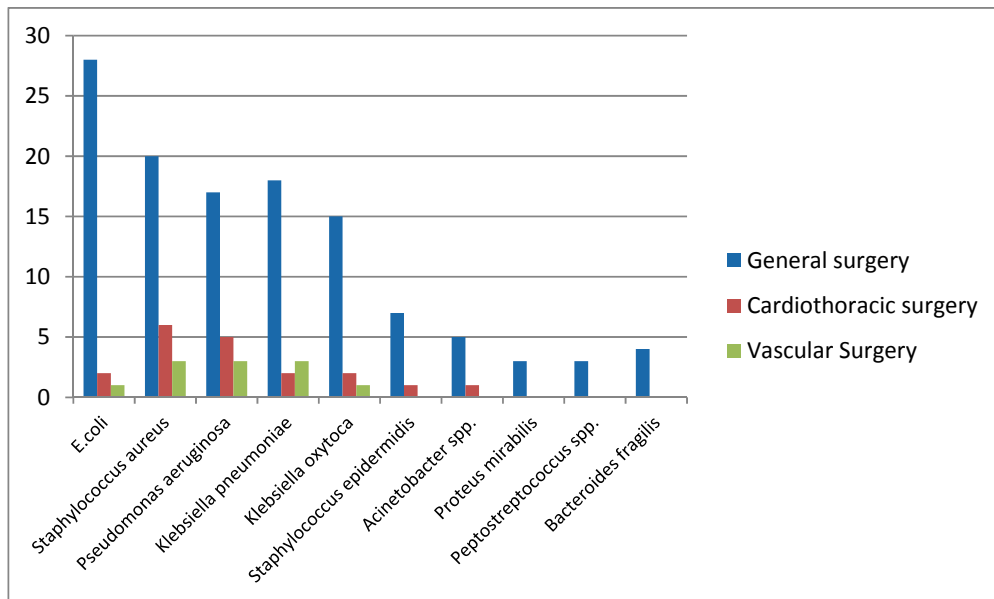
**Table:12 FREQUENCY OF VARIOUS PATHOGENS CAUSING SSI IN RELATION TO VARIOUS SPECIALITIES**

Among the total 150 isolates Causing SSI, bacterial isolation rate was higher in General surgery 120/150 (80%) compared to Cardiothoracic surgery 19/150(12.66%) and Vascular surgery 11/150(7.33%).

Organisms	General surgery	Cardiothoracic surgery	Vascular Surgery	Total	Percentage of various organisms (n= 150)
<b>Aerobic isolates:</b> <i>E.coli</i>	28	2	1	31	20.66%
<i>Staphylococcus aureus</i>	20	6	3	29	19.33%
<i>Pseudomonas aeruginosa</i>	17	5	3	25	16.67%
<i>Klebsiella pneumoniae</i>	18	2	3	23	15.3%
<i>Klebsiella oxytoca</i>	15	2	1	18	12.%
<i>Staphylococcus epidermidis</i>	7	1	0	8	5.33%
<i>Acinetobacter spp.</i>	5	1	0	6	4%
<i>Proteus mirabilis</i>	3	0	0	3	2%
<b>Anaerobic isolates:</b> <i>Peptostreptococcus spp.</i>	3	0	0	0	2%
<i>Bacteroides fragilis</i>	4	0	0	0	2.67%
Total	120(80%)	19(12.67%)	11(7.33%)	150	100%



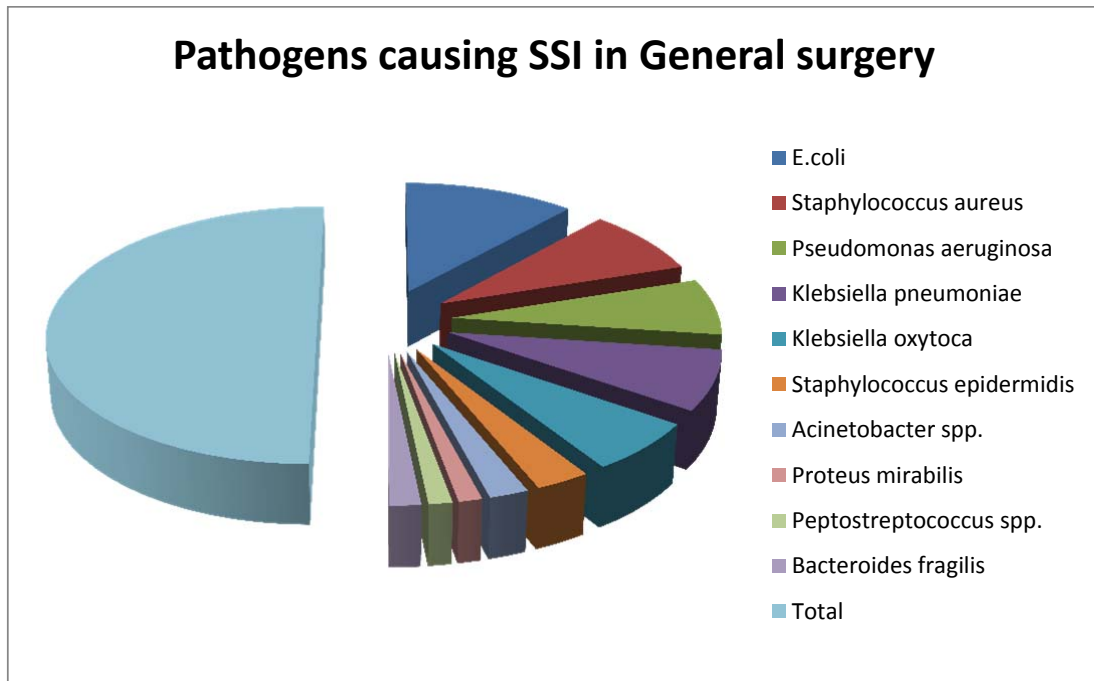
In this study, *Escherichiae coli* 31/150(20.66%) was the most frequent pathogen isolated from the Surgical site infection. The least common were anaerobes (*Peptostreptococcus spp.* 3/150( 2%) and *Bacteroides* 4(2.67%).



**TABLE: 13 PATHOGENS CAUSING SSI IN GENERAL SURGERY**

Organisms	General surgery			Percentage n= 120
	T*	M*	P*	
<i>E.coli</i>	28	26	2	23.33%
<i>Staphylococcus aureus</i>	20	18	2	16.67%
<i>Pseudomonas aeruginosa</i>	17	17	0	14.17%
<i>Klebsiella pneumoniae</i>	18	17	1	15%
<i>Klebsiella oxytoca</i>	15	15	0	12.5%
<i>Staphylococcus epidermidis</i>	7	7	0	5.83%
<i>Acinetobacter spp.</i>	5	5	0	4.17%
<i>Proteus mirabilis</i>	3	1	2	2.5%
<i>Peptostreptococcus spp.</i>	3	0	3	2.5%
<i>Bacteroides fragilis</i>	4	0	4	3.33%
Total	113	106	14	100%



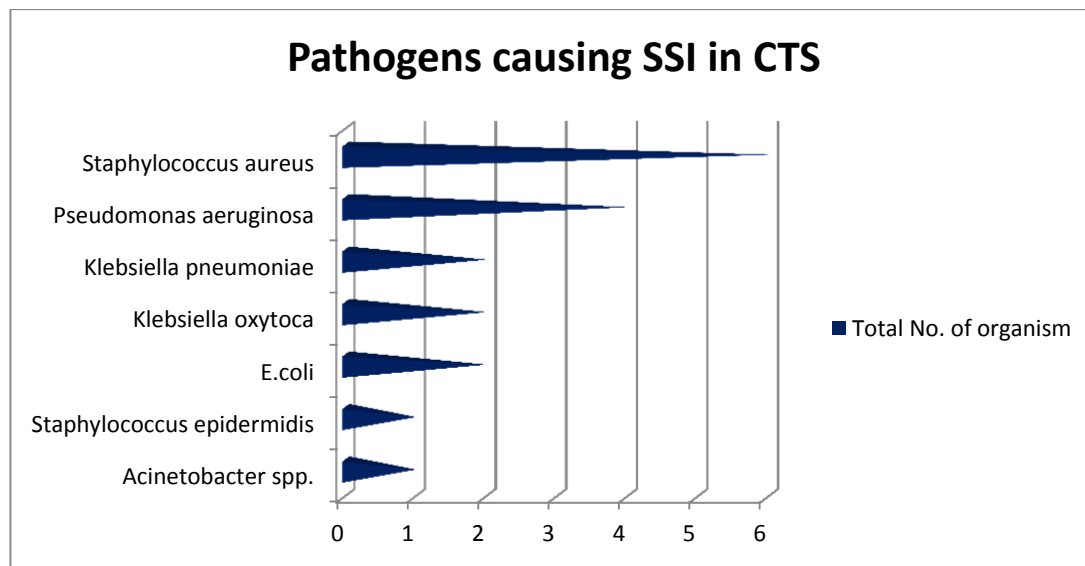


In General surgical wound culture, *E.coli* (28/120) was the commonest isolate followed by *Staphylococcus aureus*(20/120), *Pseudomonas aeruginosa*(17/120) and *Klebsiella pneumoniae*(18/113) and the least common was *Proteus mirabilis* (3/120).

Anaerobes in General surgical patients, were isolated in combination with aerobes like *E.coli* and *Proteus mirabilis*.

**TABLE: 14 PATHOGENS CAUSING SSI IN CARDIOTHORACIC SURGERY**

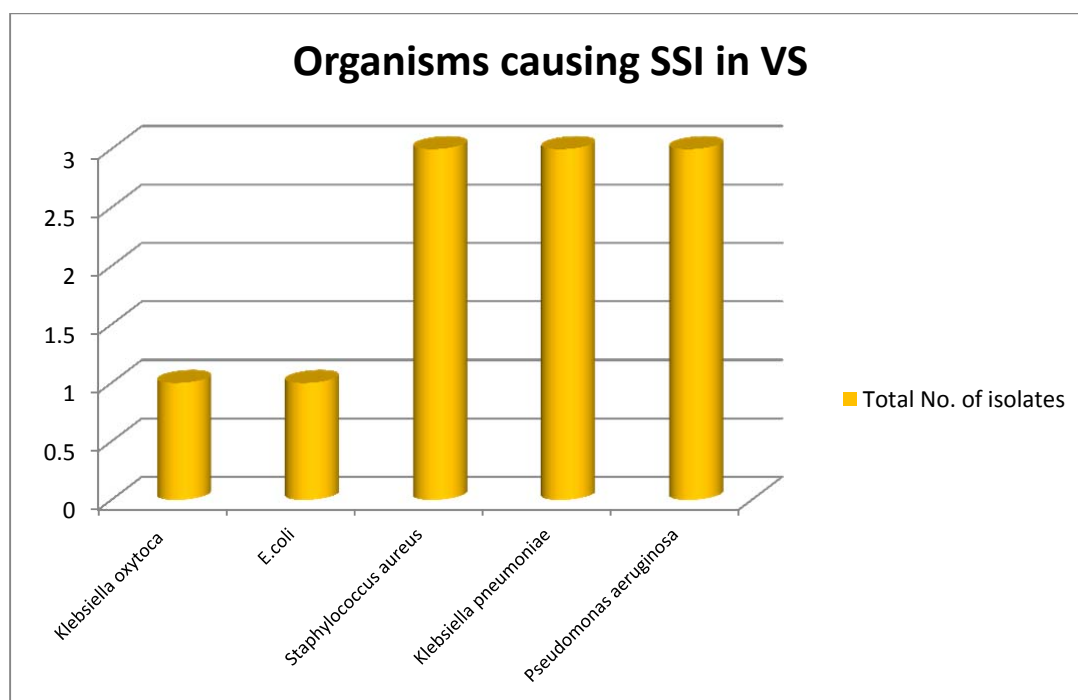
<b>Organisms</b>	<b>Cardiothoracic surgery</b>	<b>Percentage (n=19)</b>
<i>E.coli</i>	2	10.52%
<i>Staphylococcus aureus</i>	6	31.57%
<i>Pseudomonas aeruginosa</i>	5	26.31%
<i>Klebsiella pneumoniae</i>	2	10.52%
<i>Klebsiella oxytoca</i>	2	10.52%
<i>Staphylococcus epidermidis</i>	1	5.26%
<i>Acinetobacter spp.</i>	1	5.26%
<b>Total</b>	<b>19</b>	<b>100%</b>



In cardiothoracic surgery, *Staphylococcus aureus* 6/19(31.57%) was the most common isolate followed by *Pseudomonas aeruginosa* 5/19(26.31%) and *Klebsiella pneumoniae* 2/19(10.52%) . The least common isolate was *Acinetobacter spp.* 1/19(5.26%).

**TABLE : 15 PATHOGENS CAUSING SSI IN VASCULAR SURGERY**

Organisms	Vascular surgery	Percentage (n=11)
<i>E.coli</i>	1	9.09%
<i>Staphylococcus aureus</i>	3	27.27%
<i>Pseudomonas aeruginosa</i>	3	27.27%
<i>Klebsiella pneumoniae</i>	3	27.27%
<i>Klebsiella oxytoca</i>	1	9.09%
Total	11	100%



In vascular surgery, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (27% each) were common pathogens, whereas *Klebsiella oxytoca* and *E.coli*(9.09% each) were less common isolates.

**TABLE 16 : ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM  
POSITIVE BACTERIAL ISOLATES:**

<b>Antibiotics</b>	<b>MSSA n=21</b>		<b>MRSA n= 8</b>		<i>Staphylococcus epidermidis</i> n= 4	
	<b>Sensitive</b>	<b>%</b>	<b>Sensitive</b>	<b>%</b>	<b>Sensitive</b>	<b>%</b>
Amikacin	16	76%	7	87.5%	3	75%
Ciprofloxacin	12	57.14%	4	50%	2	50%
Penicillin	2	14.28%	0	0%	0	0%
Erythromycin	16	76.19%	4	50%	2	50%
Chloramphenicol	20	95.24%	7	87.5%	3	75%
Vancomycin	21	100%	8	100%	4	100%

All the Gram positive cocci were 100% sensitive to vancomycin and least sensitive to penicillin.

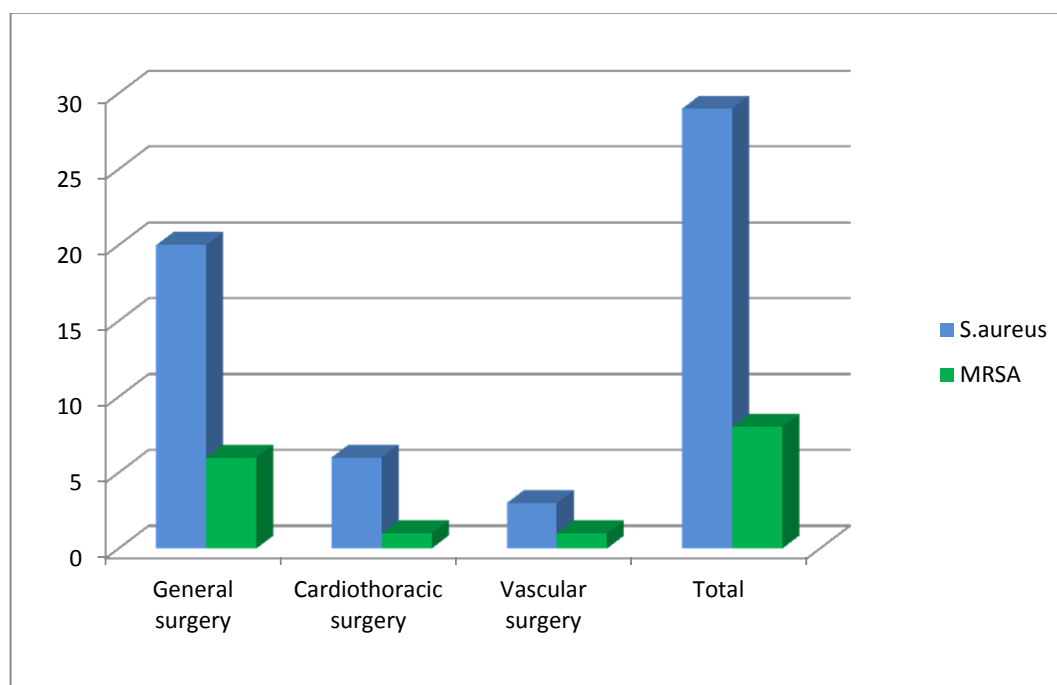
**TABLE 17: ANTIMICROBIAL SENSITIVITY PATTERNS OF GRAM NEGATIVE BACTERIAL ISOLATES**

<b>Organism</b>	<b>No. of isolates</b>	<b>AK</b>	<b>CZ</b>	<b>Cip</b>	<b>Gm</b>	<b>PT</b>	<b>Imip</b>
<i>Escherichia coli</i>	31	93.5% (29)	22.58% (7)	41.93% (13)	35.48% (11)	90.32% (28)	100% (31)
<i>Klebsiella pneumoniae</i>	23	69.56% (16)	65.21% (15)	52.17% (12)	43.47% (10)	87% (20)	100% (23)
<i>Klebsiella oxytoca</i>	18	67% (12)	38.88% (7)	33.33% (6)	16.66% (3)	89% (16)	100% (18)
<i>Pseudomonas aeruginosa</i>	25	76% (19)	28% (7)	84% (21)	44% (11)	88% (22)	100% (25)
<i>Acinetobacter spp</i>	6	83.33% (5)	0% (0)	66.66% (4)	50% (3)	83.33% (5)	100% (6)
<i>Proteus mirabilis</i>	3	100% (3)	67% (2)	67% (2)	0% (0)	100% (3)	100% (3)

All the Gram negative bacilli were 100% sensitive to Imipenem and were least sensitive to Ceftazidime and Gentamycin.

**TABLE 18: METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SSI**

Speciality	<i>S.aureus</i>	MRSA	MSSA	Percentage of MRSA
General surgery	20	6	14	30%
Cardiothoracic surgery	6	1	5	16.66%
Vascular surgery	3	1	2	33%
Total	29	8	21	



Out of 29 *Staphylococcus aureus* isolates from SSI, 8 isolates(27.58%) were Methicillin resistant and 21 isolates were methicillin sensitive(71.41%).

**TABLE 19 : INTERPRETATION OF MIC OF VANCOMYCIN FOR  
METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* BY  
MACROBROTH DILUTION METHOD**

<b>Number of MRSA</b>	<b>MIC value</b>	<b>Interpretation</b>
8	$\leq 2\mu\text{g/l}$	Sensitive

$\leq 2\mu\text{g/ml}$  – Susceptible

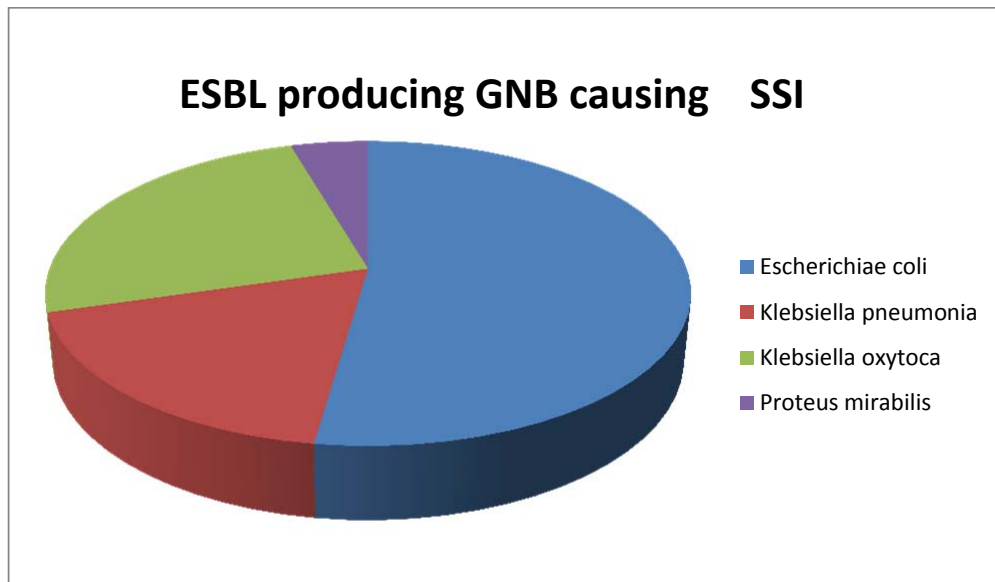
4-8 $\mu\text{g/ml}$  – Intermediate

$\geq 16\mu\text{g/ml}$  – Resistant

All the eight Methicillin Resistant *Staphylococcus aureus* isolates were sensitive to Vancomycin by Macrobroth dilution method .

**TABLE 20: EXTENDED SPECTRUM BETA LACTAMASE(ESBL)  
PRODUCERS CAUSING SSI:**

<b>Organisms</b>	<b>Total isolates</b>	<b>ESBL</b>	<b>Percentage of ESBL producers (n=75)</b>
<i>Escherichiae coli</i>	31	23	31%
<i>Klebsiella pneumonia</i>	23	8	11%
<i>Klebsiella oxytoca</i>	18	11	15%
<i>Proteus mirabilis</i>	3	2	3%
Total	75	44	59%



Among 75 enteric Gram negative bacilli 44(59%) were ESBL producers.

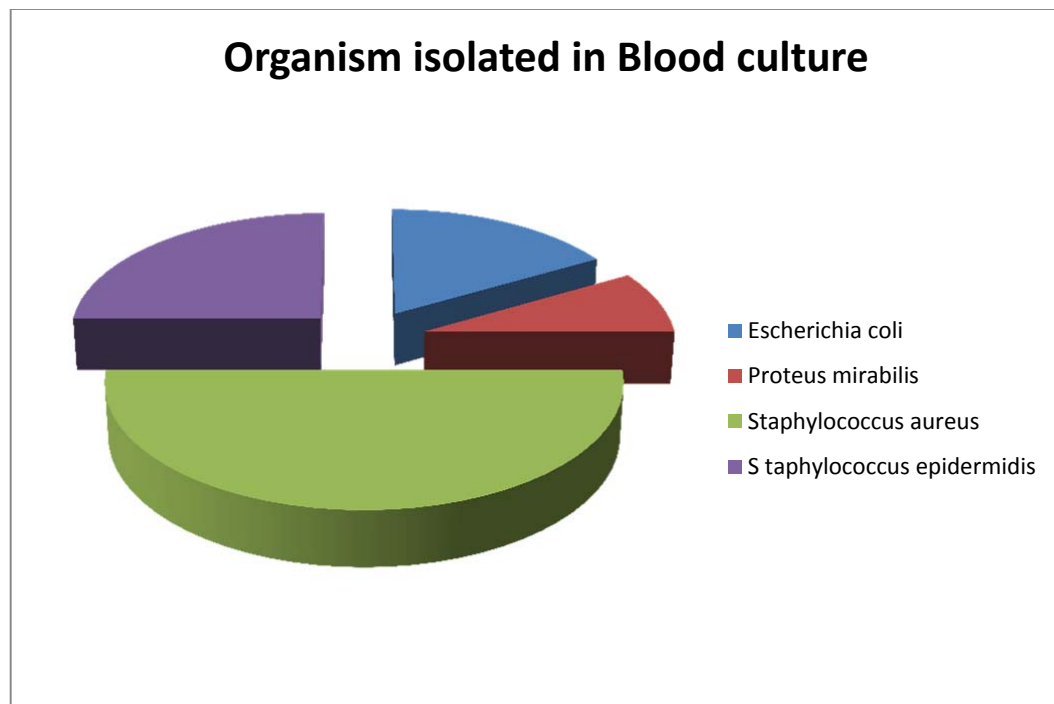
**TABLE 21: CORRELATION OF ISOLATES FROM BLOOD AND WOUND CULTURE AMONG VARIOUS SURGERIES:**

Among the total 200 patients who were clinically diagnosed with SSI, 12 were found to be blood culture positive. The most common isolate was *Staphylococcus aureus*(6) followed by *S taphylococcus epidermidis*(3), *Escherichia coli*(2) and *Proteus mirabilis*(1).



<b>Diagnosis</b>	<b>Surgery</b>	<b>Wound culture</b>	<b>Blood culture</b>	<b>Organism in Blood culture</b>
Left Postlat Bronchiectasis	Left lower lobectomy	NG	Positive	<i>Staphylococcus epidermidis</i>
Right Bronchiectasis	Right pneumonectomy	<i>Staphylococcus aureus(MSSA)</i>	Positive	<i>Staphylococcus aureus(MSSA)</i>
Mass Rt Colon	Right hemicolectomy	NG	Positive	<i>Staphylococcus aureus</i>
Incisional hernia	Mesh repair	<i>Staphylococcus aureus(MSSA)</i> ,	Positive	<i>Staphylococcus aureus(MSSA)</i>
Carcinoma Head of Pancreas	Whipple's procedure	<i>E.coli</i>	Positive	<i>E.coli</i>
Pheochromocytoma	Adrenalectomy with Nephrectomy	<i>Staphylococcus aureus(MSSA)</i>	Positive	<i>Staphylococcus aureus(MSSA)</i>
Intestinal obstruction with umbilical hernia	Laprotomy with mesh repair	<i>Klebsiella pneumoniae(ESBL)</i>	Positive	<i>Staphylococcus aureus (MSSA)</i>
Acute Appendicitis	Appendisectomy	NG	Positive	<i>Staphylococcus epidermidis</i>
Incisional hernia	Laprotomy & Mesh repair	<i>Staphylococcus aureus(MRSA)</i> ,	Positive	<i>Staphylococcus aureus (MRSA)</i>
Incisional hernia	Mesh repair	<i>Proteus mirabilis(ESBL)</i>	Positive	<i>Proteus mirabilis(ESBL)</i>
Carcinoma Rt breast	Modified radical mastectomy	<i>Klebsiella oxytoca</i>	Positive	<i>Staphylococcus epidermidis</i>
Subacute intestinal obstruction	Ileal resection and anastomosis	NG	Positive	<i>E.coli</i>

Both wound and blood culture isolates were similar in 6 patients. In the rest 6 patients the blood culture isolates could have routed from other source of infection. Nine isolates were from patients who underwent abdominal surgeries, 2 isolates from Cardiothoracic surgery and 1 from Modified radical mastectomy.



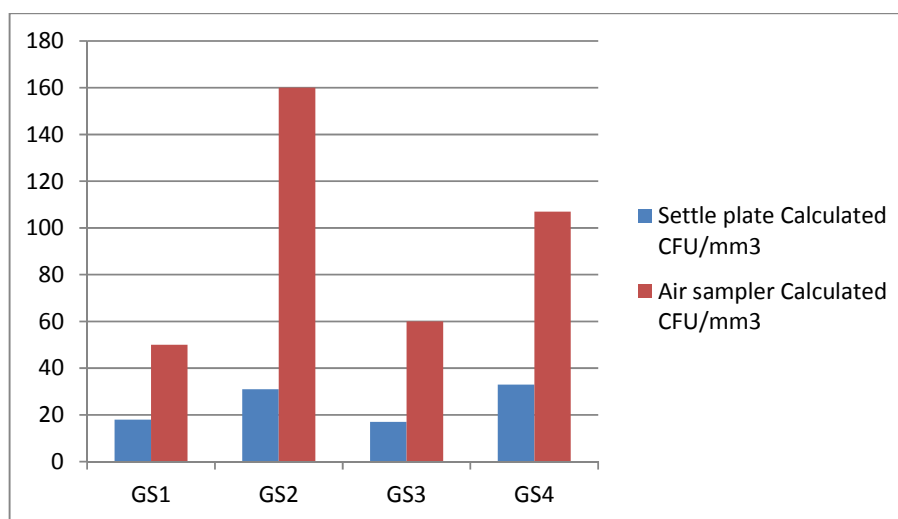
### Environmental study:

Organisms isolated in Operation theatres and postoperative wards by active and passive air sampling:

### General surgery:

**Table 22: DESCRIPTIVE VALUES OF CFU COUNT BY ACTIVE AND PASSIVE AIR SAMPLING IN GENERAL SURGERY OT**

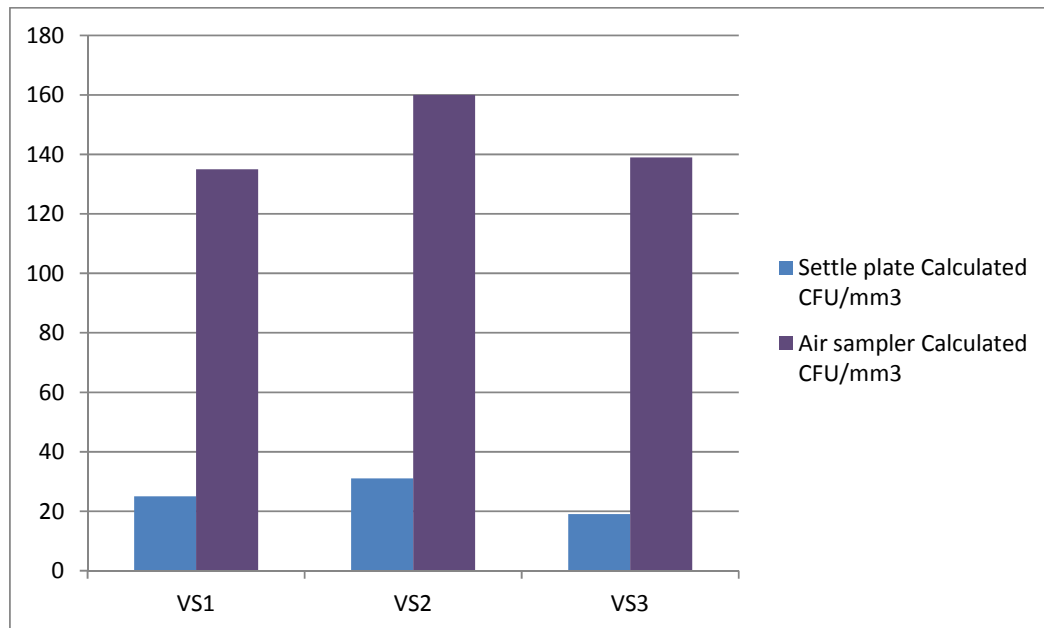
OT No.	Settle plate(Passive)		Air sampler(Active)	
	No. of colonies	Calculated CFU/mm <sup>3</sup>	No. of colonies	Calculated CFU/mm <sup>3</sup>
GS1	12	9	14	50
GS2	20	16	45	160
GS3	11	9	17	60
GS4	21	17	30	107
Mean		12.75		125.66



*Methicillin sensitive Staphylococcus aureus(MSSA) and Staphylococcus epidermidis* were isolated from General surgery OT and postoperative wards.

**TABLE 23: DESCRIPTIVE VALUES OF CFU COUNT BY ACTIVE AND PASSIVE AIR SAMPLING IN VASCULAR SURGERY OT:**

OT No.	Settle plate(Passive)		Air sampler(Active)	
	No. of colonies	Calculated CFU/mm <sup>3</sup>	No. of colonies	Calculated CFU/mm <sup>3</sup>
VS1	16	13	38	135
VS2	20	16	45	160
VS3	12	10	39	139
Mean		13		145

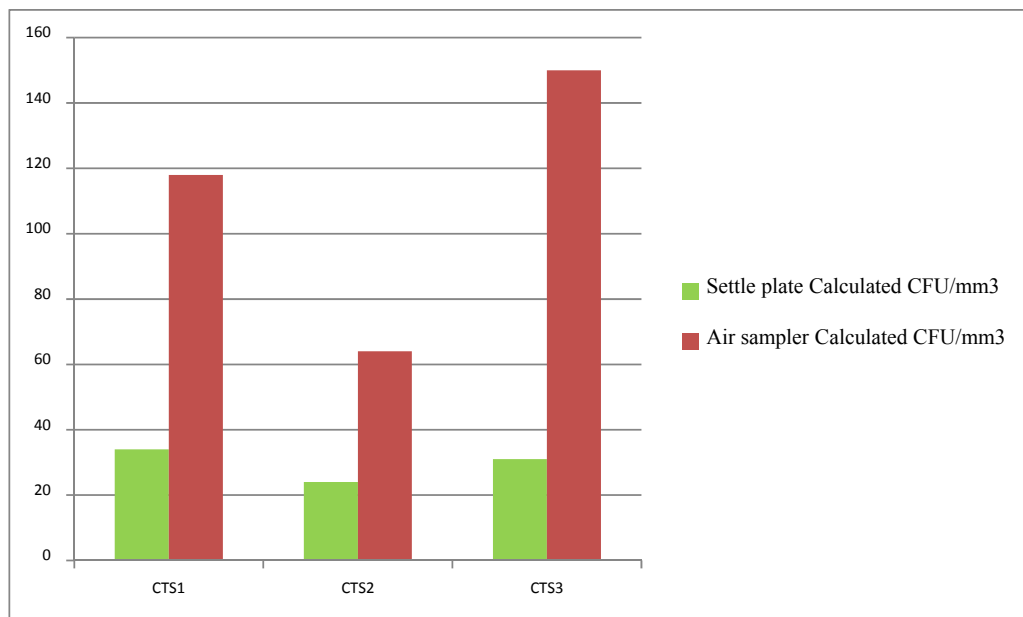


**Vascular surgery:**

Methicillin sensitive Staphylococcus aureus , Staphylococcus epidermidis and Acinetobacter baumannii were isolated from Vascular surgery OT.

**TABLE 24: DESCRIPTIVE VALUES OF CFU COUNT BY ACTIVE AND PASSIVE AIR SAMPLING IN CARDIOTHORACIC SURGERY OT**

OT No.	Settle plate(Passive)		Air sampler(Active)	
	No. of colonies	Calculated CFU/mm <sup>3</sup>	No. of Colonies	Calculated CFU/mm <sup>3</sup>
CTS1	21	11	33	118
CTS2	15	12	18	64
CTS3	20	16	42	150
Mean		13		111



**Cardiothoracic surgery:**

Methicillin sensitive Staphylococcus aureus and Acinetobacter baumannii were isolated from Cardiothoracic surgery OT.

**Surface swabs** collected in RCM from all OTs were negative for Clostridium tetani and anaerobes.

**TABLE 25: DESCRIPTIVE VALUES OF CFU COUNT BY ACTIVE AND PASSIVE AIR SAMPLING IN POSTOPERATIVE WARDS:**

Post operative wards	Settle plate(Passive)		Air sampler(Active)	
	No. of colonies	Calculated CFU/mm <sup>3</sup>	No. of Colonies	Calculated CFU/mm <sup>3</sup>
General surgery	219	172	250	892
CTS	134	105	155	553
VS	94	73	105	375
		116		606

**TABLE 26:ORGANISMS ISOLATED FROM VARIOUS POSTOPERATIVE WARDS BY AIR SAMPLING:**

<b>Postoperative wards</b>	<b>Isolates</b>
General surgery	<i>Methicillin sensitive Staphylococcus aureus(MSSA), Methicillin resistant Staphylococcus aureus (MRSA) Micrococcus spp</i>
CTS	<i>Methicillin sensitive Staphylococcus aureus(MSSA)</i>
VS	<i>Staphylococcus epidermidis and Micrococci</i>

*Methicillin sensitive Staphylococcus aureus* was the most common isolate in the postoperative wards.

**TABLE 27:ORGANISMS ISOLATED FROM NASAL SWABS AND HAND IMPRINT CULTURE:**

<b>Organisms isolated</b>	<b>Total No. of isolates</b>	<b>Percentage(n=30)</b>
<i>MSSA</i>	<b>4</b>	<b>13.33%</b>
<i>Staphylococcus epidermidis</i>	<b>6</b>	<b>20%</b>
<i>Micrococcus spp.</i>	<b>4</b>	<b>13.33%</b>

*Staphylococcus epidermidis* was the most common isolate from the anterior nares swabs and hand imprint culture collected from 30 staff working in postoperative wards.

**Table 28: COMPARISON OF AIR SAMPLING BY SETTLE PLATE METHOD AND AIR SAMPLER SYSTEM:**

<b>Area sampled</b>	<b>Settle plate CFU/mm<sup>3</sup> (Mean)</b>	<b>Air sampler system CFU/mm<sup>3</sup> (Mean)</b>	<b>Test</b>	<b>P Value</b>	<b>Significance</b>
General surgery OT	12.75	125.66	Pearson Chi-Square	0.001	S*
Cardiothoracic surgery OT	13	145	Pearson Chi-Square	0.001	S
Vascular surgery OT	13	111	Pearson Chi-Square	0.001	S
General surgery PO** ward	172	892	Pearson Chi-Square	0.001	S
Cardiothoracic surgery PO ward	105	553	Pearson Chi-Square	0.001	S
Vascular surgery PO ward	73	375	Pearson Chi-Square	0.001	S

\* Significant, \*\*Postoperative



The mean CFU count in the operation theatres and postoperative wards using settle plate technique was low compared to the mean calculated by using the Air sampling device. This difference in the mean CFU count between the two methods was found to be of high statistical significance ( $P < 0.001$ )

**Table 29: 16S rRNA GENE AMPLIFICATION AND AMPLIFIED RIBOSOMAL DNA RESTRICTION ANALYSIS (ARDRA) DONE IN THE METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES:**

Lane M : 100 bp ladder

Lane 1,4, 7 & 10 : SSI-1

Lane 2, 5, 8 & 11 : SSI2

Lane 3, 6, 9, 12 : OT

<b>Restriction enzymes used</b>	<b>Lanes</b>	<b>Banding patterns produced by 16S rRNA products of SSI 1, SSI 2, OT in ARDRA</b>
<i>Hinf</i> I	Lane 1,2,3	All three were similar
<i>Rsa</i> I	Lane 4,5,6	Dissimilar
<i>Msp</i> I	Lane 7,8, 9	Products from SSI 1 and SSI 2 isolates showed similar banding pattern but they were different from the pattern of that from Operation theatre.
<i>Alu</i> I	Lane 10, 11, 12	Products from SSI 1 and SSI 2 isolates had similar banding pattern but were different from that of Operation theatre.

# COLOUR PLATES

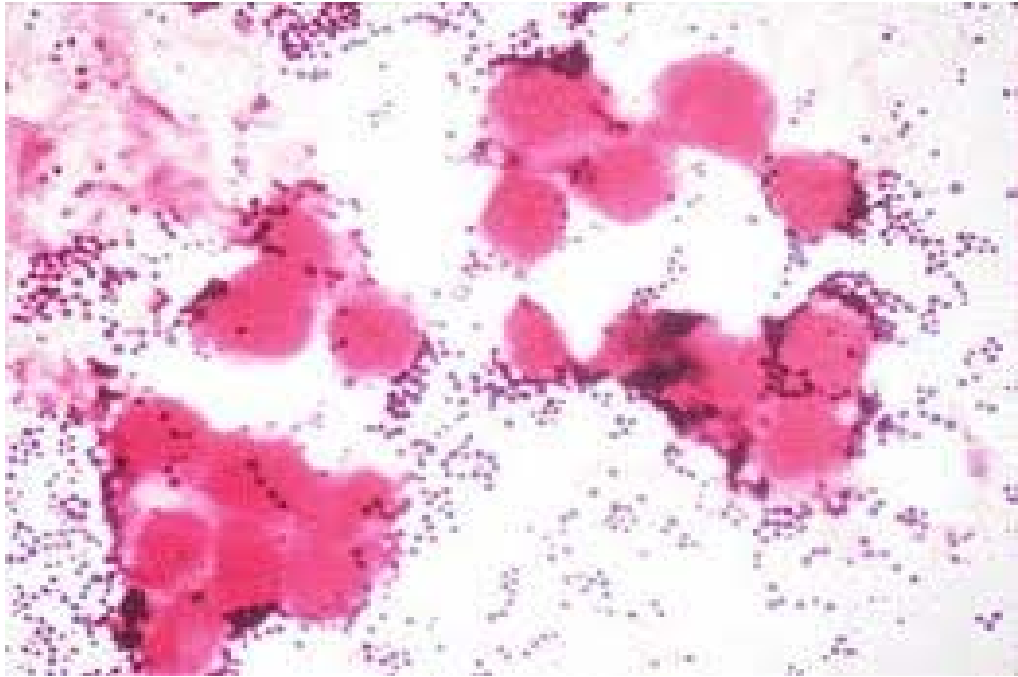
**FIGURE 1: SURGICAL SITE INFECTION WITH WOUND DEHISCENCE  
IN A LAPAROTOMY PATIENT**



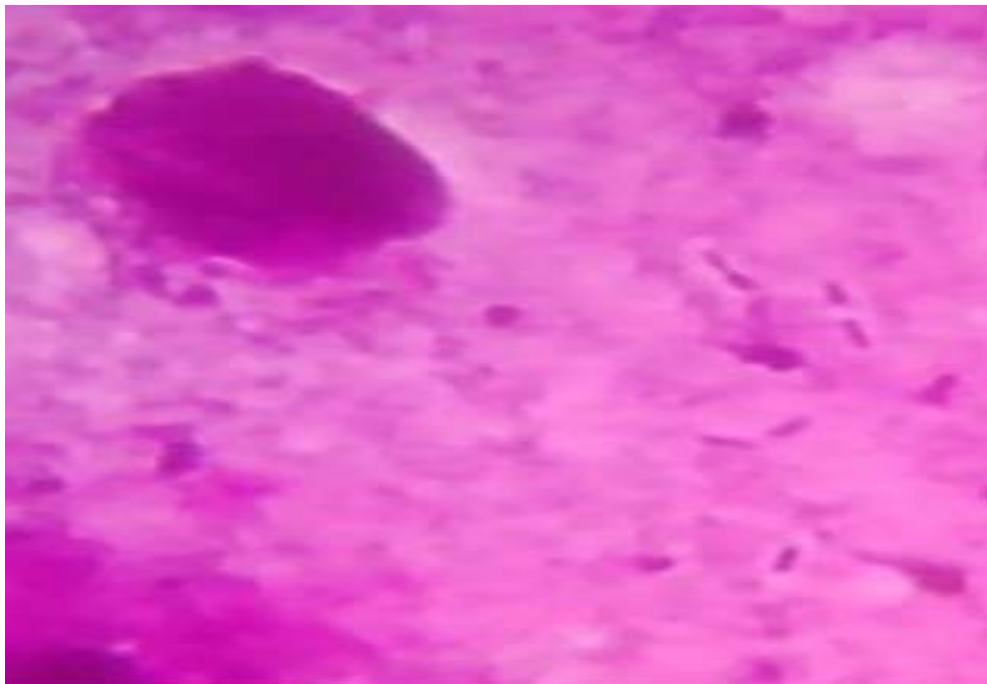
**FIGURE 2 : SUPERFICIAL SURGICAL SITE INFECTION  
IN A LAPOROTOMY PATIENT**



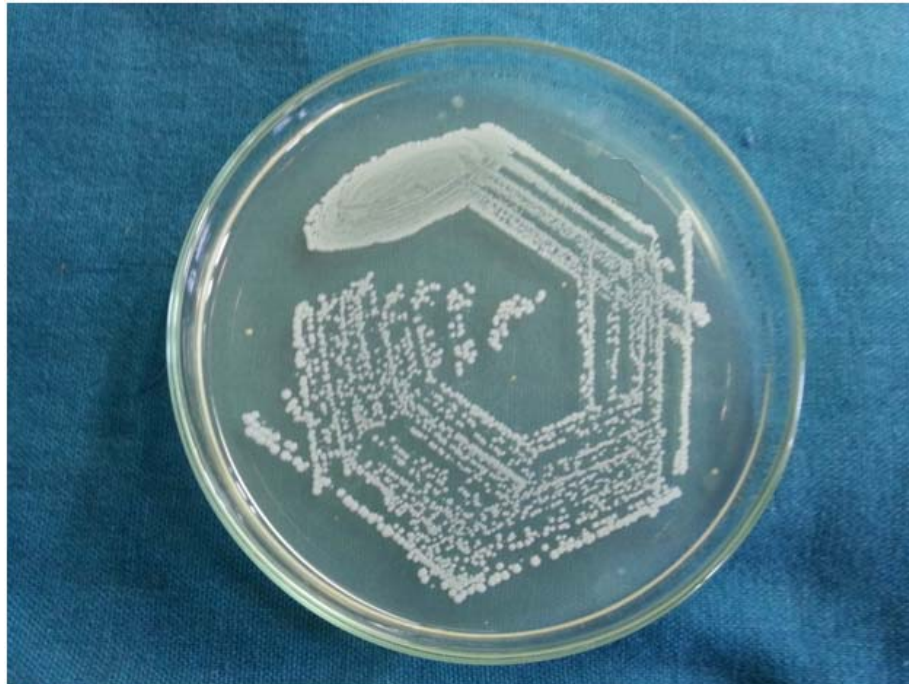
**FIGURE 3: DIRECT GRAM STAIN OF PUS SHOWING  
GRAM POSITIVE COCCI IN CLUSTERS**



**FIGURE 4: DIRECT GRAM STAIN OF PUS SHOWING  
GRAM NEGATIVE BACILLI:**



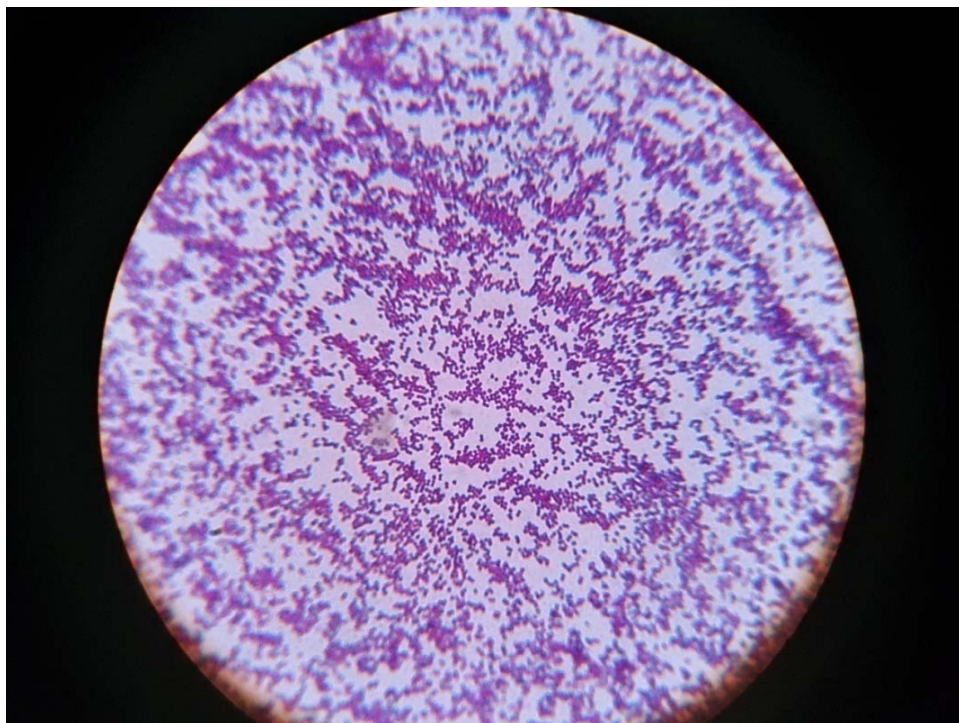
**FIGURE 5: STAPHYLOCOCCUS AUREUS COLONIES  
ON NUTRIENT AGAR PLATE:**



**FIGURE 6: STAPHYLOCOCCUS AUREUS ON  
MANNITOL SALT AGAR PLATE**



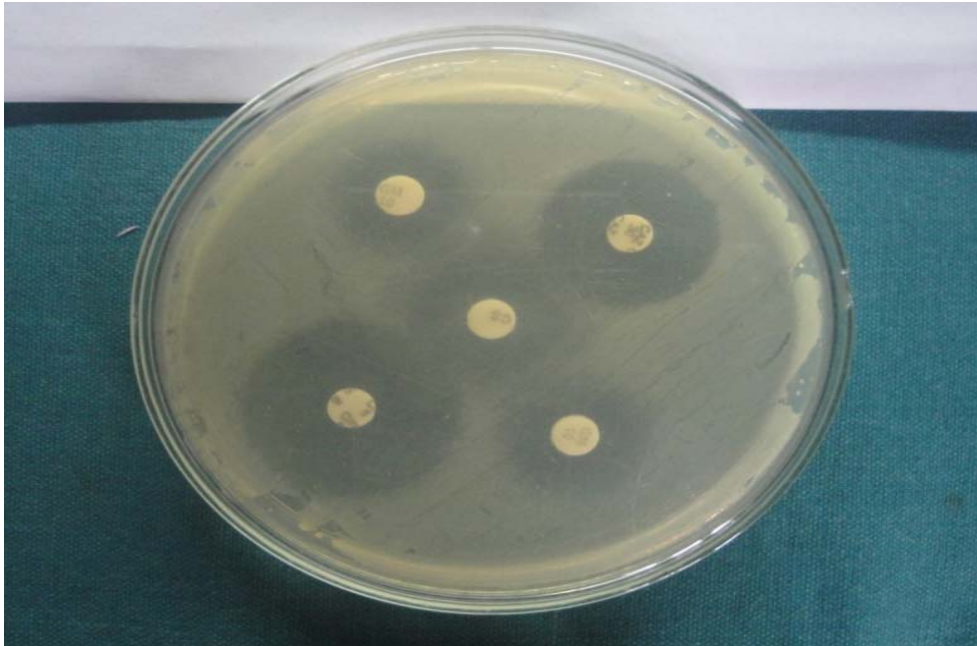
**FIGURE 7: CULTURE SMEAR OF STAPHYLOCOCCUS AUREUS**



**FIGURE 8 : METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS**



**FIGURE 9: ANTIMICROBIAL SENSITIVITY PATTERN OF ESCHERICHIA COLI**



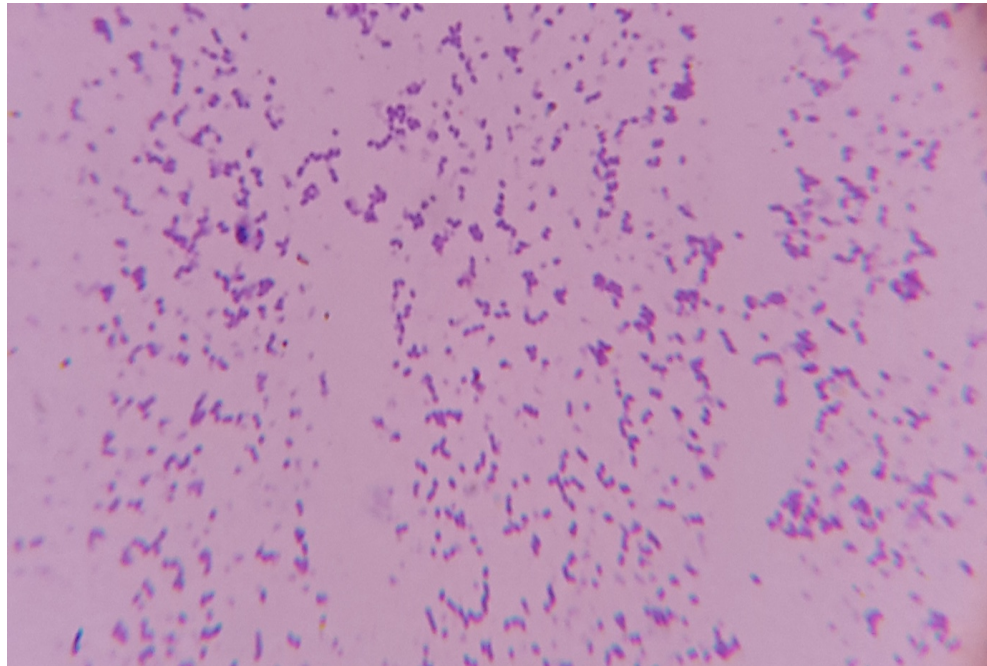
**FIGURE 10 : PHENOTYPIC CONFIRMATION DISC DIFFUSION TEST FOR ESBL PRODUCTION**



**FIGURE 11 : MCINTOSH FILDES ANAEROBIC JAR  
AND GAS PACK**

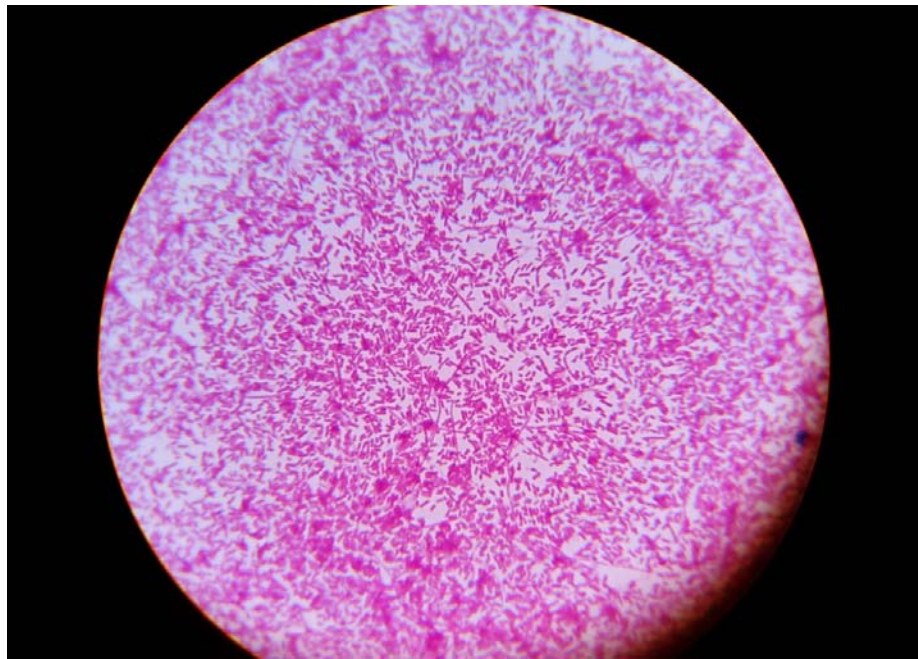


**FIGURE 12 : GRAM STAIN SHOWING ANAEROBIC  
GRAM POSITIVE COCCI**





**FIGURE 11 : GRAM STAIN SHOWING ANAEROBIC GRAM  
NEGATIVE BACILLI**



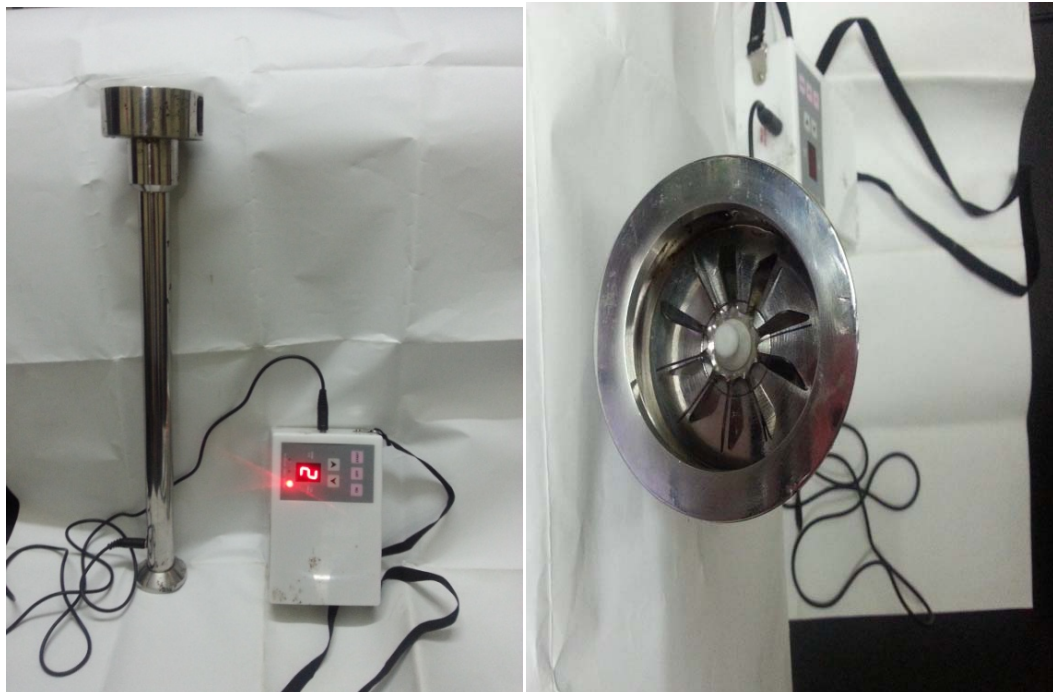
**FIGURE 12 : MINIMUM INHIBITORY CONCENTRATION OF  
STAPHYLOCOCCUS AUREUS BY MACRO BROTH DILUTION  
METHOD – 1  $\mu\text{g/ml}$**



**FIGURE 13 : AIR SAMPLING DONE IN  
OPERATION THEATRE USING AIR SAMPLER SYSTEM**



**FIGURE 14 : AIR SAMPLER SYSTEM**



**FIGURE 15 : AIR SAMPLING IN OPERATION THEATRE  
BY SETTLE PLATE( PASSIVE METHOD)**



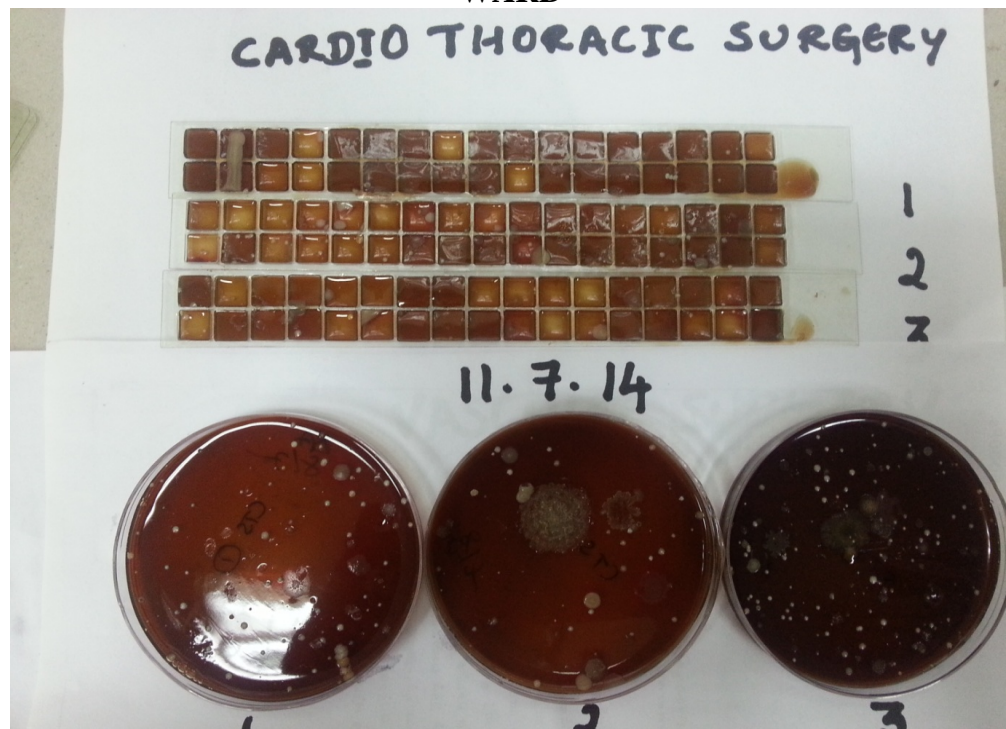
**FIGURE 16 : BLOOD AGAR STRIPS USED IN ACTIVE AIR  
SAMPLING IN GENERAL SURGERY OT AND WARD**



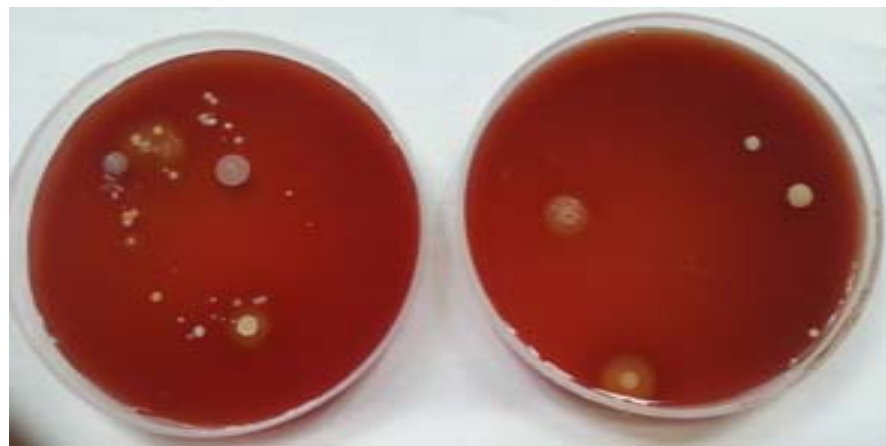
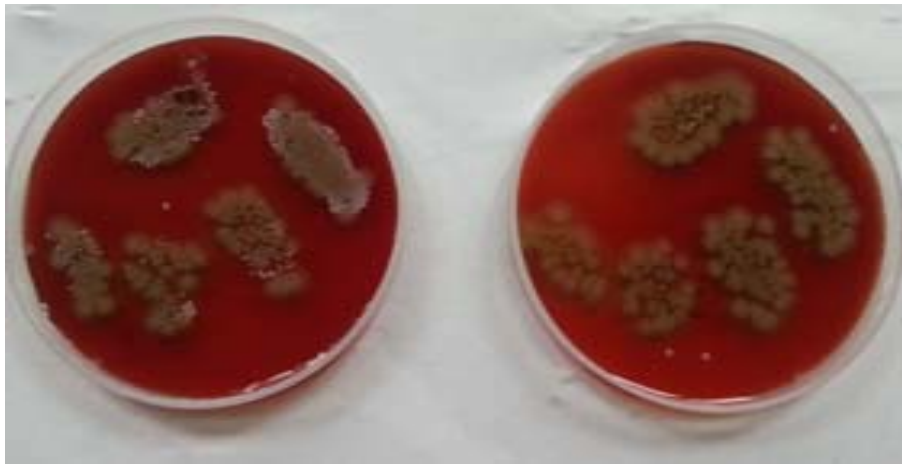
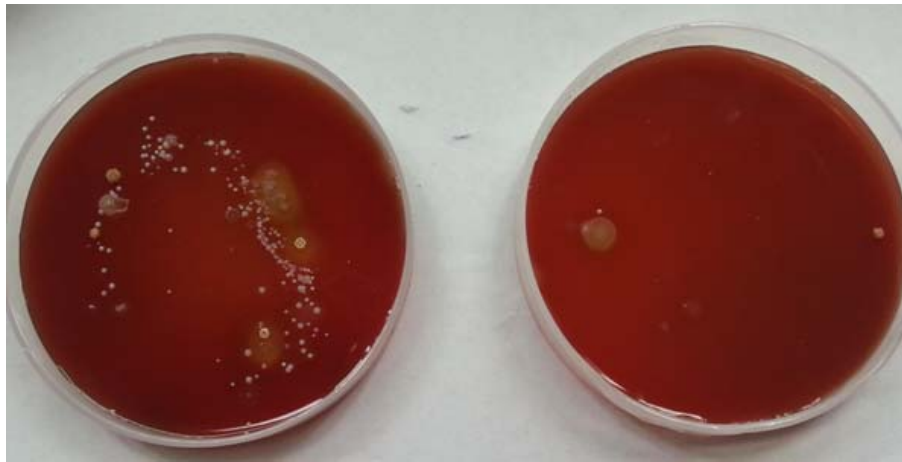
**FIGURE 17 : BLOOD AGAR STRIPS AND SETTLE PLATES USED IN ACTIVE AND PASSIVE AIR SAMPLING IN VASCULAR SURGERY OT AND WARD**



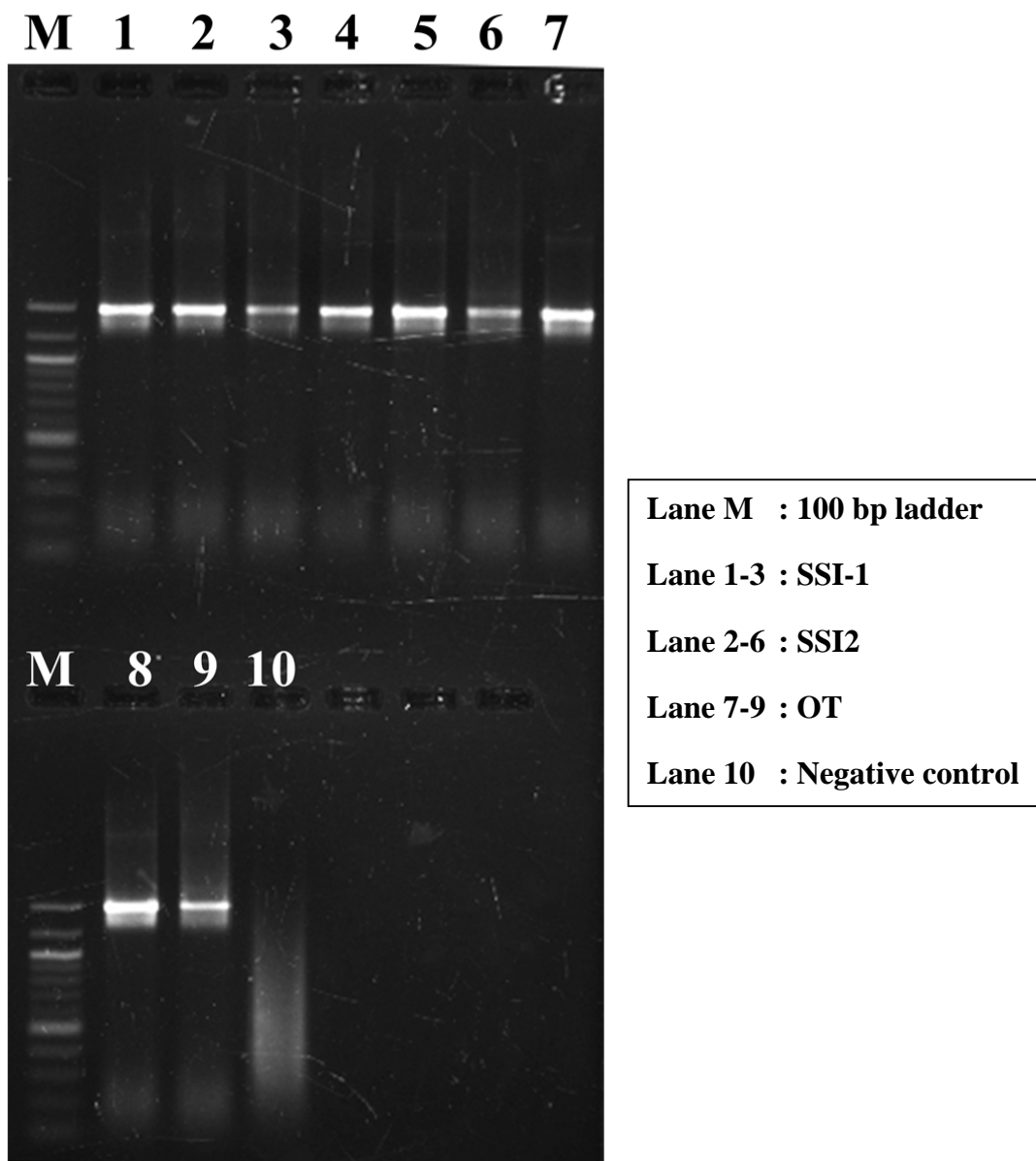
**FIGURE 18 : BLOOD AGAR STRIPS AND SETTLE PLATES USED IN ACTIVE AND PASSIVE AIR SAMPLING IN CTS OT AND WARD**



**FIGURE 19 : HAND IMPRINT CULTURE OF STAFF IN POSTOPERATIVE WARDS**

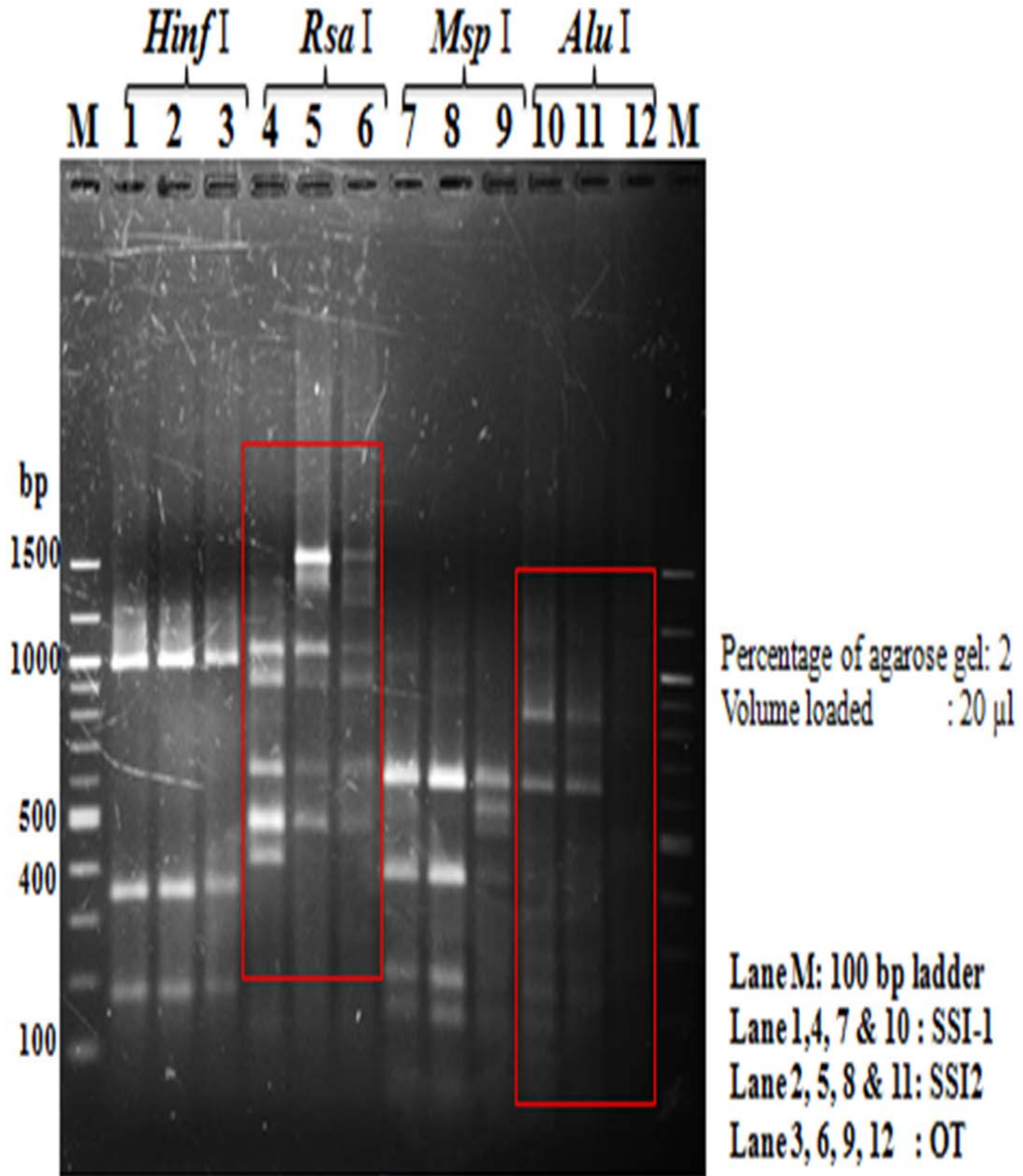


**FIGURE 20 : 16S rRNA GENE AMPLIFICATION OF  
*STAPHYLOCOCCUS AUREUS* ISOLATES**



**Percentage of agarose gel : 1.5**  
**Volume loaded : 7  $\mu$ l**

ARDRA PATTERN OF *STAPHYLOCOCCUS AUREUS* ISOLATES AFTER DIGESTION WITH  
FOUR DIFFERENT RESTRICTION ENZYMES



# DISCUSSION



## DISCUSSION

In a developing country like ours, SSI continues to be a major burden in health care facilities resulting in increased hospital stay, excess cost, substantial morbidity and less frequently mortality.

The present study was conducted at the Institute of Microbiology, Madras Medical College in association with departments of General surgery, Cardiothoracic surgery and Vascular surgery of Rajiv Gandhi Government General Hospital, Chennai. A total number of 200 patients with clinically diagnosed SSI satisfying the inclusion criteria were included in the study. Of these 200 patients, 158 patients belonged to General surgery, 31 patients from Cardiothoracic surgery and 11 patients from vascular surgery (Table 1). This disproportion among various departments in the number of patients taken into the study is due to relatively higher number of patient admissions and surgeries in General surgery (33,377 admissions & 6645 surgeries/year) compared to Cardiothoracic surgery (940 admissions & 683 surgeries/year) and Vascular surgery (1252 admissions & 807 surgeries/year).

In this study, 900 patients who underwent various procedures in General surgery, Cardiothoracic surgery and vascular surgery were analysed. Of these 200 (22.22%) patients were diagnosed clinically with SSI, out of which 140 (15.66%) patients were found to be wound culture positive and 60 were wound culture negative. The overall SSI rate was 15.66%.

In similar studies conducted at Sudan by Mohamed Issa Ahmed et al 2012<sup>(90)</sup>, the SSI rate was 23.15% which is higher than the present study and Anand Saxena et al <sup>(89)</sup> in India showed SSI rate of 14.33% which is similar to the present study. Anvikar AR et al <sup>(96)</sup> documented a SSI rate of 6.1% in their study which was lower than the present study.

In this study, among the 140 culture positive patients, males had a higher SSI rate (64.29%) compared to females (35.71%) [Table 2]. Similar predominance of SSI among males were observed by Shriyan et al (2010)<sup>(94)</sup>. In their study among 100 patients with SSI, 72% were males and 28% were females.

However, Nwankwo EO et al <sup>(92)</sup> and Maksum Radji et al <sup>(91)</sup> in their study documented predominance of SSI among females 64.9% and 75.15% compared to 35.3% and 24.85% in males respectively.

In the present study, among the wound culture positive patients, the SSI rate was higher in clean contaminated wound (10.22%) compared to clean wounds (5.3%) (Table 3). This observation is comparable to the study by Patel Sachin<sup>(95)</sup> et al where, the percentage of SSI in clean contaminated wounds was 11.4% as against 3% in clean wounds.

In this study, patients who developed SSI were divided into six age groups. Among these, the rate of SSI was highest (25%) in age group of 51-60 years which is comparable to the Study by S.M Patel<sup>(81)</sup> et al. In their study, patients who were

> 55yrs of age had the highest rate of SSI[Table4] . This could be due to their poor immune response , existing co morbidities and reduced compliance with treatment.

In the present study, the SSI rates were higher (45.72%) among patients with ASA score of more than 2[Table5]. This observation is similar to the study by Patel Sachin<sup>(95)</sup> et al . The SSI rates were directly proportional to the ASA score. The rates steadily increased from 22.14% in patients with ASA score 2 to 45.72% in those with ASA score 4. This correlation is attributed to the associated risk factors with the increasing ASA score. namely, Diabetes mellitus, malnutrition and other infection.

A prolonged preoperative stay in the hospital exposes the patients to the hospital environment for a longer time and hence increase the rate of SSI. In this study[Table:6] the SSI rate was highest(65.5%) among patients with a pre-operative hospital stay 7-13 days and lowest(7.5%) among those with a stay of 0-1 days. In a similar study by Sachin Patel<sup>(95)</sup> et al, the SSI rate was 33.3% in patients with Preoperative hospital stay of 7-13 days compared to 5.5% in those with 0-1 day stay.

The SSI rates were found to be higher in patients with longer duration of surgery[Table:7]. In patients who underwent surgery for less than 30 minutes the SSI rate was only 0.7% whereas the rate was 52% in those with more than 2 hours of surgery. A similar observation has been reported by SP Lilani <sup>(113)</sup> et al in their study, where the SSI rate was low(1.47%) in patients who underwent surgery for less than 30 minutes and high (38.46%) in those with more than 2 hrs of surgery .

This increase in SSI rate with increasing duration of surgery may be due to longer exposure time leading to more contamination and hence more damage to the tissues, and also due to fatigue in workers leading to breaks in sterile technique<sup>(95)</sup>.

In the present study, the rate of wound infection has been found to be increase with the postoperative days[Table8]. 10.72% of SSI have occurred in 2<sup>nd</sup> - 3<sup>rd</sup> postoperative day(POD), 48% in 4<sup>th</sup>-5<sup>th</sup> POD and 55% of infections have occurred after the 6<sup>th</sup> POD. This is similar to the study by Jahanara Rahman et al<sup>(101)</sup>, where 22.5% of the SSI had appeared within 5<sup>th</sup> day, 52.5% in 6<sup>th</sup> to 10<sup>th</sup> day and 5% beyond 10<sup>th</sup> day.

According to this study[Table 9], the SSI rates were found to be highest in patients who had undergone appendicectomy (13.29%) followed by laparotomy with mesh repair(10.13%) and hernioplasty (7.6%). The rate of wound infection were least in thyroidectomy(1.3%) , adrenalectomy (1.3%) and hysterectomy surgeries (1.3%). This observation is similar to that of Abhijit Awari et al, where the SSI rate was highest in patients who underwent Appendicectomy (15.2%) followed by Laparotomy (12.12%) and the least rate of infection were in patients who underwent surgery for hydrocoele(2.04%) and hysterectomy(3.40%).<sup>(97)</sup> In another study, Anvikar et al also demonstrated a higher SSI rate in appendicectomy(14.20%) and lower rates in hernia(4.10%) and hydrocoele (1.72%). Monomicrobial isolates predominated over polymicrobials in general surgical patients.

In a study conducted by Neelam Abdulrauf Bagwan et al <sup>(99)</sup>, the rate of SSI was higher in esophageal, bowel and gastric surgeries(30.77%) whereas in thyroidectomy and adrenalectomy it was nil(0%). The observation in these studies are similar to the present study.

The SSI rate in Cardiothoracic surgery[Table 10] was found be higher in patients who underwent lobectomy(25.8%) followed by pneumonectomy(13%) and least in CABG(6.45%). In a study of 454 open heart surgery patients by F.C.Wells<sup>(102)</sup> et al, the percentage incidence of infection was 12% in intracardiac surgeries, 12% in thoracotomies and 39% in CABG.

In vascular surgery[Table11], the SSI rate was high in arterial bypass graft surgery 8/11(72.72%) compared to amputation2/11 and arterial cystectomy1/11 (9.09%).

Among the total 150 isolates Causing SSI[Table 12], bacterial isolation rate was higher in General surgery120/150 (80%) compared to Cardiothoracic surgery19/150(12.66%) and Vascular surgery11/150(7.33%). The commonest aerobic isolate among various wound infections was *Escherichiae coli* (20.66%) followed by *Staphylococcus aureus* (19.33%), *Pseudomonas aeruginosa*(16.67%), *Klebsiella pneumonia*(15.3%) and the least common was *Proteus mirabilis*(2%). Anaerobes were isolated only in general surgical patients.

In the General surgery patients[Table 13], *Escherichiae coli* was the commonest isolate accounting for 23.33% of SSI followed by *Staphylococcus*

*aureus* (16.67%), *Pseudomonas aeruginosa*(14.17%), *Klebsiella pneumonia*(15%), *Klebsiella oxytoca*(12.5%), *Staphylococcus epidermidis*(5.83%), *Acinetobacter spp.*(4.17%) , *Proteus mirabilis* (2.5%), *Peptostreptococcus spp.*(2.5%) and *Bacteroides fragilis*(3.33%).

Similar to the present study, S.M. Patel et al demonstrated *Escherichia coli*(35.7%) as the most common pathogenic isolate followed by *Staphylococcus aureus*(21.4%) , *Pseudomonas aeruginosa*(14.3%) and *Klebsiella spp.*(14.3%).

In similar study in north India, Barnali Kakati et al<sup>(98)</sup> observed that *Escherichia coli*(41.17%) as the most common bacteria isolated , followed by *Staphylococcus aureus* (13.72%), *Klebsiella pneumoniae*( 9.80 %), *Pseudomonas aeruginosa* ( 7.84 %) and *Enterococcus faecalis* ( 7.84 %)

Varsha Shahane et al , Barnali Kakati et al and S.M Patel.et al, have demonstrated *Escherichia coli* as the commonest isolate in their studies, whereas, Abhijit Awari et al, Shriyan A et al and Cathy A. Petti et al have observed *Staphylococcus aureus* as the commonest pathogen causing SSI in their respective studies.

Anaerobes contributed to 4.67% of the total isolates causing SSI, of which *Peptostreptococcus spp.* amounted to 2% and *Bacteroides fragilis* to 2.67%. These anaerobes occurred only in combination with aerobes namely *Escherichiae coli* , *Proteus mirabilis* and were isolated from patients who underwent appendisectomy, modified radical mastectomy and bowel resection surgery.

Similar reports were documented by Itzhak Brook and Edith H. Frazier<sup>(110)</sup>. In their study the predominant anaerobes isolated were *Bacteroides fragilis* group (n=9) and *Peptostreptococcus* sp. (n = 6). In another study, Di Rosa et al reported 37% isolation of anaerobes of which 33% were in combination with aerobes and 4% were isolated alone. This isolation rate is very high compared to the present study.

In patients who developed SSI after cardiothoracic surgery (Table 15) *Staphylococcus aureus* was the most common isolate accounting for 31.57%. The next common isolate was *Pseudomonas aeruginosa* (26.31%) followed by *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* accounting for 10.52% each and the least was *Staphylococcus epidermidis* and *Acinetobacter species* which accounted for 5.26% of SSI each.

Similar reports were documented by Daisy Jonkers et al, where the most frequently isolated organism was *Staphylococcus aureus* (26%) followed by *Pseudomonas aeruginosa* (10%), *Staphylococcus epidermidis* (8%), *Escherichia coli* (7.7%), *Proteus mirabilis* (6.9%) and *Enterobacter cloacae* (5.4%).

In a study by A. Softah et al, among 136 Cardiothoracic surgery patients with SSI, the predominant isolate was *Staphylococcus aureus* (33.3%) and the next common was *Staphylococcus epidermidis* (31%), followed by *Escherichia coli* (5.8%), *Enterobacter spp.* (1.4%), *Pseudomonas aeruginosa* (0.7%), *beta-hemolytic streptococcus* (0.7%) and *Proteus spp.* (0.7%).

In the present study, among patients who underwent vascular surgery (Table 16) *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (27.27% each) were predominant isolates followed by *E. coli* and *Klebsiella oxytoca* (9.09% each)

Turtiainen J et al<sup>(104)</sup> and Bandyk DF et al<sup>(105)</sup> similar to the present study have documented *Staphylococcus aureus* as the leading pathogen isolated from wound culture. Other predominant isolates were *Staphylococcus epidermidis* (17%-24%), *Pseudomonas aeruginosa* (3%-20%), *Escherichia coli* (2-9%), *Streptococcus* (19%), *Enterococcus* (6%-21%) and *Enterobacter* (3%-9%).

Among the Gram positive cocci, all the *Staphylococcus aureus* isolates were sensitive to Vancomycin by macrobroth dilution method, majority of them showed high sensitivity to chloramphenicol (75%-96%) and amikacin (75%-90%). Some had moderate sensitivity to erythromycin (50%-75%) and ciprofloxacin (54.54%-57.7%) but least sensitive to Penicillin (0-11.5%). Of all the *Staphylococcus epidermidis* isolated 100% were sensitive to Vancomycin by macrobroth dilution method, 75% were sensitive to amikacin and chloramphenicol, 50% were sensitive to erythromycin and none was sensitive to penicillin [Table 17].

Among the various gram negative bacterial isolates from wound culture [Table 18], all the enteric Gram-negative isolates were sensitive to imipenem (100%). They showed moderate to high level resistance to ciprofloxacin (33.33% - 67%). Most of them were found to be highly resistant to 3<sup>rd</sup> generation cephalosporins but were susceptible to Betalactam and Betalactam inhibitor



combinations(83.33%-100%) . Around 67%- 93.5% of the enteric Gram negative bacilli were susceptible to amikacin but were least susceptible to Gentamycin(16.66%-43.47%). The alarmingly high resistance of these isolates to 3<sup>rd</sup> generation cephalosporins may be due to their frequent use in surgical prophylaxis. In the present study it was observed that majority of patients had received surgical antimicrobial prophylaxis on the day of surgery and the same had been continued for more than 5 days. According to the current guidelines regarding surgical antimicrobial prophylaxis for prevention of SSIs , the antimicrobial agent should be administered immediately prior to surgery and should be discontinued soon after surgery . In the present study Cefotaxime was used in 126 patients and Ceftriaxone in 53 patient as surgical prophylaxis, either alone or in combination with metronidazole or gentamycin . Cefotaxime used most commonly as surgical prophylaxis was found to be less effective against common wound pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and other Gram negative bacilli. This emphasizes the need of using antimicrobial sensitivity pattern of the isolates to guide the choice of antibiotics.

*Pseudomonas aeruginosa* isolates were found to be 100% sensitive to Imipenem They showed high sensitivity to ciprofloxacin(84%) and Amikacin(76%) and moderate sensitivity to Gentamycin. These isolates were least sensitive to 3<sup>rd</sup> generation cephalosporins(28%) but highly sensitive to Piperacillin Tazobactam (88%).

*Acinetobacter spp.* isolates were highly sensitive to imipenem(100%) , amikacin(83.33%) and Piperacillin Tazobactam(83.33%). They were moderately sensitive to Ciprofloxacin (66.66%) and Gentamycin(50%).

In general surgical patients of all the *Staphylococcus aureus* isolates 27.58% were Methicillin resistant [Table 19]. All these MRSA [Table 20] were sensitive to Vancomycin by macrobroth dilution method [MIC Value sensitive range : <2 µg/l]. This observation is similar to the MRSA rate of 28.5% in a study by Farrin<sup>(107)</sup> and his colleagues regarding the association of MRSA with SSI. In another study by Joel Manyahi<sup>(93)</sup>, the rate of MRSA was 44% which is very high compared to the present study.

In patients who underwent cardiothoracic surgery, 16.66% of the *Staphylococcus aureus* isolates were methicillin resistant. According to Walsh<sup>(109)</sup> and his colleagues, MRSA accounted for 54% of all SSI after Cardiac surgeries in a 520 bedded hospital at New York. As a result, preventive measures were followed in the above hospital which included, preoperative screening for colonization of MRSA, intravenous administration of Vancomycin prophylaxis to all the identified carriers , application of intranasal mupirocin ointment in all patients preoperatively for 5 days and also application of mupirocin at chest tube sites during removal.

In patients who underwent Vascular surgery, 1 out of 3 *Staphylococcus aureus* isolates was Methicillin resistant . Taylor and Napolitano<sup>(106)</sup> in a study on the increasing prevalence of MRSA in Vascular surgery patients found that out of 119

patients who developed SSI, 73 were due to *Staphylococcus aureus*(60.9%), of which 42 were *Methicillin resistant Staphylococcus aureus*. Two of these patients required graft removal and 40% required amputation. But in the present study the patient with *MRSA* responded to the antibiotic well.

In the present study 58.66% of the enteric Gram negative bacilli were Extended spectrum beta Lactamase(ESBL) Producers[Table 21]. ESBL production was observed in 74.19% strains of *Escherichiae coli*, 34.74% strains of *Klebsiella pneumonia*, 61.11% strains of *Klebsiella oxytoca* and 66.66% of *Proteus mirabilis*. These ESBL producers were found to be sensitive to the combination of beta lactam and beta lactamase inhibitors.

Shriyan and his colleagues<sup>(94)</sup> in a similar study documented ESBL production by 64.2% of enteric Gram negative bacilli. ESBL production was observed in 60% strains of *E.coli* and 75% strains of *Klebsiella* species.This observation is similar to the present study.

Joel Manyahi<sup>(93)</sup> in his study documented an ESBL production of 88% by the enteric Gram negative bacilli. ESBL production was documented in 92.3% strains of *E. coli* and 69% strains of *Klebsiella pneumoniae*. This percentage is very high compared to the present study.

In the present study,the predominant isolate from blood culture[Table 21] was *Staphylococcus aureus* (6/12), the next common isolates were *Staphylococcus epidermidis*(3/12), *Escherichia coli* and *Proteus mirabilis*(1/12).

In general surgical patients, abdominal surgeries showed more association with bacteraemia and the most common isolate was *Staphylococcus aureus*(5/10) followed by *Proteus mirabilis*(1/10) , *Escherichia coli*(1/10) and *Staphylococcus epidermidis*(1/10).

In Cardiothoracic surgery *Staphylococcus aureus*(MSSA) and *Staphylococcus epidermidis* were isolated from lung surgeries. In a study by Gottlieb et al<sup>(120)</sup>, source of 67% of *Staphylococcus aureus* bacteraemia was an SSI because of their virulence factors which helps them evade the immune system better than other organisms. This is comparable to the present study.

In this study, **the index of microbial air contamination** was analyzed by active and passive air sampling methods. In General surgical OT[Table 22], the mean CFU by settle plate method was 12.75 CFU/m<sup>3</sup> , whereas the corresponding mean by air sampler system was 125.66 CFU/m<sup>3</sup>. which is statistically significant(0.05).

The isolates from General surgery OT were *Methicillin Resistant Staphylococcus aureus*(MRSA) and *Staphylococcus epidermidis*. MRSA isolate was sensitive to Erythromycin, Amoxicillin clavulanic acid and Gentamycin . *Staphylococcus epidermidis* was sensitive to Penicillin, Amoxicillin- Clavulanic acid and Ciprofloxacin

In the Vascular surgery OT[Table 23], the mean CFU calculated by settle plate and air sampler system were 13 and 145 CFU/m<sup>3</sup> respectively. This was also statistically significant(<0.05).

*Methicillin sensitive Staphylococcus aureus* , *Staphylococcus epidermidis* and *Acinetobacter baumannii* were isolated from Vascular surgery OT.

*Methicillin sensitive Staphylococcus aureus* was sensitive Amikacin, Amoxicillin-Clavulanic acid and Vancomycin.

*Staphylococcus epidermidis* was sensitive only to Ciprofloxacin. *Acinetobacter spp.* was sensitive to Amikacin, Ciprofloxacin, Piperacillin Tazobactam and Imipenem.

In Cardiothoracic surgery OT[Table 24], the mean CFU calculated by settle plate and air sampler system were 13 and 111 CFU/m<sup>3</sup> respectively. This was statistically significant.

*Methicillin sensitive Staphylococcus aureus* and *Acinetobacter spp.* were isolated from Cardiothoracic surgery OT. *Methicillin sensitive Staphylococcus aureus* was sensitive to Amikacin, Ciprofloxacin and Amoxicillin-Clavulanic acid. *Acinetobacter spp.* was sensitive to Amikacin, Gentamycin, Piperacillin Tazobactam and Imipenem.

According to Fisher<sup>(48)</sup> et al, the CFU calculated by settle plate method using a 9cm blood agar containing Petri dishes , left open to the air in operation theatre

and post surgical ward by the scheme 1/1/1 should be within 180 and 450CFU/mm<sup>3</sup>/ hr respectively. In all OTs and postsurgical wards under this study, the Index of Microbial contamination was found to within acceptable limits [Table 25].

*MSSA, MRSA and Micrococcus spp.* were isolated from general surgery ward postoperative ward[Table 26] *MSSA* alone was isolated from CTS ward and *Staphylococcus epidermidis* and *Micrococcus spp.* from vascular surgery ward. *MSSA* in general surgery postoperative ward was sensitive to Erythromycin, Penicillin and Amoxicillin clavulanic acid. *MRSA* was sensitive to Ciprofloxacin and Amoxicillin- Clavulanic acid. In Vascular surgery postoperative ward, *Staphylococcus epidermidis* was isolated and it was sensitive to Erythromycin, Penicillin, Amoxicillin clavulanic acid and Ciprofloxacin. *MSSA* isolated from Cardiothoracic postoperative ward was sensitive to Penicillin, Amikacin, Amoxicillin clavulanic acid and Ciprofloxacin.

The *Methicillin sensitive Staphylococcus aureus* , *Staphylococcus epidermidis* and *Micrococci* were isolated from the anterior nares and hand imprint culture of staff working in postoperative wards[Table 27]. The *MSSA* were sensitive to Amikacin and Ciprofloxacin .

The mean CFU observed by air sampler system in comparison to settle plate method in operation theatres and wards of General surgery, Cardiothoracic surgery and Vascular surgery showed high statistical significance(<0.05) by Pearson's Chi Square test[Table 28]. In a similar study by Dr. A. G. Prathab<sup>(75)</sup> et al, the mean CFU

was 17.11 and 22 CFU/ mm<sup>3</sup> by settle plate method as against 137.83 and 164.1122 CFU/mm<sup>3</sup> respectively which showed high statistical significance . This is comparable to the present study.

The antimicrobial resistance pattern of a strain of *Methicillin resistant Staphylococcus aureus* isolated from General surgery OT was similar to that of 2 MRSA's isolated from the wound culture of general surgical patients. SSI 1 isolate belonged to a female patient who had undergone laparotomy with mesh repair and SSI 2 belonged to a male patient who had undergone hernioplasty. These 3 isolates (SSI 1, SSI 2, OT) were subjected to DNA extraction, 16S rRNA gene amplification and Amplified Ribosomal DNA restriction analysis (ARDRA). The 16 S rRNA products were digested with four selected restriction enzymes (*Hinf* I, *Rsa* I, *Msp*I and *Alu*I) in separate reactions and were run on a 2% agarose gel. The gel was stained with Ethidium Bromide, visualized under UV transilluminator and documented [Table 29].

The gel image of ARDRA revealed similar banding pattern of lanes 1 (SSI-1), 2 (SSI-2) and 3 (OT) loaded with *Hinf*I digested 16S rRNA PCR products. The lanes 4 (SSI-1), 5 (SSI-2) and 6 (OT) loaded with *Rsa*I digested products showed distinct variation in the banding pattern of the three isolates.

Banding pattern of *Alu* I digested products loaded in lanes 10 (SSI-1) and 11 (SSI-2) were similar but they were different from that in lane 12 (OT). *Msp*I digested products also showed similar patterns in lane 7 (SSI 1) and 8 (SSI 2) but were different from lane 9 (OT).

With these observations, it is pertinent to state that the *MRSA* isolates from (SSI-1) and (SSI-2) were genetically distinct from the OT strain. Hence the *MRSA* strain isolated from the OT was not the source of any of the above Surgical site infections. For further confirmation of the genetic disparity, DNA sequencing of 16 S rDNA gene fragments of the above strains could be done. J. D. Carroll et al<sup>(117)</sup>, in their study regarding methicillin- and gentamicin-resistant *Staphylococcus aureus* (*MGRSA*) isolated in a Dublin hospital, used Hinf I and Hind III to digest DNA plasmids for restriction enzyme digest pattern analysis, . This study demonstrated that the “new” *MGRSA* Dublin isolates, were completely distinct and unrelated to the *MGRSA* strains which were responsible for many previous nosocomial infections in the same hospital .

In a similar study by Afaf I. Shehata<sup>(115)</sup>, *Lactobacillus* isolates were subjected to Amplified Ribosomal DNA Restriction Analysis (ARDRA) by using Alu I , Mbo I and Msp I restriction enzymes and also 16S rDNA gene sequencing was done. This study revealed that 7 unique patterns were prevalent among the isolates and some isolates had similar patterns.

In a study by Anitha et al<sup>(118)</sup>, *Pseudomonas spp.* isolated from HIV patients were subjected to 16SrDNA PCR using universal primers and the amplicons were digested with restriction enzymes namely, Hae III, Alu I and Rsa I for ARDRA analysis. ARDRA banding pattern revealed 14 groups and 10 clones among the 72 *pseudomonas* isolates studied.



Johannes G. M. Koeleman<sup>(119)</sup> et al used *Alu I*, *RsaI*, *MspI*, *Mbo I* and *CfoI* as restriction enzymes for performing ARDRA for differentiating *Acinetobacter* at the species and strain level.

In the present study, an apparent source of nosocomial infection could not be identified. This could be due to transient carriage of the organisms or inadequacy in frequency of sampling. Further study with more extensive and frequent sampling would pave a way to identify the source of nosocomial infection.

# SUMMARY

## SUMMARY

- A total number of 200 postoperative inpatients with clinically diagnosed SSI in the department of General surgery(158), Cardio Thoracic surgery(31) and Vascular Surgery(11) were included in this study.
- The overall Surgical site infection(SSI) rate was 15.55%
- Males had higher preponderance(64.29%) compared to females(35.71%)
- Class II clean contaminated wounds (10.22%) had a higher surgical site infection rate compared to Class I clean wounds(5.3%).
- The Surgical site infection was relatively high in the age group of 51-60 years.
- The SSI rate was found to be higher in patients with ASA score >2.
- The rate of SSI increased with prolonged duration of preoperative hospital stay. The rate was maximum in patients with a preoperative stay of more than 7 days (65.5%)
- SSI rate was higher among patients with postoperative stay of more than 6 days (55%) compared to a stay of 2-3days(10.71%).
- Among 158 General surgery patients who were clinically diagnosed with SSI, 113 were culture positive, of which appendicectomy had the highest rate of infection(13.29%) followed by laparotomy with mesh repair(10.13%) . SSI rate was least(1.3%) in patients who underwent thyroidectomy, adrenelectomy and hysterectomy.

- Out of 31 Cardiothoracic surgery patients who were clinically diagnosed to have SSI, 18 were culture positive. Among these culture positive patients, lobectomy patients(25.8%) formed the highest proportion.
- Among 11 patients with clinically diagnosed SSI, 10 were culture positive and patients who underwent Bypass graft(72.72%) had higher rate of SSI.
- The predominant aerobic organisms were *Escherichiae coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Klebsiella oxytoca*.
- The predominant anaerobes isolated were *Bacteroides fragilis* followed by *Peptostreptococcus spp.*
- Blood culture was positive in 12 patients (8.57%) only
- All the Gram positive organisms were highly sensitive to Vancomycin, Amikacin and least sensitive to Ciprofloxacin and Penicillin.
- Among 29 *Staphylococcus aureus* isolated 8 were methicillin resistant.
- All Gram negative bacilli were 100% sensitive to Imipenem, highly sensitive to Amikacin and Piperacillin Tazobactam and least sensitive to Cephalosporins and Gentamycin.
- Among the enteric Gram negative bacilli, 58.67% were ESBL producers.
- The environmental study revealed that the index of microbial air contamination in both operation theatres and postoperative wards were within acceptable limits but not optimal limits .

- The mean CFU count in the operation theatres and postoperative wards using settle plate technique was low compared to the mean calculated by using the Air sampling device. This difference in the mean CFU count between the two methods was found to be of high statistical significance ( $P < 0.001$ )
- 16S rRNA gene amplification and Amplified Ribosomal DNA Restriction Analysis (ARDRA) performed on the *MRSA* isolates with similar antibiotic resistant pattern, revealed that the isolates from SSI were distinct from the one isolated from the OT.

# CONCLUSION

## CONCLUSION

Aerobic and anaerobic bacteria causing surgical site infection in various postoperative patients were studied at the Institute of Microbiology, Rajiv Gandhi Government General Hospital and Medical college. This study included 200 clinically diagnosed SSI patients where 158 patients belonged General surgery, 31 to Cardiothoracic surgery and 11 to Vascular surgery. The higher proportion of SSI patients from General surgery compared to other departments is due to relatively high inpatients(IP) strength and surgeries done in General surgery compared to Cardiothoracic and vascular surgery departments.

- The rate of postoperative surgical site infection was 15.56%
- Significant increase in the rate of SSI was seen in patients with prolonged preoperative hospitalisation, increased duration of surgery and in patients with an ASA score more than 2.
- There was predominance of Gram negative bacilli among the wound culture isolates, with *Escherichia coli* being the most common isolates.
- Majority of the Gram negative isolates were sensitive to Amikacin and Piperacillin Tazobactam but were resistant to commonly prescribed antimicrobial agents namely Cephalosporins, Gentamycin and Ciprofloxacin.
- The present study also observed an increase in SSIs caused by ESBL producing enteric Gram negative bacilli.

- Cefotaxime and Ceftriaxone, the commonly used third generation cephalosporins in the present study as surgical prophylaxis to prevent SSIs were found to be less effective against most of the gram negative organisms.
- Air sampler system was found to measure the microbial burden more accurately compared to settle plate method.
- Environmental sampling done in the operation theatres and postoperative wards revealed that the Index of Microbial contamination of air were within acceptable limits.



# RECOMMENDATIONS

## RECOMMENDATIONS

The conclusion drawn from the present study indicates the necessity for implementing the following guidelines.

- a. Perform routine wound culture and sensitivity (including MRSA and ESBL screening test) for all patients with suspected SSI and the test results to guide choice of antibiotics.
- b. Continuous surveillance should be established to monitor the antimicrobial susceptibility pattern of common isolates causing SSI.
- c. Periodic review and adherence to Hospital infection control policy and guidelines.
- d. Though the number of anaerobic bacteria among the total isolates causing SSI is negligible, they should also be considered as a cause in all patients with SSI.
- e. Identification of relevant gene responsible for antibiotic resistance should be done as and when required, as an epidemiologic measure to eliminate the source and thus prevent further spread of the same.
- f. Limit the use of third generation cephalosporin in surgical prophylaxis as they were found to be less effective on common wound isolates.

- g. Perform routine surveillance of all Operation Theatres once in every two months instead of once in four months done presently.
- h. Adhere strictly to the OT protocol on asepsis and prefer newer less toxic disinfecting agents (namely hydrogen peroxide, Peracetic acid and other chemical compounds of formaldehyde).

# APPENDICES

## APPENDIX – I

### ABBREVIATIONS

SSI	Surgical Site Infection
NNIS	National Nosocomial Infection Surveillance
CDC	Centre for Disease Control
MSSA	Methicillin Sensitive <i>Staphylococcus aureus</i>
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
VRE	Vancomycin resistant <i>Enterococcus</i>
ESBL	Extended spectrum Beta Lactamases
MBL	Metallobetalactamases
ATCC	American Type Culture Collections
CLSI	Clinical & Laboratory Standards Institute
ASA	American Society of Anaesthesiologists
REA	Restriction Enzyme Analysis
PFGE	Pulse Field Gel Electrophoresis
ERIC	Enterobacterial Repetitive Intergenic Consensus
AST	Antimicrobial Susceptibility Test
ATCC	American Type Culture Collection
CFU	Colony Forming Units
GS	General Surgery
CTS	Cardio Thoracic Surgery
VS	Vascular Surgery

## APPENDIX -II

### A. STAINS AND REAGENTS

#### 1. Gram Staining

Methyl Violet (2%)	10g Methyl Violet In 100ml Absolute Alcohol In 1 Litre Of Distilled Water (Primary Stain)
Grams Iodine Acetone Carbol Fuchsin 1%	10g Iodine In 20g Ki (Fixative) Decolourising Agent Secondary Stain

### B. MEDIA USED

#### 1. Mac Conkey Agar

Peptone	20g
Sodium Taurocholate	5 G
Distilled Water	1 Ltr
Agar	20 G
2% Neutral Red In 50% Ethanol	3.5ml
10% Lactose Solution	100ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. adjust PH to 7.5. Add lactose and neutral red, shake well and mix.heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

#### 2. Blood Agar (5% Sheep Blood Agar)

Peptone	10g
Nacl	5g
Distilled Water	1 L
Agar	10g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust PH to 7.4.

#### 3. Selective anaerobic Blood Agar: 1 µg/ml menadione and 20 µg/ml gentamicin added

#### 4. Chocolate Agar

Sterile defibrinated Blood	10 ml
Nutrient agar (Melted)	100 ml

Melt the desired amount of nutrient agar; cool it to 75°C, add the sterile blood with constant agitation and allow the medium to remain at 75°C till the blood becomes chocolate brown in colour within about 10 mins. Then cool it to about 50° C and pour about 15ml into petri dish with sterile precautions.

**5. Mueller- Hinton Agar**

Beef Infusion	300ml
Caesein Hydrolysate	17.5g
Starch	1.5g
Agar	Log
Distilled Water	Lltr

Ph = 7.4

Sterilise By Autoclaving At 121°C For 20 Mins

**6. Robertson's Cooked Meat Broth**

Fresh Bullock Heart	5 00g
Water	500ml
Sodium Hydroxide, Lmol/1	1.5ml
Liquid Filtered From Cooked Meat	500ml
Peptone	2.5g
Nacl	1.25g

**7. Thioglycollate broth**

Pancreatic digest of casein	15gms
Yeast extract	5gms
Dextrose (Glucose)	5.5gms
Sodium chloride	2.5gms
L-Cystine	0.5gms

Autoclaved at 15 lbs pressure (121°C) for 20 minutes.

Note : If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or until the pink colour disappears.

## **C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION**

### **1. Oxidase Reagent**

Tetra Methyl P-Phenylene Diamine Dihydrochloride- 1% Aqueous Solution.

### **2. Catalase**

3% Hydrogen Peroxide

### **3. Indole Test**

Kovac's Reagent

Amyl Or Isoamyl Alcohol 150ml Para Dimethyl Amino Benzaldehyde Log Concentrated Hydrochloric Acid 50ml

Dissolve The Aldehyde In The Alcohol And Slowly Add The Acid. Prepare In Small Quantities And Store In The Refrigerator. Shake Gently Before Use.

### **4. Christensen's Urease Test Medium**

Peptone	Lg
Sodium Chloride	5g
Dipotassium Hydrogen Phosphate	2g
Phenol Red	6ml
Agar	20g
Distilled Water	1 Ltr
10% Sterile Solution Of Glucose	10ml
Sterile 20% Urea Solution	100ml

Sterilize The Glucose And Urea Solutions By Filtration. Prepare The Basal Medium Without Glucose And Urea, Adjust To Ph 6.8-6.9 And Sterilize By Autoclaving In A Flask At 121°C For 30min. Cool To About 50°C, Add The Glucose & Urea, And Tube The Medium As Slopes.

### **5. Simmon's Citrate Medium**

Koser's Medium	1 Ltr
Agar	20 G
Bromothymol Blue 0.2%	40ml

Dispense, Autoclave At 121°C For 15 Min And Allow To Set As Slopes

### **6. Triple Sugar Iron Medium**

Beef Extract	3g
Yeast Extract	3g
Peptone	20g



Glucose	Lg
Lactose	10 G
Sucrose	L0g
Ferric Citrate	0.3g
Sodium Chloride	5g
S Odum Thiosulphate	0.3g
Agar	12g
Phenol Red 0.2% Solution	12ml
Distilled Water	1 Ltr

Heat To Dissolve The Solids, Add The Indicator Solution, Mix And Tube. Sterilize At 121°C For 15 Min And Cool To Form Slopes With Deep Butts.

### 7. Glucose Phosphate Broth

Peptone	5g
Dipotassium Hydrogen Phosphate	5g
Water	1 Ltr
Glucose 10% Solution	50ml

Dissolve The Peptone And Phosphate And Adjust The Ph To 7.6. Filter Dispense In 5ml Amounts And Sterilize At 121°C For 15min. Sterilize The Glucose Solution By Filtration And Add 0.25ml To Each Tube.

### Methyl Red Reagent

Methyl Red	L0mg
Ethyl Alcohol	30ml
Distilled Water	20ml

### Voges Proskauer Reagent

Reagent A: Alpha Naphthol	5g
Ethyl Alcohol	100ml
Reagent B: Potassium Hydroxide	40g
Distilled Water	100ml

### 8. Peptone Water Fermentation Test Medium.

To The Basal Medium Of Peptone Water, Add Sterilised Sugars Of 1% Indicator Bromothymol Blue With Durham's Tube. Basal Medium Peptone Water Sugar Solutions:

Sugar	1ml
Dislilled Water	100ml
Ph = 7.6.	

### 9. Mannitol Motility Medium

Agar	5g
Peptone	1g
Potassium Nitrate	1g
Mannitol	2g
Phenol Red Indicator	
Distilled Water	1000ml
Ph	7.2

### 10. Phenolphthalein Diphosphate Agar

- Sterilize A 1% Aqueous Solution Of Sodium Phenolphthalein Diphosphate By Filtration And Store At 4°C
- Add 10ml Of This Solution To 1000ml Melted Nutrient Agar Cooled To 50°C And Pour Plates
- Grow The Staphylococcus Overnight At 37°C On The Medium
- Invert The Plate And Pour A Few Drops Of Ammonia Solution Sg 0.88 Into The Lid
- Read As Positive A Culture Whose Colonies Turn Bright Pink Within A Few Minutes. The Colour Soon Fades.

### 11. Potassium Nitrate Broth

Potassium Nitrate (Kno <sub>3</sub> )	0.2gm
Peptone	5.0gm
Distilled Water	100ml

The Above Ingredients Were Mixed And Transferred Into Tubes In 5 Ml Amount And Autoclaved.

### 12. Phenyl Alanine Deaminase Test

Yeast Extract	3g
DL-Phenylalanine	2 g
Disodium Hydrogen Phosphate	1 g
Sodium Chloride	5 g
Agar	12g
Distilled Water	1 Lr
Ph	7.4

Distributed In Tubes And Sterilized By Autoclaving At 121° C For 15 Minutes, Allowed To Solidify As Long Slopes.

### **13. Sugar Fermentation Medium**

Peptone	15g	
Andrade's Indicator		10 Ml
Sugar To Be Tested		20g
Water		1 Litre

Andrade's Indicator Is Prepared From 0.5% Aqueous Acid Fuchsin To Which Sufficient 1m Sodium Hydroxide Has Been Added To Turn The Colour Of The Solution Yellow.

Dissolve The Peptone And Andrade's Indicator In 1 Litre Of Water And Add 20g Of The Sugar; Sugars To Be Tested Generally Include Glucose, Sucrose, Lactose And Maltose. Distribute 3ml Amounts In Standard Test Tubes Containing An Inverted Durham Tube. Sterilize By Steaming At 100 Degree C For 30 Min On 3 Consecutive Days.

# ANNEXURES

# ANNEXURE-I

## **INSTITUTIONAL ETHICS COMMITTEE** **MADRAS MEDICAL COLLEGE, CHENNAI-3**

EC Reg No. ECR 270/Inst./TN/2013

Telephone No : 044 25305301

Fax : 044 25303970

### **CERTIFICATE OF APPROVAL**

To

**Dr.C.Abba Ruba Sunanthini,**  
Post Graduate in MD Microbiology,  
Institute of Microbiology,  
Madras Medical College, Chennai-3.

Dear **Dr. C.Abba Ruba Sunanthini,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **“Prevalence of Nosocomial Wound Infection Among Postoperative Patients and Antimicrobial susceptibility Patterns in a tertiary care hospital”** No.19122013.

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

- |                                                                                           |                     |
|-------------------------------------------------------------------------------------------|---------------------|
| 1. Dr. G. Sivakumar, MS FICS FAIS                                                         | -- Chairperson      |
| 2. Prof. B. Kalaiselvi, MD<br>Vice Principal, MMC, Ch-3                                   | -- Member Secretary |
| 3. Prof. Ramadevi,<br>Director i/c, Instt. of Biochemistry, Chennai.                      | -- Member           |
| 4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy<br>Prof. Instt. of Pathology, MMC, Ch-3 | -- Member           |
| 5. Thiru. S. Govindasamy, BABL                                                            | -- Lawyer           |
| 6. Tmt. Arnold Saulina, MA MSW                                                            | -- Social Scientist |

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

  
Member Secretary, Ethics Committee

MEMBER SECRETARY  
INSTITUTIONAL ETHICS COMMITTEE  
MADRAS MEDICAL COLLEGE  
CHENNAI-600 003

## ANNEXURE-II

### PROFORMA

- Name :
  - Age:
  - Sex:
  - Occupation:
  - Address:
- IP no:  
Ward:

- DOA:
- DOD:
- Diagnosis:
- Procedures /Surgery:
- Date of surgery:

- Emergency/Elective surgery/ Re-operation:

- ASA score

- Duration of operation (minutes) \_ \_ \_

- Days of postoperative stay in hospital:

- Presenting complaints:

- History of any infection/ fever on admission:

- Risk factors:
  - Diabetes mellitus
  - Neoplasm
  - Trauma
  - Other immunosuppressive conditions

- Physical examination :

Temperature:

- Local examination: Infection involving skin/ subcutaneous tissue/ muscle above fascia/ Wound dehiscence/ Erythema / tenderness at surgical site organ/space surgical site infections

- Infection site : superficial / deep/ organ/space

- Date of infection (dd/mm/yy) \_ \_ \_ \_ \_

- Antimicrobial prophylaxis Yes/ No

- Microbiological investigation:

Direct examination: Gram's stain

Culture : Bacterial culture

Other special tests:

Antimicrobial sensitivity pattern:-

## ANNEXURE-III

### PATIENT CONSENT FORM

**STUDY TITLE : “Prevalence of Nosocomial Infection in Surgical wounds among Postoperative Patients and their Antimicrobial susceptibility pattern”**

I....., hereby give consent to participate in the study conducted by Dr.C.Abba Ruba Sunanthini, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Specimen (blood/pus/ serous discharge/ fluid aspirate etc.) for further investigations.I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression

Place

Date

Of the patient/ relative

Patient Name & Address:

Signature of the investigator:

Signature of the guide

## ANNEXURE – IV MASTER CHART

S.No	Age	Sex	Ward	DOA	DOS	Diagnosis	Surgery	Type of surgery	duration of operation	Postop days stay	presenting C/O	ASA	fever on admission	Comorbid (conid/Neoplasm/immunossupp resied)	Temperature	Local Examination	DOI	Drugs	Bacterial culture	Anerobic culture	Blood culture	Amikacin	Cefazidime	Cip	Gentamycin	Piperacillin Tacobactum	Co-tri	Imipenem	Penicillin	Erythromycin	Cefoxitin	Chloramphenicol	Vancomycin		
1	65	M	CTPO 1	1.11.13	5.11.13	Fibrocavitary	Left lower lobectomy	Elective	320	7	Breathlessness	3	No	No	N	Superficial	12.11.13	Inj.Ceftriaxone 1g IV BD	Pseudomonas aeruginosa	Negative	Negative	R	R	R	R	S									
2	18	M	VS1	2.11.13	3.11.13	TAO	Left Femoro popliteal	Elective	160	3	Claudication pain	3	no	no	N	Superficial	6.11.13	Inj.Cefotaxime 1g IV BD	Pseudomonas aeruginosa	Negative	Negative	S	R	S	S	S									
3	18	F	GS 133	3.11.13	4.11.13	Appendicitis	Appendisectomy	Emergency	45	2	Abdominal pain	3	No	No	N	Wound	6.11.13	Inj.Ceftriaxone 1g IV BD	Pseudomonas aeruginosa	Negative	Negative	S	R	S	R	S									
4	48	M	GS 224	5.11.13	6.11.13	Appendicitis	Appendisectomy	Elective	54	4	Abdominal pain	2	No	No	N	Superficial	10.11.13	Inj.Cefotaxime 1g IV BD	E.coli(ESBL),	Peptostre	Negative	R	R	R	R	S									
5	55	M	VS 49	5.11.13	9.11.13	Right	RightFemoro popliteal	Elective	120	10	claudication pain	2	No	No	N	Superficial	18.11.13	Inj.Cefotaxime 1g IV BD	Klebsiellaoxytoca	Negative	Negative	R	R	R	S	S									
6	60	M	CTPO 1	8.11.13	10.11.13	Right	Right pneumonectomy	Elective	320	4	Breathlessness	4	No	No	N	superficial	12.11.13	Inj.Cefotaxime 1g IV BD	Staphylococcus	Negative	Negative	S	S	S	S					R	R	S	S	S	
7	66	F	VS1	3.11.13	5.11.13	Left lower limb ischemia	Through knee amputation	Elective	140	6	Discolouration left toe	3	No	No	N	Superficial	8.11.13	Inj.Ceftriaxone 1g IV BD	Staphylococcus aureus(MRSA)	Negative	Negative	S	R					R	R	S	S	S	S		
8	62	M	VS1	5.11.13	7.11.13	Left Aortic occlusion	Ileo Femoral Bypass	Elective	180	15	Left great toe gangrene	3	No	No	N	Superficial	10.11.13	Inj.Ceftriaxone 1g IV BD	Staphylococcus aureusS-CK-Vanco, R- Pen, Cio, Gm	Negative	Negative									R	R	R	S	S	
9	50	M	VS1	21.1.13	25.11.13	Aortollic	AortoFemoral Bypass	Elective	260	4	Claudication pain	3	No	No	N	Superficial	29.3.13	Inj.Piptaz	Klebsiella pneumoniae(ESBL)	Negative	Negative	R	R	R	R	S									
10	46	M	CTPO 1	25.11.13	30.11.13	Left Postlat	Left lower lobectomy	Elective	310	6	Breathlessness	4	No	No	N	Superficial	3.12.13	Inj.Piptaz	Klebsiella pneumoniae	Negative	Negative	S	R	R	R	S									
11	55	M	GS 232	8.11.13	17.11.13	Superior	Surgery	Elective	130	20	Abdominal pain	4	No	No	N	Deep wound		Inj.Cefotaxime 1g IV BD	NG	Negative	Negative	S	R	R	R	S									
12	30	M	GS 251	21.11.13	27.11.13	Duodenal	Lap & omental patch	Elective	180	4	Abdominal pain	4	No	No	N	Superficial	1.12.13	Inj.Cefotaxime 1g IV BD	Klebsiella pneumoniae	Negative	Negative	S	S	S	R	S									
13	40	M	GS 131	22.11.13	25.11.13	Bilateral Ovarian mass	Transabdominal Hysterectomy with Bilateral oopherectomy	Elective	130	8	Abdominal mass	3	No	No	N	Superficial	30.11.13	Inj.Cefotaxime 1g IV BD	NG	Negative	Negative														
14	40	M	GS 251	23.11.13	25.11.13	Duodenal perforation	Lap & omental patch	Elective	143	5	Abdominal pain	4	No	No	N	Superficial	28.11.13	Inj.Cefotaxime 1g IV BD	Klebsiella pneumoniae	Negative	Negative	S	S	S	S	S									
15	32	M	CTPO 2	25.11.13	30.11.13	Right Bronchiectasis	Right pneumonectomy	Elective	340	6	Breathlessness, cough	4	No	No	N	Superficial	3.11.13	Inj.Piptaz	Staphylococcus aureus	Negative	Negative	S	R					R	R	R	S	S			
16	19	M	GS 224	3.12.13	4.12.13	Appendicitis	Appendisectomy	Emergency	45	2	Abdominal pain	2	No	No	N	Superficial	6.12.13	Inj.Cefotaxime 1g IV BD	Klebsiella oxytoca(ESBL)	Negative	Negative	S	R	R	R	S									
17	58	M	GS2 224	3.12.13	5.12.13	Varicose veins		Elective	125	3	Dilated veins, pain over Right leg	3	No	No	N	Superficial	8.12.13	Inj.Cefotaxime 1g IV BD	S. epidermidis	Negative	Negative	S	R					R	R	R	S	S			
18	45	M	GS 233	2.12.13	3.12.13	Hollow viscus perforation	Omental patch closure	Emergency	140	5	Abdominal pain	4	No	No	N	Deep wound infection	7.12.13	Inj.Cefotaxime 1g IV BD	E.coli(ESBL)	Negative	Negative	S	R	R	R	S									
19	30	M	CTPO2	13.11.13	28.11.13	Mitral stenosis/Regurgitation		Elective	310	7	K/C/O RHD, Breathlessness	4	No	No	N	Superficial	3.12.13	Inj.Ceftriaxone 1g IV BD	Acinetobacter species	Negative	Negative	S	S	S	S	S									
20	30	F	GS 132	5.12.13	6.12.13	Incisional hernia	Mesh repair	Elective	140	4	swelling in abdomen	3	No	No	N	Superficial	10.12.13	Inj.Cefotaxime 1g IV BD	S. epidermidis	Negative	Negative	S	R					R	R	R	S	S			
21	65	M	CTPO 2	2.10.13	1.12.13	Bronchopulmonary fistula	Left lower lobectomy	Elective	360	15	Breathlessness	4	No	K/C/O PT on treatment for 6 months	N	Superficial	16.12.13	Inj.Piptaz	Pseudomonas aeruginosa	Negative	Negative	S	S	R	R	S									
22	45	M	GS 251	29.11.13	2.12.13	Right Inguinal Hernia	Rt Hernioplasty	Elective	50	5	Swelling in right inguinal region	2	No	No	N	Superficial	12.12.13	Inj.Cefotaxime 1g IV BD	Proteus mirabilis	Negative	Negative	S	S	S	R	S									
23	73	M	GS 233	6.11.13	27.11.13	Bilateral Hydrocoele	Hydrocoele reduction	Elective	70	4	Swelling in both scrotum	3	No	HT for 5 yrs	N	Wound dehiscence/Deep	1.12.13	Inj.Cefotaxime 1g IV BD	NG	Negative	Negative														
24	45	M	GS 221	1.12.13	3.12.13	Left inguinal hernia/ Left hydrocoele	Hernioplasty/Lord's Plication	Elective	45	7	Swelling in left inguinal region	2	No	No	N	Superficial	11.12.13	Inj.Ceftriaxone 1g IV BD	Staphylococcus aureus(MSSA)	Negative	Negative	S	R					R	R	R	S	S			
25	62	M	GS 245	25.11.13	3.12.13	Carcinoma oesophagus	Transhiatal oesophagectomy	Elective	185	7	Dysphagia	4	No	No	N	Superficial	9.12.13	Inj.Ceftriaxone 1g IV BD	Pseudomonas aeruginosa	Negative	Negative	S	S	S	S	S									
26	60	M	GS 132	25.11.13	29.11.13	Carcinoma Left breast	Left radical Modified Mastectomy	Elective	130	12	Lump left breast	4	No	No	N	Deep wound infection/wound dehiscence	7.12.12	Inj.Cefotaxime 1g IV BD	Staphylococcus aureus(MSSA)	Negative	Negative	S	S					S	R	S	S	S	S		























# BIBLIOGRAPHY

## BIBLIOGRAPHY

1. Prescott Textbook of Medical Microbiology 8<sup>th</sup> edition pg 369-374.
2. Murray Rosenthal Pfaller Medical Microbiology 7<sup>th</sup> edition pg 360-363.
3. Mandell, Douglas and Bennett's Principles and Practice of Infectious diseases.7<sup>th</sup> edition ,Edited by Gerald L.Mandell, John E Bennett, Raphael Dolin. Chapter 296, Surgical and Trauma related infections.
4. Mackie and McCartney Practical Medical Microbiology 14<sup>th</sup> edition pg 559-562.
5. Guideline for Prevention of Surgical Site Infection, 1999, Healthcare Infection Control Practices Advisory Committee (HICPAC)
6. Topley and Wilson's Microbiology and Microbial Infections Bacteriology 10<sup>th</sup> edition, Vol-2 pg 1865-1884.
7. CDC/NHSN surveillance definitions of healthcare-associated infection.
8. Mim's Medical Microbiology 5<sup>th</sup> edition –Richard V Goering, Hazelm Dockrell.
9. Consensus Guidelines for the prevention of infections in the operating room. Hospital Infection Society- Mumbai Forum.
10. 10.Bailey & Love's short practices of Surgery -24<sup>th</sup> edition.
11. Edward S.Won.Surgical Site Infections. Chapter 11 in text book of Hospital Epidemiology and Infection Control by Williams & Wilkins C. Glen Mayhall.
12. Koneman's Color Atlas and Textbook of Diagnostic Microbiology (Color Atlas & Textbook of Diagnostic Microbiology) 6<sup>th</sup> edition pg 1024-1026.

13. Textbook of Medical Microbiology Jawetz, Melnick and Adelberg's Medical Microbiology Edition VI Normal Microbial Flora of the Human Body Chapter 11.
14. Edwin A. Deitch M.D. Surgical infections. Surgical Clinics of North America 19. Betty A. Forbes, Daniel F. Sahn, Alice S. Weissfeld Bailey and Scott's Diagnostic Microbiology, Ed:12, Pg 539-54.
15. Textbook of Surgery by Sabiston. Surgical infections and choice of Antibiotics Chapter 11.
16. Textbook of Surgical Infections by Donald E Fry. The Microenvironment of Infection. Chapter 4.
17. Revathi Gunturu, Sam kariuki, Abdul Hakeem et al. Pattern of Pathogens and Their Sensitivity Isolated from Surgical Site Infections at the Aga Khan University Hospital, Nairobi, Kenya. Ethiopian Journal of Health science Jul 2013; 23(2): 141–149.
18. Ardeshriri M, Faritius, Effect of obesity on mortality and morbidity after coronary artery bypass grafting surgery in Iranian patients. NCBI 2014 May 4; 4(2): e18884.
19. Sam Chen, Matt V Anderson et al Diabetes Associated with Increased Surgical Site Infections in Spinal Arthrodesis. Clin Orthop Relat Res. Jul 2009; 467(7): 1670–1673.
20. Ata A, Vallerian Bt et al, The effect of diabetes mellitus on surgical site infections after colorectal and non colorectal general surgical operations. Am surg 2010 Jul; 76(7): Page 697-702.
21. Debra L. Malone, Thomas Genuit, J. Kathleen Tracy et al, Surgical Site Infections: Reanalysis of Risk Factors Journal of surg Research Vol 103, Iss1, Pages 89-95, March 2002

22. Stephane Leung Wai Sang, Rakesh Chaturvedi, Ahsan Alam, Preoperative hospital length of stay as a modifiable risk factor for mediastinitis after cardiac surgery; *Journal of Cardiothoracic Surgery* 2013, 8:45 .
23. de Boer AS, Mintjes-de Groot AJ, Severijnen AJ et al . Surgical Site Infection in the Elderly Following Orthopaedic Surgery, *Infect Control Hosp Epidemiol* 1999;20:402-7.
24. Keith S. Kaye, Kenneth E. Schmader, and Robert Sawyer Surgical Site Infection in the Elderly Population *Clinical Infectious Diseases* Volume 39, Issue 12 Page. 1835-184.
25. Alavi K, Sturrock PR, Sweeney WB, Maykel JA, Cervera-Servin JA, Tseng J, Cook EF. A simple risk score for predicting surgical site infections in inflammatory bowel disease. *NCBI*, 2010 Nov;53(11):1480-6.
26. Durand F, Berthelot P, Cazorla C, Farizon F, Lucht F. Smoking is a risk factor of organ/space surgical site infection in orthopaedic surgery with implant materials. *Int Orthop.* 2013 Apr;37(4):723-7.
27. Boyce JM, Potter-Bynoe G, Opal SM, Dziobek L, Medeiros AA A common source outbreak of *S. epidermidis* infection among patients undergoing cardiac surgery. *The Journal of Infectious Diseases*/ Vol 161/ Iss 3/ Pg 493-498.
28. *Medical Microbiology*, 12<sup>th</sup> Edition, Volume II, Robert Cruickshank, *Bacteriology of air*, Pg 297-299.
29. Malone DL, Genuit T, Tracy JK, Gannon C, Napolitano LM. Surgical site infections: reanalysis of risk factors. *J Surg Res.* 2002 Mar;103(1):89-9.
30. Roser González Baulies, V. Balasso, S. Uriona, J.A. Rodrigo, A. Asensio, V. Pastor, J. Vaqué, and EPINE Working Group Diabetes mellitus and Nosocomial surgical site Infection in elderly patients in Spain. *Medicina Preventiva* 2008; XIV(1 Pt2): 31-36.

31. C. Pasquarella, O. Pitzurra and A. Savino The index of microbial air contamination *Journal of Hospital Infection* (2000) 46: 241–256.
32. Ananthanarayanan and Panicker. Bacteriology of air. Textbook of Microbiology ninth edition pg 628 –629.
33. Christian Napoli, Vincenzo Marcotrigiano and Maria Teresa, Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatre.
34. Dr. John de Campco, Microbial air sampling in operation theatres, operational Directives and InformationCirculars.2006.
35. Whyte W. The Casella Slit sampler or the BiotestCentrifugal sampler – which is the more efficient? *JHosp Infect* 1981; 2: 297–299.
36. Zimmerman NJ, Reist PC, Turner AG. Comparisonof two biological aerosol sampling methods. *ApplEnviron Microbiol* 1987; 53: 99–104.
37. Kang YJ, Frank JF. Evaluation of air samplers forrecovery of biological aerosols in dairy processingplants. *J Food Protect* 1989; 52: 655–659.
38. Verhoeff AP, Wijnen JH, Boleij JSM, Brunekreef B,Reenen-Hoekstra E, Samson RA. Enumeration andidentification of airborne viable mould propugales inhouses; a field comparison of selected techniques.*Allergy* 1990; 45: 275–284.
39. Kang YJ, Frank JF. Biological aerosols: a reveiw of airborne contamination and its measurement in dairy processing Plants. *J Food Protect* 1989; 52: 512–524.
40. V Robert Latham, Md; Ava D. Lancaster, Rn; Janet F. Covington, Rn; John S. Pirolo, Md; Clarence S. Thomas, Jr, MD .The Association Of Diabetes And Glucose Control With Surgical-Site Infections Among cardiothoracic Surgery Patients. *Infection control and Hospital epidemiology*, 2001;22:607-612.

41. Morikane K, Honda H, Yamagishi T, Suzuki S, Aminaka M. Infect Control Hosp Epidemiol. Factors associated with surgical site infection in colorectal surgery: the Japan nosocomial infections surveillance 2014. Jun;35(6):660-6. doi: 10.1086/676438. Epub 2014 Apr 22.
42. Mahdi H, Gojayev A, Buechel M, Knight J, SanMarco J, Lockhart D, Michener C, Moslemi-Kebria M. Surgical site infection in women undergoing surgery for gynecologic cancer. Int J Gynecol Cancer. 2014 May;24(4):779-86.
43. Daniel J Lex, Roland Tóth, Zsuzsanna Cserép, Tamás Breuer, Erzsébet Sági, András Szatmári, János Gál and Andrea Székely, Postoperative differences between colonization and infection after pediatric cardiac surgery- a propensity matched analysis, Journal of cardiothoracic surgery 2013, 8:166
44. O.A. Akingbade, S.A. Balogun, D.A. Ojo, R.O. Afolabi, B.O. Motayo, P.O. Okerentugba, I.O. Okonko Plasmid Profile Analysis of Multidrug Resistant *Pseudomonas aeruginosa* Isolated from Wound Infections in South West, Nigeria, World Applied Sciences Journal 20 (6): 766-775, 2012
45. Abdalla O. A. Ahmed, Alex van Belkum, [...], and Henri A. Verbrugh Nasal Carriage of *Staphylococcus aureus* and Epidemiology of Surgical-Site Infections in a Sudanese University Hospital, JCM, Dec 1998, 36(12), 3614-3618
46. Jan Kluytmans, Hans Berg, and Alex van Belkum Outbreak of *Staphylococcus schleiferi* Wound Infections: Strain Characterization by Randomly Amplified Polymorphic DNA Analysis, PCR Ribotyping, Conventional Ribotyping, and Pulsed-Field Gel Electrophoresis JCM, Aug 1998, 36(8), 2214-2219
47. P Y Liu, Z Y Shi, Y J Lau, B S Hu, J M Shyr, W S Tsai, Y H Lin, and C Y Tseng Epidemiological typing of *Flavimonas oryzihabitans* by PCR and pulsed-field gel electrophoresis. J Clin Microbiol. Jan 1996; 34(1): 68-70.

48. Fisher G, Fodré S, Nehéz M. Das Ergebnis der Untersuchungen zur Feststellung von Gesamtkeimzahl-Grenzwerten in der Luft von Operationsräumen. *Z Ges Hyg* 1972; 18: 729–733.
49. Pitzurra M, Savino A, Pasquarella C. II Monitoraggio ambientale microbiologico (MAM). *Ann Ig* 1997; 9: 439–454.
50. Desai S N, Kikani K M, Mehta S, J Microbiological Surveillance of Operation Theaters & Intensive Care Units of Teaching Hospital in Surendranagar, Gujarat.
51. S. Poongodi @lakshmi, N. Palaniappan, M.Kannan, Nithya gomatheeswari Microbiological Surveillance of Operation Theatre : Why... What...How ...Where...Which...?" *IJBMS* , volume 5, issue 1, April 2014.
52. Manual of Himedia Air sampler system LA002/182/2306.
53. Christian Napoli<sup>1</sup>\*, Vincenzo Marcotrigiano<sup>2</sup> and Maria Teresa Montagna<sup>1</sup> Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. *BMC Public Health* 2012, 12:594.
54. Kelkar U, Kelkar S, Bal AM, Kulkarni S, Kulkarni S. Microbiological evaluation of various parameters in ophthalmic operating rooms. The need to establish guidelines. *Indian J Ophthalmol* 2003;51:171-6.
55. Cheesbrough M. *District Laboratory Practice in Tropical Countries*. 2nd ed. New York: Cambridge University Press; 2006.
56. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Ninth Edition. CLSI. M2-A9; 26: 1.
57. Sterilisation Of Operating Theatres Published: 16th Dec 2013 in AMH Magazine.



58. M Olson, M O'Connor, and M L Schwartz Ann Surgical wound infections. A 5-year prospective study of 20,193 wounds at the Minneapolis VA Medical Center. *Annals of Surgery*. Mar 1984; 199(3): 253–259.
59. David E Reichman, MD and James A Greenberg, MD; Reducing Surgical Site Infections: A Review, *Reviews in Obstet & Gynecol* 2009;2(4) 212-221.
60. Narendra Patwardhan , Uday Kelkar, Disinfection, sterilization and operation theater guidelines for dermatosurgical practitioners in India, *IJDVL, Dermatosurgery SPECIALS*,2011, vol :77, Iss :1, Page 83-93.
61. M.E.E. van Kasteren, J. Mannien, A. Ott, B.J. Kullberg, A.S. de Boer, and I.C. yssens Antibiotic prophylaxis and the risk for surgical site infections following total hip rthroplasty. Timely administration is the most important factor ,*Clinical Infectious Diseases* 2007;44: 921-927.
62. G. Peersman, MD, R. Laskin, MD, [...], and T. Richart, MD, Prolonged Operative Time Correlates with Increased Infection Rate After Total Knee Arthroplasty, *HSS, J*. Feb 2006: 2(1); 70-72.
63. Kim ES, Kim HB, Song KH, Kim YK, Kim HH, Jin HY, Jeong SY, Sung J, Cho YK, Lee YS, Oh HB, Kim EC, Kim JM, Choi TY, Choi HJ, Kim HY; Prospective nationwide surveillance of surgical site infections after gastric surgery and risk factor analysis in the Korean Nosocomial Infections Surveillance System (KONIS). *Infect Control Hosp Epidemiol*. 2012 Jun;33(6):572-80. doi: 10.1086/665728. Epub 2012 Apr 19.
64. Hulam Asghar Channa, Taranum Ruba Siddiqui, Waquaruddin Ahmed; Frequency and risk factors of surgical site infections in general surgery ward of a tertiary care hospital of Karachi, *pakistanwww.ijic.info* ISSN 1996-9783 doi10.3396/ijic.V7i3.019.11.
65. C. Glen Mayhall, *Hospital Epidemiology and Infection Control*, 4<sup>th</sup> edition, Chapter 21, Surgical site infection, Pg 289.

66. Nitin Goel Insan, Nikhil Payal, Mahesh Singh, Amod Yadav, B.L Chaudhary, Ambrish Srivastava, Post operative wound infection: bacteriology And antibiotic sensitivity pattern, Department of Microbiology, MGM Medical College and Hospital, IJCRR, Vol 05 issue 13.
67. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd edition. New York: Cambridge University Press; 2006.
68. Washington C, Allen S. Koneman's color atlas and textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
69. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S24. Vol. 31 No. 1. January 2014.
70. Suggested Groupings of Antimicrobial Agents With FDA Clinical Indications that should be Considered for Routine Testing and Reporting on Nonfastidious Organisms by Clinical Microbiology Laboratories. CLSI document M100-S24. Table 1A. January 2014.
71. Zone Diameter and Minimal Inhibitory Concentration (MIC) Interpretive Standards for Enterobacteriaceae, Pseudomonas, Acinetobacter and staphylococcus respectively. CLSI document M100-S24. Table 2A&2B &2C. January 2014.
72. Screening and confirmatory test for ESBL's in Enterobacteriaceae isolates. CLSI document M100-S24. Table 3A. Jan 2014.
73. Workshop manual –Antimicrobial susceptibility testing methods. Dept of Microbiology, JSS Medical College & Hospital, Mysore, India.
74. Screening Tests for  $\beta$ -Lactamase Production, Oxacillin Resistance, mecA Mediated Oxacillin Resistance Using Cefoxitin, MIC  $\geq 8 \mu\text{g/mL}$ , Inducible Clindamycin Resistance, and High-Level Mupirocin Resistance in the

Staphylococcus aureus Group. CLSI document M100-S24. Table 2C-S4. Jan2014.Vol 34.No.1

75. Dr. A. G. Prathab, Dr. C. Lalitha Microbiological surveillance of air quality in Operation Theatres - comparison of the conventional settle plate techniques vs use of an air sampling device. JEMDS 2012Month :October2278-4802.
76. Dr.T.V.Rao MD Air sampling for microbes in hospitals.
77. Friberg,B., Friberg,S.,Burman,L.G., Correlation between surface and air counts of particles carrying aerobic bacteria in operating rooms with turbulent ventilation:an experimental study. J Hosp Infect, 1999;42:61-68.
78. Hunter JG, Padilla M, Cooper-Vastola S. Late Clostridium perfringens breast implant infection after dental treatment. Ann Plast Surg 1996; 36(3):309-312.
79. Stuesse DC, Robinson JH, Durzinsky DS A late sternal wound infection caused by hematogenous spread of bacteria. Ann Thoracic Surg 1995 Dec;108(6):1742-1743.
80. Vicente Monge Jodra, PhD; Lourdes Sainz de los Terreros Soler, MD; Cristina Dí'az-Agero Pe'rez, MD;Carmen Mari'a Saa Requejo, MD; Nieves Plana Farra's, MD Excess Length of Stay Attributable to Surgical Site Infection Following Hip Replacement: A Nested Case-Control Study infection control and hospital epidemiology december 2006, vol. 27, no. 12.
81. S.M. Patel, D.M. Kinariwala, S.D. Patel, P.A.Gupta; Study of risk factors including NNIS risk index in surgical site infections in abdominal surgeries. Guj Med Jour, 2011, Vol.66 No.1.
82. Hart D. Pathogenic bacteria in air of operating rooms. Arch Surg:1938:37:4:521-530.

83. Whyte,W., Hambraeus, A., Laurell,G., Hoborn, J. The relative importance of routes and sources of wound contamination during general surgery. II . airborne . *J. Hosp Infect*, 1992; 22: 41-54.
84. Patwardhan N. Hospital associated infections :Epidemiology, prevention and control. First ed.2006:107-126.
85. Curtis SE, Balsbaugh RK, Drummond JG.Comparison of Andersen eight-stage and two-stage viable air sampler. *Appl Environ Microbiol* 1978; 35:208–209.
86. Delmore RP, Thopson WN. A comparison of air sampler efficiencies. *Med Dev Diagn Ind* 1981; 53:45–48.
87. Nakhla LS, Cummings RF. A comparative evaluation of a new centrifugal air sampler (RCS) with a slit air sampler (SAS) in a hospital environment.*J Hosp Infect* 1981; 2: 261–266.
88. Seyd Mansour R., Mohammad I., Ahmad S.K., Ali J.Abdominal surgical site infections: Incidence and risk factors at an Iranian teaching hospital. *BMC Surgery* 2005; 5(2):1-5.
89. *Anand Saxena*, Surgical site Infection among postoperative patients of tertiary care centre in Central India - A prospective study, *Asian Journal of Biomedical and Pharmaceutical Sciences* , Volume 3, No.17 (2013).
90. Mohamed Issa Ahmed,Prevalence of Nosocomial Wound Infection Among Postoperative Patients and Antibiotics Patterns at Teaching Hospital in Sudan, *North Am Journal of Medical Sciences*,Jan 2012, 4(1), 29-34.
91. Maksum Radji *Et Al*, Evaluation Of Surgical Antibiotic Prophylaxis In Tertiary Care Hospital In Jakarta Indonesia *The Experiment*, 2014., Vol. 18(4), 1292-1296.

92. Nwankwo EO, Ibeh IN, Enabulele O, Incidence and risk factors of surgical site infection in a tertiary health institution in Kano, Northwestern Nigeria, *International Journal of Infection Control*, v8i4.035.12.
93. Joel Manyahi Bacteriological Spectrum Of Post Operative Wound Infections And Their Antibioqram In A Tertiary Hospital, Dar Es Salaam, Tanzania, Muhimbili University Of Health And Allied Sciences, October, 2012.
94. Shriyan A, Sheetal R, Nayak N. Aerobic Micro-Organisms In Post-Operative Wound Infections And Their Antimicrobial Susceptibility Patterns. *Journal Of Clinical And Diagnostic Research* 2010 December; 4:3392-3396.
95. Patel Sachin M, Patel Mitesh H2, Patel Sangeeta D3, Soni Sumeeta T, Kinariwala Dipa M, Vegad Mahendra M, Surgical Site Infections: Incidence And Risk Factors In A Tertiary Care Hospital, Western India *National Journal Of Community Medicine Vol 3 Issue 2 April-June 2012*.
96. Anvikar A.R., Deshmukh A.B., Karyakarte R.P., Damle A.S., Patwardhan N.S., Malik A.K., et al. A one year prospective study of 3280 surgical wounds. *Indian J Medical Microbiology* 1999; 17(3):129-132.
97. Abhijit Awari, Sunita Nighute, Sachin Deorukhkar *Journal of Clinical and Diagnostic Research. Surgical Wound Infections: A Prospective Hospital Based Study* 2011 November (Suppl-2), Vol-5(7): 1367-1370.
98. Barnali Kakati, Ashish Kumar, Pratima Gupta, PK Sachan, Bhaskar Thakuria Surgical site abdominal wound infections: Experience at a north Indian tertiary care hospital; *JACM* 2013; 14(1): 13-9.
99. Neelam Abdulrauf Bagwan , Sanjay More , Vivek Gujar; Study of Bacteriology of Post-Operative Wound Infection; *JKIMSU*, Vol. 3, No. 2, July-Dec 2014 , ISSN 2231-4261.

100. Dr. Varsha Shahane, Dr. Saikat Bhawal, and Mr. Upendra Lele, Surgical site infections: A one year prospective study in a tertiary care center; Int J Health Sci (Qassim). Jan 2012; 6(1): 79–84.
101. *Jahanara Rahman, Nasreen Sultana, Munir Hasan, Hosne Ara Begum*; Factors of Post-operative wound infection in abdominal surgeries of Obstetrics and Gynaecology department Journal of Dhaka National Med. Coll. Hos. 2012; 18 (01): 39-42.
102. F.C. Wells 1, S.W.B. Newsom , Christine Rowlands, Wound Infection In Cardiothoracic Surgery, The Lancet, Volume 321, Issue 8335, Pages 1209 - 1210, 28 May 1983.
103. Ali Asghar Moinipoor, Mohammad Abbasi, Ahmad Amouzeshi, Jamil Esfahanizadeh,, Shahram Amini; Deep sternal wound infection following cardiac surgery; Epidemiology and causative germs;Journal of Surgery and Trauma 2013; 1(1):21-25www.Jsurgery.bums.ac.ir.
104. Turtiainen J, Saimanen E, Partio T, Kärkkäinen J, Kiviniemi V, Mäkinen K, Hakala T; Surgical wound infections after vascular surgery: prospective multicenter observational study.Scand J Surg. 2010;99(3):167-72.
105. Bandyk DF; Vascular surgical site infection: risk factors and preventive measures; Semin Vasc Surg. 2008 Sep;21(3):119-23.
106. Taylor MD, Napolitano LM.Methicillin-resistant *Staphylococcus aureus* infections in vascular surgery: increasing prevalence. Surg Infect (Larchmt). 2004 ;5(2):180-7.
107. Farrin A. Manian, P. Lynn Meyer, Janice Setzer, and Diane Senkel ; Surgical Site Infections Associated with Methicillin-Resistant *Staphylococcus aureus*: Do Postoperative Factors Play a Role? CID 2003:36 (1 April).

108. Daisy Jonkers, Ted Elenbaas, Peter Terporten, Fred Nieman and Ellen Stobberingh, Prevalence Of 90-Days Postoperative Wound Infections After Cardiac Surgery; *Eur J Cardiothorac Surg* (2003) 23(1): 97-102.
109. Walsh EE, Greene L, Kirshner R Sustained Reduction In Methicillin-Resistant *Staphylococcus Aureus* Wound Infections After Cardiothoracic Surgery. *Arch Intern Med*. 2011 Jan 10;171(1):68-73.
110. Itzhak Brook and Edith H. Frazier ; Aerobic and Anaerobic Microbiology of Surgical-Site Infection Following Spinal Fusion; *J Clin Microbiol*. Mar 1999; 37(3): 841–843.
111. Di Rosa R, Di Rosa E, Panichi G.J *Chemother*. 1996 Apr;8(2):91-5. Anaerobic Bacteria In Postsurgical Infections: Isolation Rate And Antimicrobial Susceptibility.
112. Wondemagegn Mulu, Gebre Kibru and Meku Damtie; Postoperative Nosocomial Infections and Antimicrobial Resistance Pattern of Bacteria Isolates among Patients Admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia; *Ethiop J Health Sci*. Mar 2012; 22(1): 7–18
113. SP Lilani, N Jangle, A Chowdhary, GB Daver; Surgical site infection in clean and clean contaminated cases, *IJMM* 2005;23(4):249-252
114. Demisew Amenu, Tefera Belachew, and Fitsum Araya; Surgical Site Infection Rate and Risk Factors Among Obstetric Cases of Jimma University Specialized Hospital, Southwest Ethiopia; *Ethiop J Health Sci*. Jul 2011; 21(2): 91–100.
115. Afaf I. Shehata; Molecular Identification Of Probiotics *Lactobacillus* Strain isolates by: Amplified Ribosomal DNA Restriction Analysis (ARDRA), *Science Journal of Microbiology* ; Volume 2012, Article ID SJMB-175, 8 Pages.

116. Bias in Template-to-Product Ratios in Multitemplate PCR; Appl. Environ. Microbiol. October 1998 vol. 64 no. 103724-3730.
117. J. D. Carroll, Harriett M. Pomeroy, R. J. Russell, J. P. Arbuthnott, C. T. Keane", Orla M. McCormick And D. C. Coleman; A new methicillin- and gentamicin-resistant *Staphylococcus aureus* in Dublin: molecular genetic analysis ; J. Med. Microbiol. -Vol. 28 (1989), 15-23.
118. Molecular characterization of *Pseudomonas* sp. isolated from lower respiratory tract infection in HIV and non-HIV population by 16S rDNA and ARDRA C Anitha, Sujatha Kabilan, N Rajinish, A Santhosh Kumar, Padma Krishnan, Illaikiam Rasikan, S Senthilkumar, S Vincent, S Senthamarai, S Sivasankari, P Gunasekaran, Rajasekharan Sikhamani, M Pushkala
119. Johannes G. M. Koeleman, Jeroen Stoof, Dennis J. Biesmans, Paul H. M. Savelkoul\*, and Christina M. J. E. Vandenbroucke-Grauls, Comparison of Amplified Ribosomal DNA Restriction Analysis, Random Amplified Polymorphic DNA Analysis, and Amplified Fragment Length Polymorphism Fingerprinting for Identification of *Acinetobacter* Genomic Species and Typing of *Acinetobacter baumannii*. J. Clin. Microbiol. September 1998 vol. 36 no. 9 2522-2529.
120. Gottlieb GS, Fowler VG, Kong LK, et al. *Staphylococcus aureus* bacteremia in the surgical patient: a prospective analysis of 73 postoperative patients who developed *Staphylococcus aureus* bacteremia at a tertiary care facility. J Am Coll Surg 2000; 190:50-7.