"ROLE OF OPERATING THEATRE ENVIRONMENT IN CAUSING SURGICAL SITE INFECTIONS - ONE YEAR LONGITUDINAL STUDY"

REG. NO. BI0113001

Dissertation

Submitted to the
KLE University, Belagavi, Karnataka
In partial fulfillment
of the requirements for the degree of

M. D.

in

MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
JAWAHARLAL NEHRU MEDICAL COLLEGE,
BELAGAVI, KARNATAKA

APRIL - 2016

KLE UNIVERSITY, BELAGAVI,
KARNATAKA

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LIST OF ABBREVIATIONS

ACH	-	Air changes per hour
AHU	-	Air handling unit
ASA	-	American Society of Anaesthesiology
ATCC	-	American type culture collection
BAP	-	Blood Agar Plate
CBD	-	Common bile duct
CDC	-	Centers for Disease Control and Prevention
CFU	-	Colony forming units
CLSI	-	Clinical & Laboratory Standards Institute
CoNS	-	Coagulase negative Staphylococci
GI	-	Gastrointestinal
GNB	-	Gram negative bacilli
GPC	-	Gram positive cocci
HAI	-	Healthcare Associated Infections
HEPA	-	High efficiency particulate air
HVAC	-	Heating, ventilation, air conditioning
ICO	-	Infection control officer
ICT	-	Infection control team
NABH	-	National Accreditation Board for Hospitals & Healthcare Providers
NHS	-	National Health Service
NNIS	-	National Nosocomial Infections Surveillance
NRC	-	National Research Council

OPA	-	Ortho-phthalaldehyde
OT	-	Operating theatre
PHMB	-	Polyhexamethylenebiguanide
SPS	-	Sodium polyanethol sulfonate
SSI	-	Surgical site infection
UCV	-	Ultraclean-ventilated

ABSTRACT

Introduction: Surgical site infections (SSIs) constitute a substantial part of surgical morbidity and mortality each year. Since air can act as a reservoir for microorganisms, airborne microbial concentration in the operating theatre (OT) needs to be studied for its role in the causation of SSIs.

Objective: The present study was done to

1. Monitor operating theatre (OT) air for bacteria, and its relation with surgical site infections (SSIs) in patients operated in that particular OT.
2. Investigate the correlation between pre/ post operative antibiotic usage and antibiotic resistance in SSI isolates in relation to the patients' condition.

Methods: Elective clean and clean-contaminated surgeries were included. Only first surgical case of a day was sampled. Pre- and post-operative air sampling was conducted by settle plate (1/1/1) method. Patients were followed up. Three swabs from surgical incision site were taken and processed for aerobic and anaerobic pathogens as per standard guidelines. A modified medium, consisting of glucose broth and sodium polyanethol sulfonate was evaluated for increased aerobic culture yield.

Results: 57 clean and 39 clean-contaminated surgeries were included. *Staphylococcus aureus* was found in six pre-op OT plates (6.25%) and ten post-op OT plates (10.42%), colony count of ten or more per plate. 87.5% of these isolates of *Staph. aureus* were susceptible to Cefoxitin, 50% to Erythromycin and 62.5% to Co-trimoxazole. 87.5% of the isolates were susceptible to Ciprofloxacin and 100% to Clindamycin. None of the isolates were susceptible to Penicillin.

Two patients pertaining to these OTs developed SSIs. Both SSIs developed following clean-contaminated surgeries. They yielded *Staphylococcus aureus* from surgical

incision site on follow up. These isolates were susceptible to Cefoxitin, Ciprofloxacin, Co-trimoxazole and Clindamycin. The isolates matched those obtained from the respective OT pre-operatively. There was no difference between culture results from moist swab and swab in the modified medium. No anaerobes were isolated.

Conclusion: The study found 6-10% of contamination of OT air. The presence of pathogenic microorganisms in the OT air in significant numbers leads to a higher risk of SSI. Patients recovered without much morbidity as they received the drugs, post-operatively as per the antibiotic sensitivity report of the OT isolates.

Surveillance of the OT environment is to be conducted vigilantly. The isolates thus obtained should be speciated and the antibiogram should be obtained to prevent post-operative infections.

Keywords: Operating theatre, air sampling, settle plate, surgical site infection, sodium polyanethol sulfonate

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INTRODUCTION

Surgical site infections are a major contributor to surgical morbidity and mortality. The problem is especially significant in developing countries. Surgical site infections (SSIs) lead to increased post-operative hospital stay, readmission or the need for re-operation. Extra nursing care and interventions, and drug treatment may be necessary, thereby increasing health care costs. The indirect costs are due to loss of productivity, patient dissatisfaction and litigation, and reduced quality of life. SSIs continue to pose a problem in spite of significant advances in infection control techniques.

SSIs can result in varied clinical outcomes, ranging from mere wound discharge to a life-threatening condition. They can lead to poor, cosmetically unacceptable scars, such as spreading, hypertrophic or keloidal scars, persistent pain and itching, restriction of movement, particularly when over joints, and a significant impact on emotional wellbeing.[(1)] Hence, it is imperative to reduce the incidence of SSIs as far as possible.

Widespread and indiscriminate use of antibiotics over a long period of time has led to increasing antibiotic resistance in pathogens associated with nosocomial infections. This further compounds the problem, by leading to more complications and making SSIs difficult to treat.

Cultures from the cases of clinical SSI, sometimes yield no growth. This creates a dilemma as to the treatment that would be most effective. Modified wound swabbing/ fluid sampling techniques can help in increasing culture yield and correctly identifying the organism responsible, thereby helping institute appropriate treatment.

Sodium polyanethol sulfonate (SPS) has been shown to be effective in increasing the yield of microorganisms when incorporated in blood culture media, by various studies.[2, 3] Hence, we also intend to evaluate the modified medium, containing glucose broth and 0.05% SPS, so as to increase the culture yield from SSI.

Various factors may be involved in the development of an SSI. Since air can act as a reservoir for microorganisms, airborne microbial concentration in the operating theatre (OT) needs to be studied for its role in the causation of SSIs. This study will be focusing predominantly on the role of OT environment in causing SSIs, as this factor has not been investigated in detail. To the knowledge of the authors, there has been no study so far, in the Indian setting to investigate the correlation between the OT environment and SSIs.

The aims of the study are to

1. Monitor operating theatre (OT) air for bacteria, and its relation with surgical site infections (SSIs) in patients operated in that particular OT.
2. Investigate the correlation between pre/ post operative antibiotic usage and antibiotic resistance in SSI isolates in relation to the patients' condition.

AIMS AND OBJECTIVES

1. Monitoring operating theatre (OT) air for bacteria, and its relation with surgical site infections (SSIs) in patients operated in that particular OT.
2. Investigating the correlation between pre/ post operative antibiotic usage and antibiotic resistance in SSI isolates in relation to the patients' condition.

REVIEW OF LITERATURE

Introduction

After five years of meticulous data collection, elimination of possibilities and the unfortunate death of a friend, in 1847, a Hungarian obstetrician – Ignaz Semmelweis – concluded that the high mortality rate due to puerperal fever in a ward at the Vienna General Hospital could only be due to the carrying of unknown cadaverous material on the hands of the examining doctors and medical students. The institution of the policy of hand washing using a solution of chlorinated lime (calcium hypochlorite) between autopsies and examination of patients, showed an immediate reduction in the incidence of fatal puerperal fever from about 10% (range 5-30%) to about 1-2%.[(4)] Despite such impressive results, the recognition of the role of the hands of a surgeon in the introduction of microorganisms to wounds was a slow and arduous process; like all good ideas.

Inspired by the germ theory of Louis Pasteur, in 1867, an English surgeon – Joseph Lister – began to experiment with solutions of carbolic acid. From spraying only his own hands before an operation, his use of phenol widened to articles in contact, surgical instruments, the hands of the team and the wound itself.[(5)] These practices and the resulting reduction in mortality rates, introduced a primordial form of asepsis to surgery.

The use of this strong chemical disinfectant was harsh on the hands of some. In 1890, on one such complain, the surgeon-in-chief at the Johns Hopkins Hospital – William Halsted – inquired with the Goodyear Rubber Company if they could create a thin rubber glove with gauntlets that would be resistant to carbolic acid. Although the

product became popular, widespread use of gloves in the operating room was not practiced well into the next century.

The standardization of aseptic practices in the operating room was gradually enforced in the twentieth century. This greatly improved the outcome of clean operative procedures, but operations involving anatomic sites with dense endogenous flora that cannot be eliminated preoperatively, such as of the colon or rectum, continued to carry a very high risk of infection. The National Research Council (NRC) organized a study to document the rate of surgical site infection (SSI) following 15,615 operations carried out over 27 months from 1959 to 1962 in sixteen operating rooms of five university hospitals. This study was one of the earliest and most convincing to document the importance of endogenous bacteria as the primary etiologic agent of SSIs. The report also introduced a classification system for wounds based on the risk of endogenous contamination (and hence, of postoperative wound infection), which provided a basis for comparing SSI statistics and was a harbinger to the currently used system of "wound class". Although more sensitive and specific wound classification systems employing additional risk factors for wound infection have been developed since the NRC study, all systems continue to incorporate elements of this original scheme. The NRC study also examined a host of other factors related to the patient and the environment that influenced the risk of postoperative wound infections. Multivariate analysis of this data provided evidence of the role of patient's age, obesity, steroid administration, malnutrition, presence of remote infections, use of drains, duration of the operation, and duration of preoperative hospitalization in increasing the risk of a postoperative infection.[(6)]

The effective use of antibiotic prophylaxis to prevent postoperative infection was only possible after John Burke conducted pioneering studies using an animal model to demonstrate the critical importance of the timing of prophylactic antibiotic administration. He showed via a guinea pig model that the appropriate antibiotics given before bacterial contamination could significantly reduce the risk of infection, whereas the same antibiotic given after bacterial contamination was much less effective. This information was used to conduct human trials, first by Bernard and Cole and then by Polk and Lopez Mayor in the 1960s. These trials showed clinically and statistically significant effects of preoperative antibiotic prophylaxis on surgical site infection.Work on prophylactic antibiotics since that time has focused on defining those procedures and circumstances most likely to benefit from the use of prophylactic antibiotics and on examining the relative efficacy of different drugs and different routes and regimens of administration.[6]

In the 1970s, the Centers for Disease Control and Prevention (CDC) began the National Nosocomial Infections Surveillance (NNIS) system. Although it included all healthcare-associated infections (HAIs), special emphasis was laid on the collection of data on postoperative infections. Data from the NNIS system provided a rich source of information about the relative incidence of surgical site infections at different sites. Also, in the 1970s, surgical groups' reports of surveillance of large numbers of procedures validated the relationship between wound class and different risks of infection as well as the beneficial effect of reporting SSI rate data to the operating surgeons on reducing the incidence of SSI.[6]

Occurrences of SSI and measurements of compliance with processes to prevent SSI can now impact a hospital's accreditation by organizations such as The

Joint Commission in the United States and the National Accreditation Board for Hospitals & Healthcare Providers (NABH) in India. Postoperative infections can prolong the length of hospitalization substantially, depending on the type of operation. They also increase the costs incurred. Cardiothoracic, orthopedic, and gastrointestinal operations are especially costly in this regard due to both pulmonary and surgical site infections. In addition to the higher direct costs of care, indirect costs include the time the patient loses from gainful employment and the possible medico-legal actions that the patient could take against the hospital or the surgical staff.

Definitions[7]

WOUND CLASS

Wound class is an assessment of the degree of contamination of a surgical wound at the time of the operation. Wound class should be assigned by a person involved in the surgical procedure, e.g., surgeon, circulating nurse, etc. Wounds are divided into four classes:

- *Clean:*An uninfected operative wound in which no inflammation is encountered and respiratory, alimentary, genital, or uninfected urinary tracts are not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow nonpenetrating (blunt) trauma should be included in this category if they meet the criteria.
- *Clean-contaminated:* Operative wounds in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination. Specifically, operations involving the biliary tract,

appendix, vagina, and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered.

- *Contaminated:* Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (e.g., open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included in this category.

- *Dirty or Infected:* Includes old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation.

SURGICAL SITE INFECTIONS

The Centers for Disease Control and Prevention (CDC) defines surgical site infections (SSIs) as:

- *Superficial incisional SSIs*

  Infection occurs within 30 days after the operative procedure ***and*** involves only skin or subcutaneous tissue of the incision, ***and*** at least **one** of the following signs or symptoms is present:

  - Purulent drainage from the superficial incision
  - Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision

- At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat; and superficial incision deliberately opened by the surgeon or attending physician
- Diagnosis of superficial incisional SSI by the surgeon or attending physician
- The following are not reported as superficial incisional SSIs: stitch abscess (minimal inflammation and discharge confined to the points of suture penetration), infection of an episiotomy or a neonate's circumcision site, infected burn wound, and incisional SSI that extends into the fascial and muscle layers

- *Deep incisional SSIs*

  Infection occurs within 30 days after the operative procedure if no implant is left in place or within 1 year if implant is in place and the infection appears to be related to the operative procedure ***and*** involves deep soft tissues (e.g., fascial and muscle layers) of the incision ***and*** at least **one** of the following signs or symptoms is present:

  - Purulent drainage from the deep incision but not from the organ/space component of the surgical site
  - A deep incision spontaneously dehisced or deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain, and tenderness unless culture of the incision gives negative results

- An abscess or other evidence of infection involving the deep incision found on direct examination, during reoperation, or by histopathologic or radiologic examination
- Diagnosis of a deep incisional SSI by a surgeon or attending physician

- *Organ/Space SSIs*

  An organ/space SSI involves any part of the anatomy (e.g., organs or spaces) other than the incision opened or manipulated during the operative procedure. Specific sites are assigned to organ/space SSIs to identify the location of the infection. The specific sites that must be used to differentiate organ/ space SSIs are:

  - Arterial or venous infection
  - Breast abscess or mastitis
  - Disk space
  - Ear, mastoid
  - Endocarditis
  - Endometritis
  - Eye, other than conjunctivitis
  - Gastrointestinal tract
  - Intra-abdominal, not specified elsewhere
  - Intracranial, brain abscess or dural infections
  - Joint or bursa
  - Mediastinitis
  - Meningitis or ventriculitis
  - Myocarditis or pericarditis
  - Oral cavity (mouth, tongue, or gums)
  - Osteomyelitis
  - Other infections of the lower respiratory tract
  - Other infections of the urinary tract

- Other male or female reproductive tract
- Sinusitis
- Vaginal cuff
- Spinal abscess without meningitis
- Upper respiratory tract, pharyngitis

Organ/space SSIs must meet the following criteria:

Infection occurs within 30 days after the operative procedure if no implant is in place and the infection appears to be related to the operative procedure ***and*** infection involves any part of the anatomy (e.g., organs or space) other than the incision opened or manipulated during the operative procedure ***and*** at least **one** of the following is present:

- Purulent drainage from a drain placed through a stab wound into the organ/space
- Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space
- An abscess or other evidence of infection involving the organ/space on direct examination, during reoperation, or by histopathologic or radiologic examination

SSIs involving more than one site:

- Infection that involves ***both*** superficial and deep incision sites is classified as deep incisional SSI

- Occasional organ/space infection draining through the incision generally not requiring reoperation and considered a complication of the incision is classified as a deep incisional SSI

n.b.

- Specific criteria are used for infected episiotomy and circumcision sites and for burn wounds.
- An implant is defined as a nonhuman-derived implantable foreign body (e.g., prosthetic heart valve, nonhuman vascular graft, mechanical heart, or hip prosthesis) that is permanently placed in a patient during an operation.
- If the area around a stab wound becomes infected, it is not an SSI but is considered a skin or soft-tissue infection, depending on its depth.

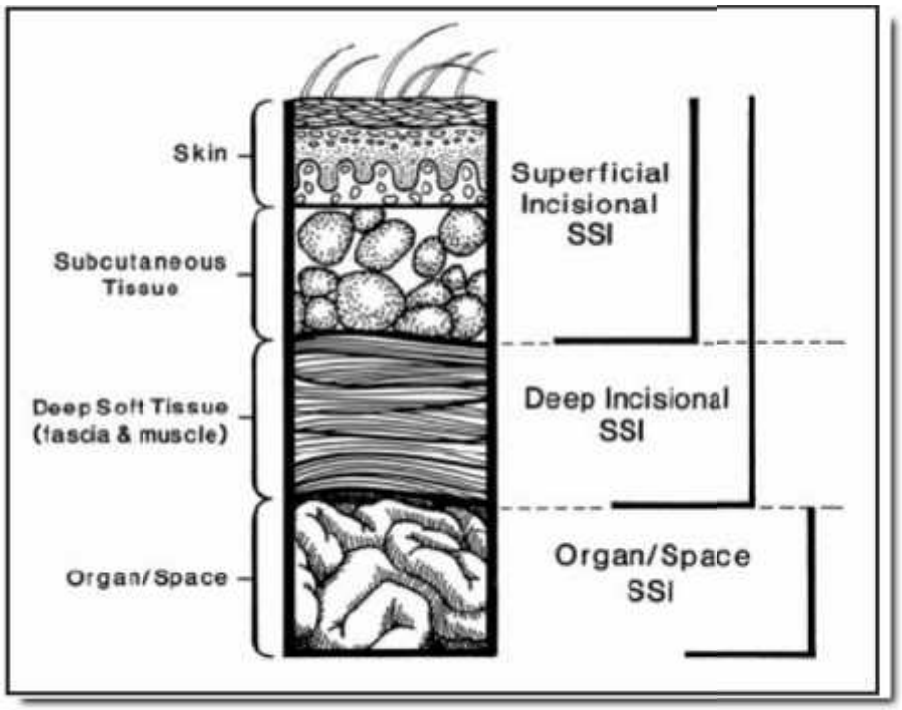


Fig. 1: Classification of surgical site infections. ***Courtesy: CDC Definitions of Nosocomial SSIs, 1992***

Antibiotic Prophylaxis[6]

Most practitioners agree that antimicrobial prophylaxis is beneficial for procedures that involve entry into the gastrointestinal tract with resulting exposure of the surgical site to endogenous intestinal bacteria. However, several controversial topics regarding antibiotic prophylaxis remain, including the use of prophylaxis for some clean operative procedures, the specific agent used in some procedures, the duration of antimicrobial administration, and the relative merits of oral antimicrobial agents, parenteral antimicrobial agents, or both for prophylaxis in colorectal procedures.

Some procedures do not enter the gastrointestinal tract, but nevertheless have a high rate of post-operative infection without prophylaxis. They include lower extremity vascular procedures, hysterectomy, primary Caesarean section, and craniotomy. Some other procedures do not have excessive SSI rates, but any SSIs that do occur have devastating consequences. These operations include joint replacement or placement of other prosthetic devices, cardiac procedures, and aortic graft placements. These procedures benefit from prophylactic antimicrobial administration.

Surgical site infection in patients who have contaminated wounds, who are immune suppressed or undergoing prosthetic surgery, is now the exception rather than the rule since the introduction of prophylactic antibiotics. The evidence for this is of the highest level. The value of prophylactic antibiotics in clean, non-prosthetic surgery remains controversial, although SSI rates after such surgery are high when judged by close, unbiased, post-discharge surveillance, using strict definitions.

The use of prophylactic antimicrobial agents for clean operations in which the SSI risk is relatively low and the consequences of infection are considered mild is

controversial. When SSI rates are low and the consequences small, the use of antimicrobial agents could expose patients to a drug to prevent a single infection, which could predispose to the development of bacterial resistance and/ or an increase in adverse drug reactions in the population being treated.

The recommendations from a recent review on antimicrobial prophylaxis are summarized in the following table.

Table 1: Recommended antibiotic prophylaxis for various types of surgeries

Type of Surgery	Common Pathogens	Recommended Antibiotics	Alternatives
Gastrointestinal			
Esophageal, gastroduodenal	Enteric GNB, GPC	Cefazolin	Clindamycin and Gentamicin or Levofloxacin or Ciprofloxacin
Biliary tract	Enteric GNB, enterococci, clostridia	Cefazolin	Levofloxacin or Ciprofloxacin
Colorectal	Enteric GNB, enterococci, anaerobes	Cefazolin and metronidazole	Clindamycin and gentamicin or cefoxitin
Gynecologic and obstetric			
Hysterectomy	Enteric GNB, enterococci, group B streptococci, anaerobes	Cefazolin or cefoxitin	Clindamycin and gentamicin
Cesarean section	Enteric GNB, enterococci, group B streptococci, anaerobes	Cefazolin	Clindamycin or vancomycin
Genitourinary	Enteric GNB, enterococci	Cefazolin or trimethoprim/ sulfamethoxazole	Clindamycin or vancomycin or ciprofloxacin
Neurosurgery	Staph. aureus, CoNS	Cefazolin and vancomycin	Vancomycin
Cardiac surgery	Staph. aureus, CoNS	Cefazolin and vancomycin or cefuroxime	Clindamycin or vancomycin
Orthopedic	Staph. aureus, Staph. epidermidis	Cefazolin and vancomycin or cefuroxime	Clindamycin or vancomycin
Vascular (Prosthetic graft)	Staph. aureus, CoNS	Cefazolin or vancomycin	Clindamycin or vancomycin
Ophthalmic	Staph. aureus, CoNS, enteric GNB, Pseudomonas spp.	Gentamicin or tobramycin or ciprofloxacin or gatifloxacin or levofloxacin or moxifloxacin	
Thoracic (Non-cardiac)	Staph. aureus, CoNS, enteric GNB	Cefazolin or cefuroxime or vancomycin	Clindamycin or vancomycin

Source: Bennett and Brachman's Hospital Infections, 2014.

Intraoperative Events[6]

Influence on infection risk and methods of prevention

DURATION OF OPERATION

The duration of the operative procedure is one of the most consistently reported factors in SSI, although the precise connection is not known. Possible reasons are that a prolonged operation would result in more desiccation of tissue, would have an increased potential for hypothermia of the patient, and increased exposure of the wound to bacteria. It is also possible, however, that a longer operative duration is a marker for other, unmeasured factors, such as the underlying difficulty of the procedure, more scarring, larger tumor, patient obesity, difficulty in exposure, or the skill or experience of the surgeon. Operations should not be prolonged unnecessarily. That being said, emphasis on the speed of operation can be misleading. An operation that is rushed could increase the risk of intra-operative contamination or of imperfect hemostasis with subsequent increased SSI risk.

TRANSFUSION AND FLUID MANAGEMENT

Repeated blood transfusions can lead to increased risk of infections. They do so by altering the body's immune response, especially macrophage functions. There is a dose-dependent correlation between blood product transfusion and increased mortality and infections in trauma patients. Additionally, crystalloids have been shown to reduce tissue oxygen supply and hence should be avoided.

HYPERGLYCEMIA

High blood sugar (~140 mg/dL), irrespective of the presence or absence of diabetes, increases the risk of SSI. However, a very aggressive approach to prevent this can cause hypoglycemia, and hence it is essential to monitor the serum glucose during the perioperative period.

Maintaining serum glucose <200 mg/dL has been demonstrated to reduce SSI following some procedures. Ata et al. showed that basal-bolus insulin regimen is preferable over sliding scale insulin as it reduces SSIs and provides good glycemic control in adult general surgery patients with Type 2 diabetes.

DELAYED PRIMARY CLOSURE

Delayed primary closure is recommended in patients with highly contaminated wounds, as it leads to improved blood flow at the wound edges and hence better delivery of functional phagocytes, resulting in decreased SSIs, especially in the first 5 to 6 postoperative days.

INTRAOPERATIVE HYPOTHERMIA

The risk of SSI may be decreased by maintaining intra-operative normothermia, particularly in colorectal surgery. Intra-operative hypothermia can cause generalized vasoconstriction leading to decreased subcutaneous blood flow and low oxygen tension and delay in wound healing.

DRAINS

Drains are a potential source of wound contamination leading to SSI. Although closed suction drains are preferred over open drains, Rao et al. demonstrated that SSIs are strongly associated with closed suction drains when left

for long durations. Hence, if it is necessary to use a drain, it should be positioned through a separate incision and for the shortest duration possible.

INTRAOPERATIVE TEAMWORK AND COMMUNICATION

Well-coordinated, seamless teamwork is essential for optimizing clinical outcomes. The lack of communication within the surgical team has shown to be associated with adverse postsurgical outcomes, including SSI, sepsis, and even death.

SURGEON SKILL AND TECHNIQUE

The skill of the surgeon has long been considered to be an important factor in determining SSI risk. However, it has not been possible to effectively measure the association between surgical skill and SSI risk. Certain surgical techniques are associated with increased SSI risk. For example, interrupted sutures lead to a higher SSI risk compared to continuous sutures owing to more tissue necrosis at the suture sites and more suture material left in the wound. Laparoscopic and robotic procedures reduce tissue trauma, shorten the duration of surgery, and result in lower SSI rates.

TYPE OF SUTURE

Monofilament and braided sutures, like silk sutures, have been demonstrated to decrease the risk of SSIs. The role of antimicrobial sutures in reducing SSI risk remains unclear. It has been proposed that antimicrobial-impregnated sutures (such as sutures impregnated with triclosan) could be helpful in reducing SSIs; however, it has not been proven yet.

PREPARATION OF THE PATIENT AND OPERATIVE SITE

- *Preoperative bathing with Antiseptic Agents*

  There is a marked reduction in surface bacterial load with preoperative bathing using chlorhexidine as compared to bathing with povidone-iodine or soap and water. Showering the evening before and the morning of the surgery is more effective than a single shower in the morning or the night before the surgery.

  Also, cleaning the body with a chlorhexidine-impregnated cloth has been shown to decrease colony count more effectively than simple showering. The role of preoperative bathing with chlorhexidine, however, remains unclear. Despite a recent review that demonstrated no protective effect, routine preoperative chlorhexidine bathing is frequently practiced. If done appropriately, it may decrease SSI risk.

- *Skin Decontamination of the Surgical Team and the Patient*

  Hand scrubs using chlorhexidine in combination with alcohol have been found to be more effective in reducing skin microbial flora compared to those with povidone-iodine. For preoperative skin preparation of the patient, chlorhexidine/alcohol and iodine povacrylex/ alcohol-based products have the highest efficacy in microbial reduction. In a randomized controlled trial, chlorhexidine with alcohol as a skin preparation agent significantly reduced SSIs compared with povidone-iodine.

- ***Hair Removal***

  Not removing hair at the operative site is associated with a lower SSI risk compared with hair removal by any means. However, when necessary, clipping of hair or a depilatory method should be preferred over shaving.

The OT Air

Airborne microorganisms pose a significant risk of causing post-operative surgical site infection. Such contaminants can enter an operating room through the air supply, shed by the operating staff (skin fragments with bacteria), through surgical activities and may be transferred from adjacent spaces.[8] Among these, the main burden of microbial air contamination in an operating theatre is due to skin fragments shed by the staff. These fragments may contain micro-colonies of resident or transient micro-flora from the individual's skin. Dispersion levels vary from person to person, but overall dispersion is directly proportional to the number and movement of personnel.[9]

Other sources of microbes in the air include an improperly filtered air supply, contaminated clothes worn by theatre staff, backtracking of contaminated air from outside the OT and microbes from the patient. The latter can become a significant source when power tools are used, as they can create aerosols from the tissues and any microorganisms within them.[9]

A properly designed, well-functioning ventilation system is required to dilute the airborne microbial contamination.

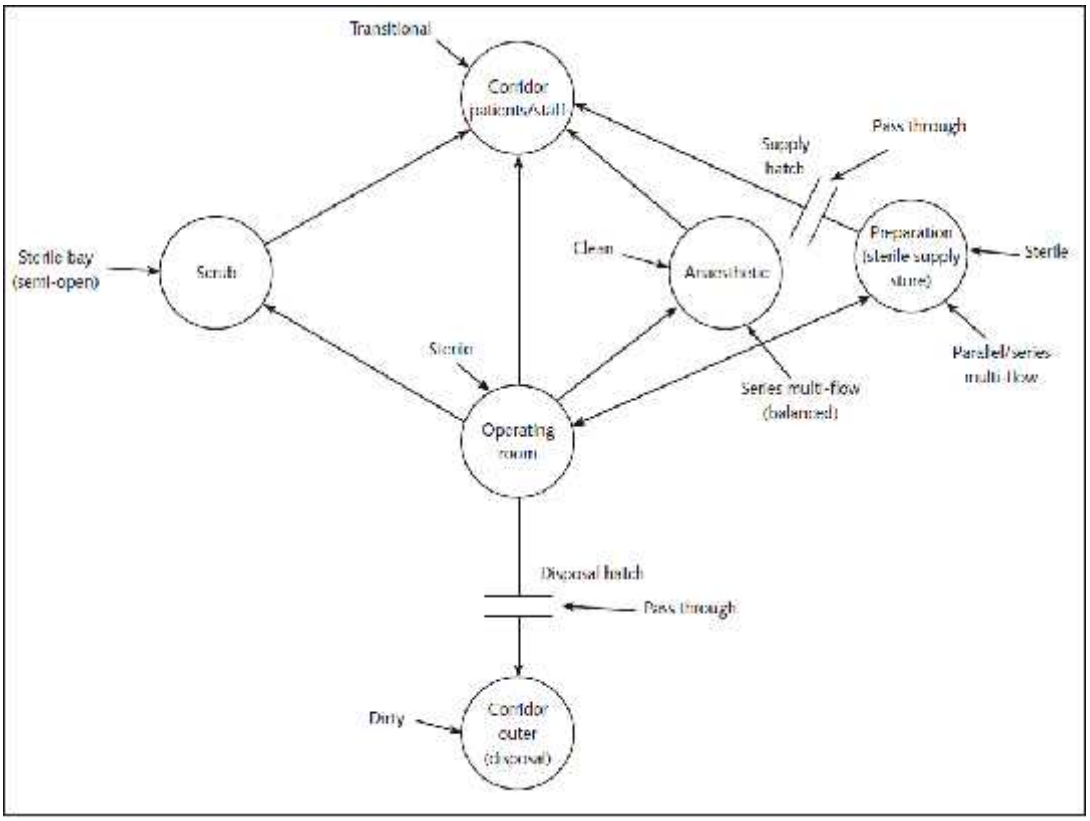


Fig. 2: Example of an airflow network. ***Courtesy: NHS Estates. Health Technical Memorandum 03-01 Part A: Specialized Ventilation in Healthcare Premises, 2007***

The key to minimizing the risk of airborne microorganisms in the ventilation of a conventionally-ventilated theatre is the filtration of the air supplied, dilution of the contaminated air in the theatre and prevention of entry of contaminated air from areas outside the theatre.[9]

The design of the operating theatre should be such that air moves from cleaner areas to less clean areas. This is of particular importance in conventionally-ventilated theatres, i.e., theatres with plenum ventilation and no high efficiency particulate air (HEPA) filters. In ultraclean-ventilated (UCV) theatres, i.e., theatres with laminar air flow and HEPA filters, it assumes relatively less significance.[9] For general operating theatres, the air supply is filtered in the Air Handling Unit (AHU) and terminal or

HEPA filters are not generally required.[10] The efficiency of ventilation of an operating theatre may be described in terms of an *air change rate*, wherein an air change is defined to have occurred when a volume of air equivalent to the volume of the room has been supplied to or removed from that room, whichever airflow is greater. The air change rate is usually denoted in terms of *air changes per hour* (ACH) and is derived from volumetric flow rate of air divided by the space volume, giving a dimensionless value for comparison and standardization. The typical air change rates in an operating theatre are around 25 ACH, with the anesthetic room at 15 ACH, and the Lay-up prep room greater than 25 ACH.[10]

The objective of air flow from cleaner to less clean areas with no backflow can be achieved by ensuring pressure differentials between the different areas. The pressure differential results from the volume of air flowing between those areas per unit time and the size of the gap through which it flows, usually measured in pascal (Pa). The desired pressure differentials between the different rooms will vary from around 9 up to 30 Pa.[9]

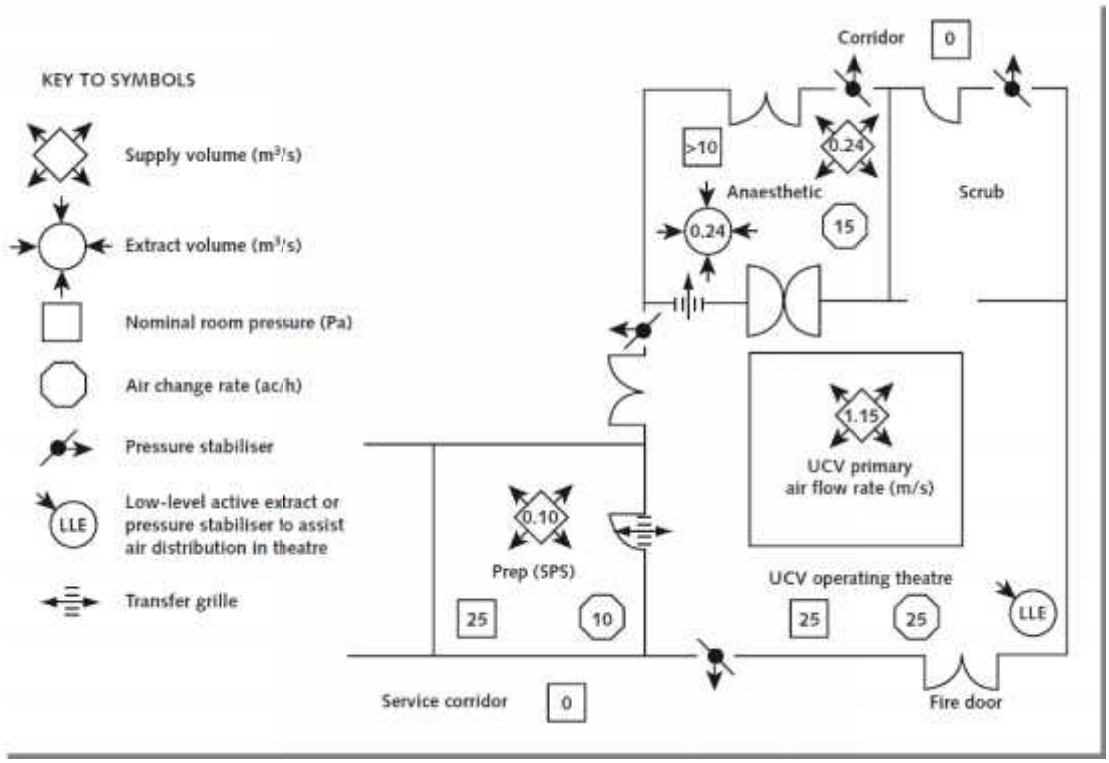


Fig. 3: Floor plan representing air change rate, pressure differentials achieving a unidirectional flow with an ACH of 25. ***Courtesy: NHS Estates. Health Technical Memorandum 03-01 Part A: Specialized Ventilation in Healthcare Premises, 2007, Appendix 7***

In ultra-clean ventilated (UCV) theatres, filtered air is supplied in a unidirectional flow over the patient, creating a *clean zone*, rapidly removing contamination generated within that zone and preventing entry of contaminated air. The large volumes of air required to maintain this zone make it necessary to recirculate air from within the theatre. Filtration of this re-circulated air is essential to prevent contaminated particles also being re-circulated which is done by employing HEPA filters. The existence of this clean zone largely negates the need for control over air movement between rooms in the operation suite. However there is still a need for the preparation room to be at positive pressure and an air change rate of 37 ACH

to other areas, so as to ensure that no contamination of sterile instruments occurs during the lay-up.[9]

For the times when the theatre is not in use, ventilation rate can be *setback* to increase the economy of the OT system. The ventilation is not turned off completely, and volumes are reduced with due consideration to the pressure relativities, maintaining the difference and direction of flow, and thus ensuring no compromise of the sterile environment. If a setback system is in place, there must be a clear visual indicator in the theatre showing whether the ventilation is on setback or on normal flow rates. Post setback mode, the theatre is usable 15 minutes after full ventilation has been restored. The control of the setback is normally on a timed basis and there should an override linked to the operating light or a movement detector so that setback does not occur when there is an unusually long OT list. There must be a setback override to allow unforeseen use of the OT. [9]

The Infection Control Officer (ICO)/ Infection Control Team (ICT) should carry out airflow visualization (smoke testing) to ensure turbulent airflow in the theatre, particularly around the operating table. A puff of smoke should disperse within seconds of creation. It should also be established that supplied air does not short-circuit, i.e., take a direct route out of the theatre, such that it fails to entrain the contamination generated in the theatre. Large volume smoke generators are useful for tracing larger airflow patterns and such airflow visualizations should be used to establish that air flows in the desired direction between rooms in the suite.[9]

Microbiological air testing in an operating theatre is the final check of the quality of air being supplied to the theatre. Such testing is required during commissioning of a new theatre and later after any maintenance work or renovation

has been done.[8] Air leaving the final diffuser or final filters should not contain more than 0.5 CFU (colony forming units)/m^3. [9]

Provided that engineering parameters are satisfactory and regularly monitored, microbiological air sampling in conventionally-ventilated theatres need not be done on a routine basis. Microbiological air sampling of empty, conventionally-ventilated theatres should be done either as part of an investigation into OT-acquired infection with a possible airborne element or after any changes that may affect airflow supply rates or distribution patterns. This would include alterations to the fabric of the theatre or changes to the ductwork distribution that may affect airflow to or within a theatre suite, but would not include routine filter changes. Such sampling should be identical to that on initial commissioning of the theatres.[9]

Recommendations state that UCV theatres be re-commissioned annually and on HEPA-filter replacement or disturbance. In empty UCV theatres, such testing is best accomplished by assessing removal of deliberately supplied inert particles around the clean zone, rather than by bacteriological testing.[9]

Air Sampling

There are two methods of air sampling: active and passive.

In *active* monitoring, a microbiological air sampler physically draws a known volume of air through or over a particle collection device which can be a liquid or a solid culture medium or a nitrocellulose membrane, and the quantity of microorganisms present is measured in CFU (colony forming units)/m^3 of air.

Passive monitoring uses *settle plates*, which are standard petri dishes containing culture media, which are exposed to the air for a given time in order to

collect biological particles which *sediment out* and are then incubated. Results are expressed in CFU/plate/time or in CFU/m^2/hour. According to some authors, passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population which falls onto a critical surface, such as in the surgical cut or on the instruments in operating theatres

Various studies have been conducted to compare these two methods of air sampling. The results were inconsistent. Some studies, such as those by Whyte[(11)], by Orpianesi et al.[(12)] and by Perdelli et al.[(13)] found a significant correlation between the results obtained by the two methods, whereas others, such as those by Petti et al.[(14)] and by Sayer et al.[(15)] found none.

Another difficulty in comparison is due to the variability shown within each technique. A study by Pasquarella et al. showed that different active air samplers showed different results when they were used in the same place at the same time. Also, there does not exist a single universal guideline regarding air sampling procedures. This makes comparison between different samplers and studies difficult.

The location of air sampling also differed in the different studies. While Whyte studied the clean-room of a pharmaceutical company, Petti et al. studied dentists' outpatient clinics. Different indoor environments have different levels of microbial contamination, different kinds of airflow, different number of people working in them who use different kinds of personal protective equipment. The timing of sampling can also vary: at rest or operational. All these factors affect the results of the sampling and also the comparison between different methods of sampling.[(16)]

Many criteria have been suggested to define unacceptable bioburden in the OT. Two of the most commonly followed ones are (Tables 2,3):

Table 2: Classification of operating theatre zones according to risk categories and limits in airborne particles and bacteria, University of Geneva Hospitals, Switzerland[17]

Class	Level of risk	Particle size (Particles per m^3)		Bacterial counts (cfu/m^3)
		≥0.5 μm	≥5 μm	
1.	Very high risk, within laminar air-flow area with HEPA filtration	10	0	<1
2.	High risk, exterior laminar air-flow, but within the operating theatre	353	10	5
3.	Medium risk, conventionally ventilated operating theatre with air filtered through terminal filters with an efficiency of 95% and above	3 530	25	25
4.	Low risk, areas with uncontrolled ventilation	NS	NS	NS

*Sampling is performed in the operating theatre whilst at rest.
cfu, Colony forming unit; NS, no specific limit.

Table 3: Air total microbial count ('Gesamtkeimzahl') according to Fisher in different hospital environments (cfu on Petri dishes 9 cm in diameter, left open to air according to the scheme 1/1/1)[18]

Place	Total microbial count (cfu/dm^2/h) ('Gesamtkeimzahl')		
	Optimal	Acceptable	Not acceptable
Medical wards	0–450	451–750	>751
Surgery	0–250	251–450	>451
Pharmacy	0–100	101–180	>181
Aseptic room	0–50	51–90	>91
Operating theatre (at rest)	0–4	5–8	>9
Operating theatre (in activity)	0–60	61–90	>91

A study conducted by Al Laham in general operating theatres at hospitals in the Gaza Strip, Palestine to determine the prevalence of bacterial contamination of different objects found that 24.7% of the swabs obtained from OT equipment, OT environment and OT personnel were contaminated with microorganisms. The equipment, environment and personnel were responsible for 45%, 48.3% and 6.7% of contamination, respectively. The rate (26.9%) of contamination in the post-operation samples was higher than in the pre-operation samples (22.6%), but the difference was not statistically significant. Of the seven bacterial genera that were recovered, the highest percentage belonged to *Staphylococcus* spp. (45.3%) followed by *Enterobacter* spp. (23.4%). [19]

In spite of taking numerous precautions, patients do develop SSI following clean and clean- contaminated surgeries. A study by Lilani et al. in Mumbai, India found 3.03% surgical site infection rate in clean surgeries and 22.41% in clean-contaminated surgeries, the overall SSI rate being 8.95%. They also found an increase in SSI with increasing duration of surgery, preoperative stay in the hospital and the use of a drain. The most common isolate was *Staphylococcus aureus*, followed by *Pseudomonas aeruginosa*. [(20)]

Similarly, a study by Mulu et al. found that out of 294 patients who had clean and clean-contaminated operation, 10.9% developed bacterial nosocomial infections. The rate of nosocomial infections among clean and clean-contaminated operations was 3.3% and 12.8% respectively. Nosocomial surgical site and blood stream infection rate was 10.2% and 2.4% respectively. *S. aureus* was the most common isolate accounting for 26.2% of the SSIs, followed by *E. coli* and *Coagulase negative Staphylococcus species*, each constituting 21.4%. Nearly 100% of Gram positive and 95.5% of Gram negative bacterial isolates showed resistance against two or more antimicrobial drugs. [(21)]

Sodium Polyanethol Sulfonate (SPS)

In some cases of SSIs, culture reports turn out to be negative for growth of microorganisms. The probable explanation in such cases would be the administration of intra- and post- operative antibiotics, anaerobic infection or intracellular organisms. Incorporation of sodium polyanethol sulfonate (SPS) into the blood culture media is known to increase the yield of the microorganisms.[(2)]

SPS is an anticoagulant which inhibits the antimicrobial systems of blood. It is used widely in blood culture media. A study by Belding and Klebanoff showed that

the addition of SPS to experimental blood cultures inoculated with small numbers of a variety of organisms caused a striking increase in recovery of these organisms. SPS completely inhibited serum antibacterial activity and serum-dependent phagocytosis by isolated leukocytes. It has also been shown to stimulate glucose C-1 oxidation in resting leukocytes and formate oxidation in both resting and phagocytosing leukocytes in serum-free systems.[(2)] A study by Traub and Fukushima showed that the addition of sodium polyanethol sulfonate to fresh human serum completely neutralized β-lysin activity for the entire observation period of 22 h.[(22)]

A study by Rosner investigated the effect of different concentrations of SPS on the recovery rates of a wide spectrum of organisms from blood cultures. Each blood culture bottle was identical except for the amount of sodium polyanethol sulfonate (SPS) present. Flasks A, B, C, and D contained SPS in final concentrations of 0.025, 0.05, 0.075, and 0%, respectively. Of 144 patients found to have clinically relevant organisms in their blood cultures, 127 had positive A flasks, 144 had positive B flasks, 140 had positive C flasks, and 110 had positive D flasks. There was no significant difference in the time required to obtain organism recovery from the A, B, or C flasks; however, the time required to obtain organism recovery from the D flask was considerably longer, ranging up to 5 days in many cases. Of the various organisms recovered, 3 of 7 strains of anaerobic streptococci and 1 of 28 strains of *Streptococcus pneumoniae* appeared to be inhibited by SPS when the concentration was 0.075%. In no case was an organism recovered from either the A or D flask but not from the B flask, indicating that a concentration of 0.05% SPS in hypertonic media does not inhibit the growth of a wide spectrum of organisms in clinical blood cultures.[(3)]

The influence of SPS on the three complement activation pathways was studied by Palarasah et al. In routinely used concentrations, SPS could inhibit the classical and alternative pathways, but not the lectin pathway. Inhibition of complement activity by SPS was found to be caused by a blocking of complement activation and was not a result of complement consumption. The classical pathway was inhibited at SPS concentrations greater than 0.1 mg/ml, and complete inhibition was seen at 0.4 mg/ml. An SPS concentration of 0.5 mg/ml completely inhibited the binding of C1q and subsequent incorporation of C3, C4, and C9. The same was observed for the alternative pathway with an inhibition at SPS concentrations from 0.1 mg/ml and a complete inhibition from 0.4 mg/ml. Here, properdin binding was completely absent, and no incorporation of C3 and C9 was observed. In contrast, the lectin complement pathway remained unaffected at these SPS concentrations, and inhibition was first observed from 0.7 mg/ml. A complete inhibition required concentrations greater than 1 mg/ml.[(23)]

In view of the aforementioned studies, we also intended to evaluate the modified medium, containing glucose broth and 0.05% SPS, so as to increase the culture yield from SSI.

Advances in Sterilization and Disinfection[(24)]

Ortho-phthalaldehyde (OPA) is a relatively new chemical sterilant that was cleared by the Food and Drug Administration in October 1999. It has proven to be an excellent microbicidal agent in in-vitro studies. It offers several advantages over glutaraldehyde: it has superior mycobactericidal activity, does not require activation, does not irritate the eyes or nasal passages, is stable over a wide pH range (pH 3-9), does not require exposure monitoring and has a barely perceptible odour. It has

excellent material compatibility, like glutaraldehyde. A disadvantage is that OPA stains proteins (including skin) grey. Thus, it must be handled with caution, i.e., using gloves, goggles and fluid resistant gowns when handling contaminated instruments or equipment and chemicals.

Another new antimicrobial agent is surfacine. It may be used on animate or inanimate surfaces. It contains a water-insoluble antimicrobial compound (silver iodide) in a surface-immobilized coating (a modified polyhexamethylenebiguanide, PHMB) that is capable of recognizing chemically and interacting with the lipid bilayer of the outer cell membrane of bacteria by electrostatic attraction. The silver is transferred directly from the coating to the organism. The silver accumulates in the microorganisms until the toxicity threshold is crossed. Dead microorganisms lyse and detach from the surface. The amount of silver present and the number of microorganisms in contact with the treated surface determine how long the coating is effective. Surfacine has demonstrated excellent activity against bacteria, especially vancomycin-resistant enterococci, yeast, fungi, and viruses. Antimicrobial activity is retained when the surface is subjected to repeated dry wiping or wiping with a quaternary ammonium compound. The advantage of surfacine over conventional, topically applied silver compounds (e.g., silver nitrate and silver sulfadiazine) is that the coating functions in a chemically intelligent way, i.e., antimicrobial response is triggered only upon microbial contact. The conventional, compounds work by generating a bactericidal level of silver ions. The ions are released into aqueous solution either by silver oxide or dissolution of the silver salt. Surfacine can be applied by dipping, brushing, or spraying without prior surface treatment. The coating does not undergo photoreduction, degradation, or color change when exposed to

intense ultraviolet irradiation. It has excellent adhesion to almost all substrates, is optically clear, and does not delaminate, flake, or crack. Permanently treated surfaces remained chemically inert and retained their biocidal activity after exposure to various physical and chemical stresses. The coated surfaces are resistant to biofilm formation. Surfacine does not cause mammalian cell toxicity.

These new chemicals can be incorporated in the routine sterilization and disinfection routine of hospitals, after some more studies prove that they are more advantageous than the chemicals being used currently.

MATERIAL AND METHODS

Source of Data

The study was carried out from January to December 2014, in KLE's Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi. Two OTs were randomly selected, and surveyed on Wednesday mornings. Details of first surgery performed, in respective OTs were studied.

Inclusion Criteria

Clean surgeries (an uninfected operative wound in which no inflammation is encountered and the respiratory, genital or uninfected urinary tracts are not entered)

Clean-contaminated surgeries (operative wounds in which the respiratory, alimentary, genital or urinary tracts are entered under controlled conditions and without unusual contamination)

Exclusion Criteria

Emergency surgeries

Operating Theater Preparation

Operating theatres were sterilized using Bacillocid Special (Raman & Weil Pvt, Ltd., Mumbai, India). Each 100g contains:

1,6-dihydroxy-2,5-dioxahexane	11.2g
Glutaraldehyde	5.0g
Benzalkonium chloride	5.0g
Alkyl urea derivatives	3.0g
Corrosion inhibitors	

At the end of surgeries each day, the OT was vacated and all the machines and equipment taken out. The floor was washed with soap and water and then left to dry with the air conditioning on. After this, the floor was mopped with 1% Bacillocid Special solution, using the 3 bucket system. The dirty mop was first put in Bucket no.1 (water) and then rinsed in Bucket no.2 (water) and then immersed in Bucket no. 3 containing prepared solution of Bacillocid. Floor was mopped liberally and kept wet for 5-10 minutes. The walls, trolleys, and OT table were also sterilized with Bacillocid Special using the wet-wipe procedure, allowing a contact time of 5-10 minutes. All surfaces were wiped with Bacillocid Special between two cases as well.

Fumigation with 1% Bacillocid Special solution was carried out in the OT every night for 45 minutes, using a fogger. To reduce evaporation, all doors and windows were closed and the air conditioner switched off before starting the fumigation. The OT was kept closed during fumigation. The fogger was switched on and off from an electric point outside the OT. The OT was left closed for 8 hours after fumigation. The next morning, air conditioning was switched on at 5 a.m., allowed to run for 3 hours, with exhaust. At 8 a.m., the OT was ready for use.

The OTs use a vertical laminar air flow system with high efficiency particulate air (HEPA) filters. Laminar air flow is a unidirectional ventilation system, delivering particle free air flow over the operating table at 300 air changes/ hour. The HEPA filters remove particles >= 0.3µm in diameter with an efficiency of 99.97%

Positive pressure was maintained in the OT. Air was introduced at the ceiling and exhausted near the floor. Humidity was maintained at 50-55% and temperature at 18-24°C.

Air Sampling

Air sampling was performed in the operating theatres using settle plates. Petri dishes containing nutrient agar were left open to the operating theatre air for one hour pre-operatively. Another set of petri dishes were similarly exposed to the OT air for one hour post operatively.

The schedule 1/1/1, a standard for measuring the microbial air contamination in hospital environments at bio-risk, was used, i.e., the Petri dish was left open to the air for 1 h, 1m above the floor, 1m from the wall.[18]

Processing of Settle Plates

The plates thus obtained were incubated aerobically at 37°C for 24h and then the colony counts obtained were recorded. In case the plates showed growth, the number and morphology of the colonies was noted. Different colonies were processed by doing gram staining. The probable pathogen or predominant colony was processed as per the standard protocol.[25]

Antimicrobial susceptibility of these isolates was tested by the Kirby-Bauer disk diffusion method, as per Clinical & Laboratory Standards Institute (CLSI) guidelines.[26]

Follow-up of patients

All the surgeries were performed by a single experienced surgeon and his team. Clinical condition was followed up, keeping track of recovery events and any complications. Antibiotic administration protocol was noted.

The patients operated in these OTs were monitored. The following details were noted: type of operation, laparoscopic vs. open surgery, duration of surgery, any

complications occurring during the surgical procedure, antibiotic prophylaxis administered, the use of non-absorbable suture, foreign bodies, copious use of subcutaneous electrocautery, excessive blood loss, and hypothermia. The date of admission, date of surgery, date of discharge, readmission within the post-discharge period, development of SSI and mortality within 6 months after the procedure was noted.

Swabs were obtained from the surgical incision site on days 1,3,5,7,9,11,13,15 post surgery. A total of three swabs were collected at each sampling:

a) One was a moist swab

b) The second was a swab in the modified medium, i.e., glucose broth and 0.05% sodium polyanethol sulfonate

c) The third swab was collected in thioglycollate medium.

The first 2 swabs were used to inoculate 5% sheep blood agar and MacConkey agar plates that were subsequently incubated aerobically at 37°C for 24h.

Smears were made and Gram stained using Hucker's modification[27] (Annexure-III) from both these swabs, for an immediate presumptive diagnosis of the number and type of microorganisms present in the sample.

The colonies obtained on the plates were observed and the isolates processed as per standard protocol for phenotypic identification using conventional tests.[25] Antimicrobial susceptibility of these isolates was tested by the Kirby-Bauer disk diffusion method, as per CLSI guidelines.[26]

Antibiotics and concentration of discs used were as follows:

Antibiotics	**Concentration per disc**
Penicillin	10U
Erythromycin	15 mcg
Cotrimoxazole	25 mcg
Cefoxitin	1 mcg
Clindamycin	2 mcg
Ciprofloxacin	5 mcg

For antimicrobial sensitivity testing, a single colony was inoculated in peptone water and incubated at 37°C for 2 hours and turbidity adjusted to 0.5 McFarland standard. Mueller Hinton Agar plate was inoculated with this broth culture by means of a cotton swab and antibiotic discs were applied. These plates were incubated overnight at 37°C. Zone of inhibition was measured. Interpretation was made according to the Kirby Bauer chart.

Control strains used were *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

Processing For Anaerobic Pathogens:

The swab in the thioglycollate medium was used to inoculate blood agar plates that were incubated anaerobically using the "internal gas generator system" method for 48h.[28] The growth thus obtained was described. Smears were prepared and Gram stained.

Procedure is as follows,

Catalyst:

Palladium pellets reactivated every time before use by drying at 160°C for 1-2 hrs.

Indicator:

Methylene blue prepared by mixing equal volumes of,

a) 6% glucose containing 1mg/ml thymol as preservative

b) Sodium hydroxide solution prepared by adding 6ml of 0.1 N NaOH to100ml of distilled water

c) Methylene blue prepared by adding 3ml of 0.5% w/v solution of methylene blue to 97ml of distilled water. Mixture was placed in anaerobic jar after it was made colourless by heating in a boiling water bath.

Principle:

In this system the hydrogen and CO_2 gas mixture required for creating anaerobiosis is obtained from the following reactions.

(1) Citric acid + Sodium bicarbonate $\longrightarrow$ Sodium citrate + Water + Carbon dioxide

$C_6H_8O_7 + 3NaHCO_3 \longrightarrow Na_3C_6H_5O_7 + 3H_2O + 3CO_2$

(2) Sodium borohydride + water $\longrightarrow$ Sodium metaborate + Hydrogen

$NaBH_4 + 2H_2O \longrightarrow NaBO_2 + 4H_2$

Operation of the gas generator:

a) 1g sodium borohydride was taken in 30 ml test tube

b) 1g sodium bicarbonate and 1g citric acid were taken in the 5 ml test tube, which was placed inside the 30 ml test tube.

c) The stem of 20 ml funnel was plugged with cotton to control the flow of water. The funnel was placed in 30 ml test tube in such a way that the stem of the funnel dipped into 5 ml test tube. Entire unit was kept inside the jar with the indicator. 20ml of distilled water was poured in the funnel just before closing the lid of the jar.

The water poured into the funnel dripped into the 5 ml test tube liberating CO_2. CO_2 being heavier stayed within the test tube, displacing the air. Once the 5 ml test tube was filled with water, it overflowed into the 30 ml test tube liberating hydrogen, which being a lighter gas, rushed out with CO_2. The palladium catalytically reduced the oxygen present within the jar to form water. Since this reaction is exothermic, warming of the lid of the jar could be felt.

After 48 hours of incubation at 37°C, the anaerobic jar was opened. The plates were examined for the types of colonies. Predominant distinct colony was subcultured to purity blood agar plate (BAP). From a pure culture on a BAP, the following were recorded[29]:

a) Colony morphology, including size of colony, shape, color, internal appearance (such as speckling) and general appearance (eg: mucoid, transparent, opaque)

b) Pigment

c) Haemolysis

d) Fluorescence

e) Pitting

The following tests were performed[29] (Annexure-III):

a) Gram stain

b) Catalase test

c) Spot indole test

d) Nitrate reduction test

Single colony of each distinct type was plated on to blood agar plates with antibiotic identification discs. Three antibiotic discs: Kanamycin 1mg, Colistin 10µg and Vancomycin 5µg were placed on the first quadrant of the purity BAP, which aid in preliminary grouping of anaerobes and serve to verify the Gram's stain. A nitrate disc was placed on the second quadrant for subsequent determination of nitrate reduction. [29].

Chocolate agar plate was inoculated for incubation in candle jar at 37^{o}C to test for aerotolerance. If the aerotolerance plate showed growth, smears were prepared and Gram staining was performed to confirm the morphology of the isolates and to co-relate with the observation from the primary plate smears. If the morphology of the organism was the same then the isolate was considered to be a facultative anaerobe. Otherwise the isolate was considered as an obligate anaerobe.

Statistical analysis was done using Two Proportion Z test and T test for two means and unknown standard deviations.

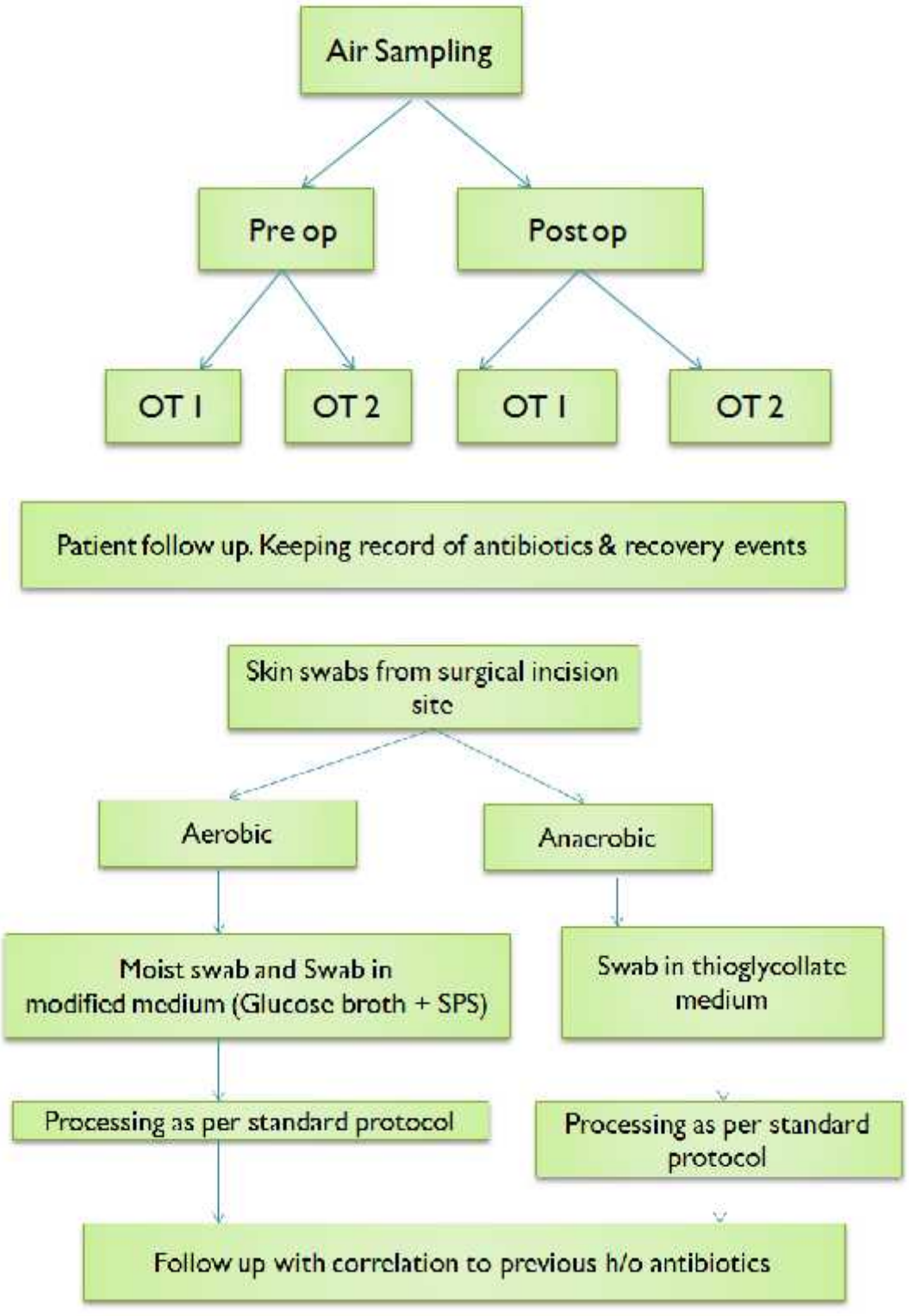
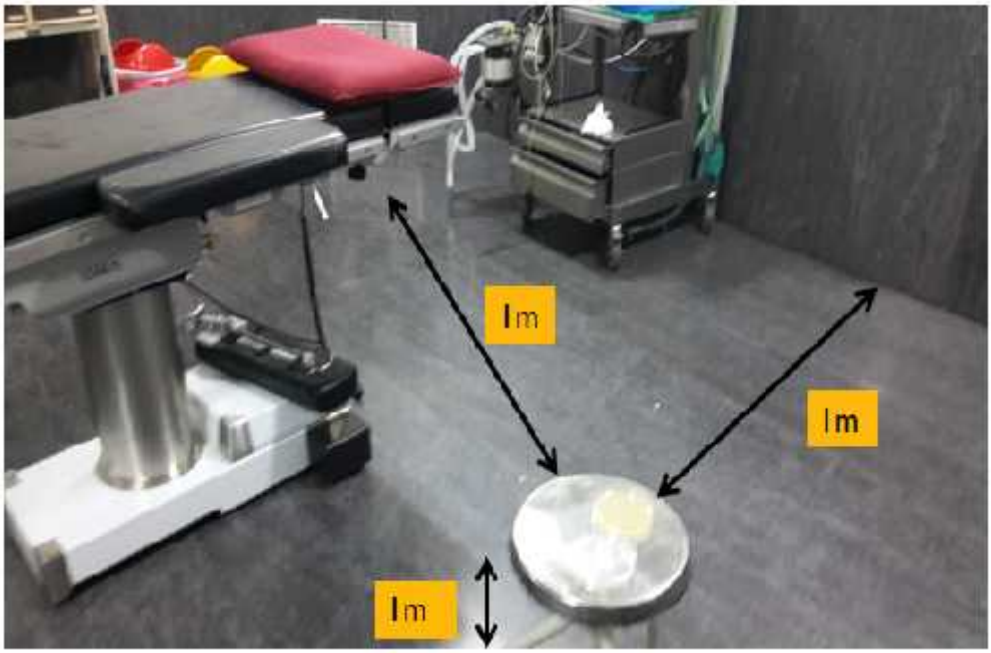


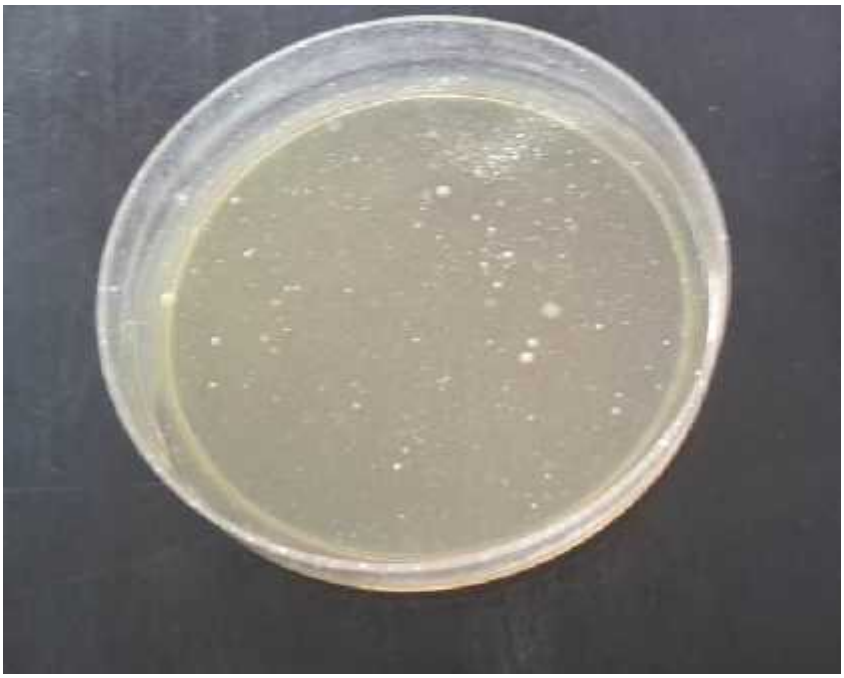
Fig. 4 : Flowchart showing the study methodology

PHOTOGRAPHS



Photograph 1: 1/1/1 scheme for passive air sampling in the OT

Photograph 2: Nutrient agar settle plate before exposure to OT air



Photograph 3: Nutrient agar settle plate with growth after exposure to OT environment

Photograph 4: Three swabs for sample collection from surgical incision site. L to R: moist swab, swab in modified medium, swab in thioglycollate medium.

Photograph 5: Biochemical reactions of *Staph. aureus.* L to R: peptone broth, urea hydrolyzed, mannitol fermented.

Photograph 6: Positive tube coagulase reaction

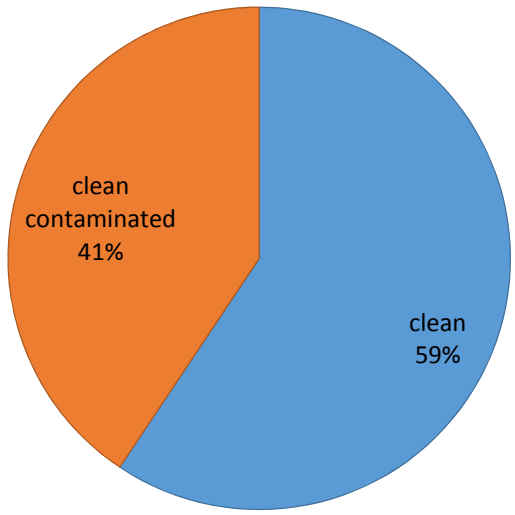
Photograph 7: Antibiotic susceptibility testing. The isolate is showing sensitivity to Cotrimoxazole, Erythromycin and Clindamycin, and resistance to Cefoxitin, Penicillin and Ciprofloxacin.

RESULTS

Types of Surgeries

Out of the total 96 surgeries included in the study, 57 (59%) were clean, and 39 (41%) were clean-contaminated. (Graph 1)

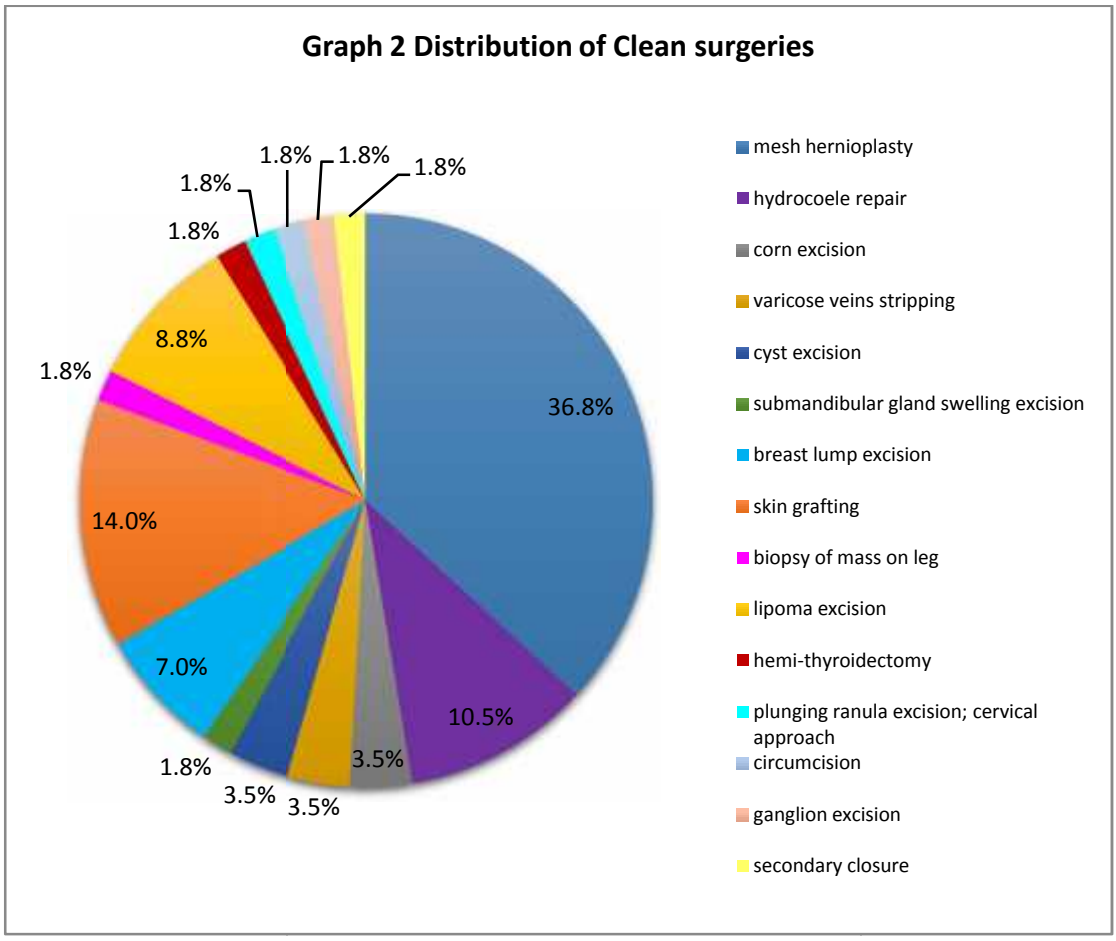
Graph 1 Distribution of Type of surgery



The clean surgeries included in the study were (Graph 2)

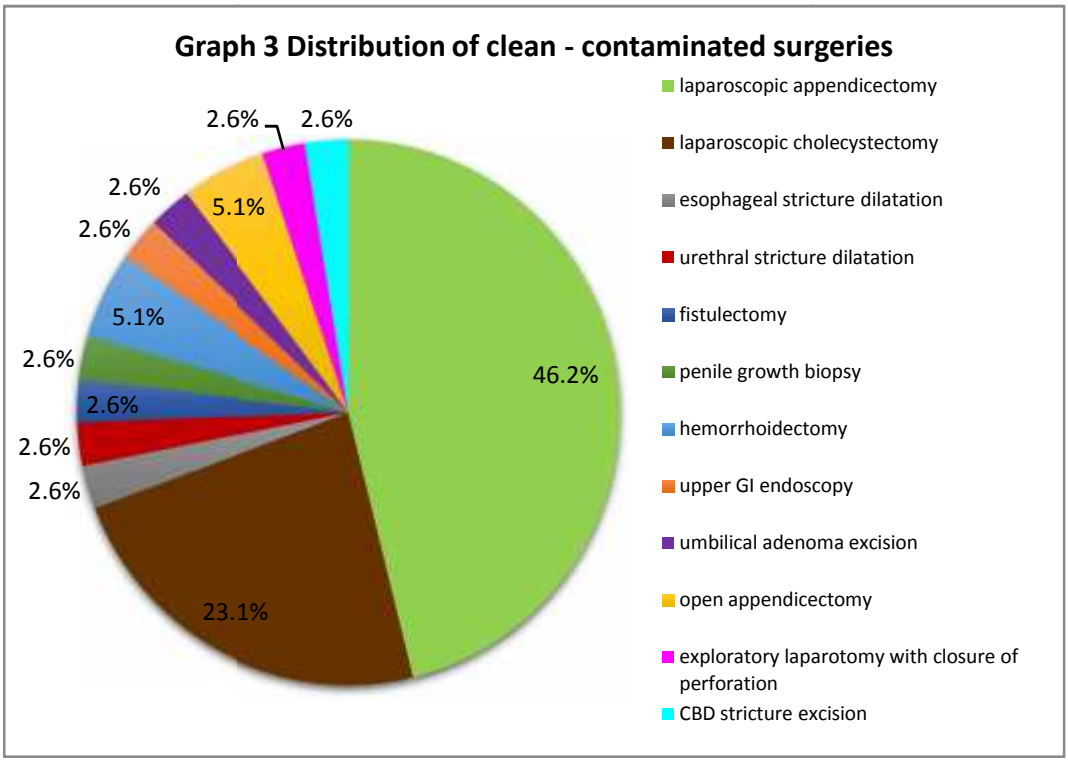
- mesh hernioplasty
- hydrocoele repair
- corn excision
- varicose veins stripping
- cyst excision
- submandibular gland swelling excision
- breast lump excision

- skin grafting
- biopsy of mass on leg
- lipoma excision
- hemi-thyroidectomy
- plunging ranula excision by cervical approach
- circumcision
- ganglion excision
- secondary closure



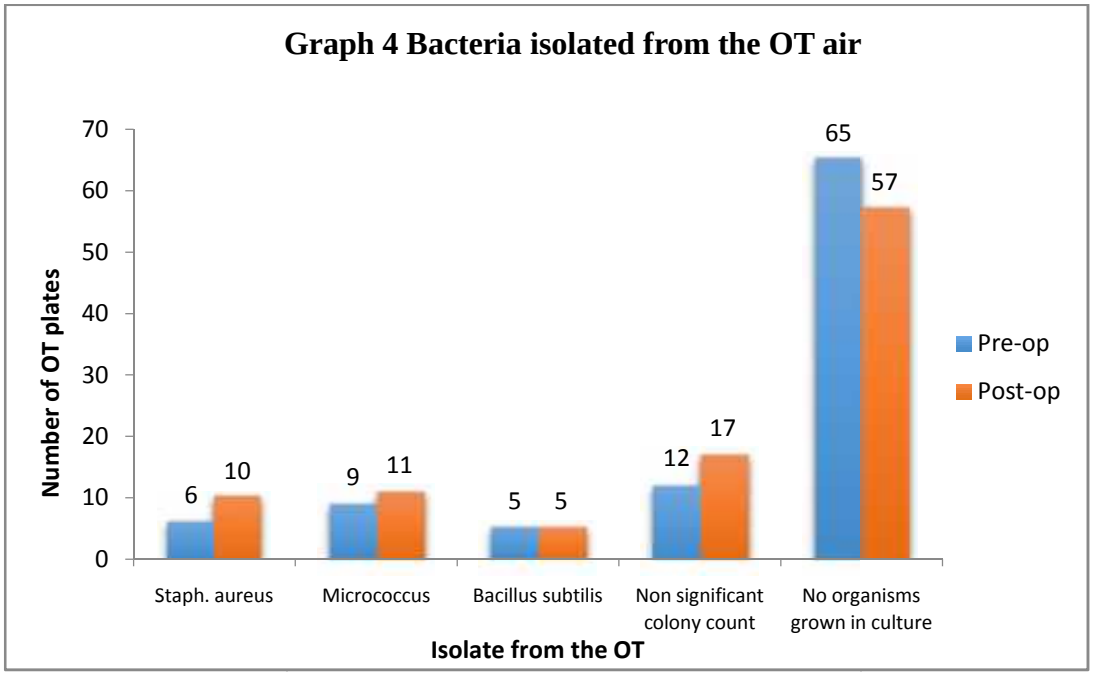
The clean-contaminated surgeries included in the study were (Graph 3)

- laparoscopic appendicectomy
- laparoscopic cholecystectomy
- esophageal stricture dilatation
- urethral stricture dilatation
- fistulectomy
- penile growth biopsy
- hemorrhoidectomy
- upper GI endoscopy
- umbilical adenoma excision
- open appendicectomy
- exploratory laparotomy with closure of perforation
- Common Bile Duct (CBD) Stricture excision



Bacteria in OT air

Our study found 6.25% (six pre-op OT plates) of significant bacterial contamination of OT air pre-operatively and 10.42% (10 post-op OT plates) post-operatively, with *Staphylococcus aureus*. Other organisms isolated were *Bacillus spp*. and *Micrococcus spp*. (Graph 4)



87.5% of these isolates of *Staph. aureus* were susceptible to Cefoxitin, 50% to Erythromycin and 62.5% to Co-trimoxazole. 87.5% of the isolates were susceptible to Ciprofloxacin and 100% to Clindamycin. None of the isolates were susceptible to Penicillin.

Effect of pre-op air on development of SSI

5.13% of patients that underwent clean-contaminated surgeries developed SSI. None of the patients that underwent clean surgeries developed SSI. SSIs developed after laparoscopic cholecystectomy and laparoscopic appendicectomy, in one case of each.

It was seen that the OT environment does play a role in the causation of SSIs. Our study showed that at a 5% level of significance (or with 95% confidence), the presence of pathogenic microorganisms in the OT air in significant numbers leads to a higher risk of SSI.

Effect of pre-op stay on development of SSI

The duration of the pre-operative stay was also found to be a factor responsible for SSIs. Patients with a longer pre-operative stay in the hospital, present with a higher rate of developing SSIs. We could say at 5% level of significance (or 95% confidence), that the pre-op stay of patients who got SSI was at least 2 days greater than that of patients who did not develop SSI.

Staph. aureus was isolated from both the patients that developed SSI. These isolates were susceptible to Cefoxitin, Ciprofloxacin, Co-trimoxazole and Clindamycin. They were resistant to Penicillin and Erythromycin. The antibiotic susceptibility of the isolates matched that of the isolates obtained from their respective OTs pre-operatively.

Culture results from the three swabs

There was no difference in the culture results obtained from the moist swab and from the swab in modified medium containing glucose broth and SPS, with two cases showing growth and 94 cases showing no growth. No anaerobes were isolated. (Table 4)

Table 4: Culture results from the three swabs

	MOIST SWAB	SWAB IN MODIFIED MEDIUM (GLUCOSE BROTH + SPS)	SWAB IN THIOGLYCOLLATE MEDIUM
NO. OF CASES WITH GROWTH	2	2	0
NO. OF CASES WITHOUT GROWTH	94	94	96

DISCUSSION

In elective surgery, infection is caused by bacteria either through the endogenous route or exogenous. The endogenous route is from the patient's own normal flora (e.g. skin or bowel). The exogenous route is mainly from the surgical staff or the environment.[30]

Whyte et al. studied the influence of airborne bacteria on surgical wound contamination during operation. They found that a 13-fold reduction in the airborne bacteria in the OT lead to a 50% decrease in wound contamination. They also found that airborne bacteria lead to significant drape contamination. Bacteria could then be transferred from the drape surface to the wound.[31]

Numerous studies have been conducted to measure microbial contamination of the air and compare active and passive sampling procedures. But since there is no international consensus on the method and type of sampling, the frequency of sampling or even the tolerable limits of bioburden in the OT, it is not feasible to compare these studies accurately.[16]

Some studies showed a significant correlation between active and passive air sampling [11-13] whereas others showed none.[14, 15]

Different active samplers used in the same place at the same time gave different results.

The location also influences the results greatly. Different indoor environments differ in their levels of bio-contamination, kinds of airflow, number of personnel working in them and the personal protective equipment that is used by these

personnel. All these factors influence the results of sampling and also the comparison between the two sampling methods.[16]

Friberg et al. conducted a study in OTs equipped with a vertical or horizontal laminar air flow, to compare the surface contamination rates (in cfu/m^2/ hour) with corresponding air contamination rates (in cfu/m^3) for aerobic bacteria. They used the Casella slit sampler for air sampling in the wound and instrument areas. Settle plates were used to determine surface contamination, in the wound, instrument areas and additionally, on the patient's chest. [32]

They found a wide variation in surface/ air ratio values. This inconsistent relationship between surface and air counts indicated that measurement of OT air contamination by active samplers is not a true indicator of surgical site contamination in laminar air flow units. They proposed that colony counts on sedimentation plates were clinically more relevant as an indicator of surgical site contamination.[32]

A study by Napoli et al. demonstrated that if a strict protocol is followed, the results of active and passive sampling of the OT air correlate in a comparable manner with the air quality, both *in operational* and *at rest*. They concluded that both methods could be used for routine surveillance of OT air. But if specific information is desired, one of these two methods must be chosen. Passive measurement is better for monitoring the risk of microbial wound contamination, as it gives a direct measure of the number of microorganisms falling onto a surface, and is hence, a better predictor of the likelihood of microbial contamination of the surgical site. On the other hand, active volumetric sampling is to be preferred to measure the concentration of all inhalable viable particles in the OT air.[16]

To summarise, settle plates are sterile, cheap, easily available and allow for the collection of many samples in different places in an environment at the same time. The results are meaningful, reliable, valid and comparable. Furthermore, the airflow is not disturbed by the sampling procedure and real conditions are reproduced.[18]

Although there is much debate on the method of air sampling, there is consensus on the fact that routine surveillance of the OT environment is not required and is a wasteful exercise. Environmental sampling should be conducted at the time of commissioning of theatres, while investigating an epidemic, to validate protocols, or when any change has been made to the materials or equipment used in the OT or the heating, ventilation, air conditioning (HVAC) system or at the time of renovation of the OT.[10, 17]

We do carryout OT air surveillance routinely. Thus, we conducted this study to investigate if the OT air plays a significant role in the causation of SSIs; and accordingly, whether to advocate the routine surveillance of OTs or not.

Two of the most commonly followed criteria to define unacceptable bioburden in the OT are mentioned in Table 2 and Table 3.

Our study found 6.25% of significant bacterial contamination of OT air pre-operatively and 10.42% post-operatively.

A study conducted by Al Laham in the Gaza Strip, Palestine to determine the prevalence of bacterial contamination of different objects in OTs found 22.6% contamination in pre-operative samples and 26.9% in post-operative samples. The considerably higher rate of contamination observed in this study, as compared to ours, could have been due to three factors. First, the Gaza Strip was under a siege for over

three years, which resulted in serious deficiencies in the quality of disinfectants, antiseptics and sterilization techniques employed in the OTs. Maintenance of instruments and equipment was also not up to the mark due to shortage of spare parts. Second, the samples were collected immediately after the last war, when the Gaza Strip was still under siege. Third, this study employed collection of swabs from the equipment, environment and the personnel, as opposed to settle plates used in our study.[19]

However, the rate of post-operative contamination was higher than that of pre-operative contamination in both the studies. It is acceptable and an expected finding that more contamination would be observed in the OT environment after performing surgery. Hence, it is standard practice to clean the OT table and other surfaces with a suitable disinfectant after finishing one surgery and before performing the next in the same OT.

Various factors may be implicated in the development of a surgical site infection – type of operation, operating surgeon's skill, insertion of foreign materials or implants, appropriateness of surgical preparation, adequacy and timing of antimicrobial prophylaxis, the immune status of the patient and contamination of the inanimate environment. Bacteriological management of OTs has been advocated to reduce the incidence of SSIs. The use of ultra clean air has been shown to reduce infection rates significantly in orthopaedic implant surgery.[17]

Lilani et al. studied the rate of SSI in clean and clean-contaminated elective cases in Mumbai. They found an overall infection rate of 8.95%; 3.03% in clean surgeries and 22.41% in clean-contaminated surgeries. Surgeries commonly presenting with SSI were mastectomies among clean surgeries. In clean-contaminated

surgeries, urinary and genital tract surgeries, and hepato-biliary surgeries commonly presented with SSIs. Surgeries where drains were used showed a higher rate of SSI.[20]

A study by Patel et al. in Gujarat found an SSI rate of 3% in clean surgeries and 11.4% in clean-contaminated surgeries. Increase in pre-operative hospital stay, ASA (American Society of Anesthesiology) score > 2, increase in surgical wound class, emergency surgeries and longer duration of surgery were associated with increased SSI rates. National Nosocomial Infections Surveillance System (NNIS) risk index was calculated for all patients and it was found that SSI rate increases with increase in NNIS risk index.[33]

In our study, we found 5.13% SSI rate in clean-contaminated surgeries, while none of the clean surgeries developed an SSI. SSIs were seen after laparoscopic cholecystectomy and laparoscopic appendicectomy, in one case of each.

Lower rates of SSI obtained at our hospital could be attributed to the use of laminar air flow and HEPA filters in the OTs studied. Also, strict antisepsis and sterilization and disinfection procedures were adhered to at all times. Since our hospital is a teaching hospital, there are a number of students that enter and exit the OT while surgeries are performed. Since their movement cannot be completely avoided, this emphasises the need of a laminar air flow system and HEPA filters. In spite of the student movement, the SSI rate was low as the students followed strict OT precautions. Emergency surgeries were excluded from the study. Drains, where used, were put in through a separate incision and only closed drains were used.

It was seen that the OT environment does play a role in the causation of SSIs. Our study showed that at a 5% level of significance (or with 95% confidence), the

presence of pathogenic microorganisms in the OT air in significant numbers, leads to a higher risk of SSI.

The duration of the pre-operative stay was also found to be a factor responsible for SSIs. Patients with a longer pre-operative stay in the hospital, present with a higher rate of developing SSIs. We could say at 5% level of significance (or 95% confidence), that the pre-op stay of patients who got SSI was at least 2 days greater than that of patients who did not develop SSI. This finding is consistent with other studies. [20, 33]

A study conducted in Taiwan by Tang et al. found *Bacillus spp*., *Micrococcus spp*., and *Staphylococcus spp*. as the predominant isolates from the OT air. A study by Shintani et al. also showed similar results. [34]

Al Laham found *Staphylococcus spp*. as the predominant isolate from the OT air. [19]

In our study, the bacteria isolated were *Staphylococcus aureus*, *Micrococcus spp*. and *Bacillus spp*.

Al Laham found the staphylococci isolated to be highly resistant to Penicillin (93.1%) and Ampicillin (86.2%). All isolates were sensitive to Vancomycin. Resistance to Rifampin and Doxycycline was 3.4% each and 24.1% of the isolates were resistant to Ciprofloxacin. Methicillin resistance was detected in 62.1% of the staphylococci.[35]

In our study, the predominant isolates were *Staph. aureus*. 87.5% of the strains were susceptible to Cefoxitin, 50% to Erythromycin and 62.5% to Co-trimoxazole.

87.5% of the isolates were susceptible to Ciprofloxacin and 100% to Clindamycin. None of the isolates were susceptible to Penicillin.

The antibiotic susceptibility of the isolates from the infected patients matched that of the isolates obtained from their respective OTs pre-operatively.

The patients recovered without much morbidity because they were administered the antibiotics that the infecting organism was susceptible to.

Thus, we recommend speciation and antibiotic susceptibility testing of the OT isolates. This would help in guiding the treatment of the patient if there is discrepancy in isolate antibiogram and post-operative antibiotic treatment of the patient.

There was no difference in the culture results obtained from the moist swab and from the swab in modified medium containing glucose broth and SPS. Previous studies had shown that SPS increases culture yield from blood samples. We could not say the same for pus samples as the cases with SSI were very few for us to make a statistically significant conclusion. Further studies utilising the modified medium with different samples may validate the use of such a medium.

No anaerobes were isolated in our study. This could be due to the fact that patients undergoing gastrointestinal tract surgeries were administered metronidazole prophylactically pre-operatively. Also, contaminated and dirty surgeries were not included in our study. Anaerobes are usually isolated in such cases. This could be another reason for no anaerobes being isolated in the present study.

Limitations of this study were that isolates from the patient and the OT were compared only phenotypically and found to be the same. Genotyping of isolates would have confirmed the relation between the isolates from the patient and the OT.

As different kinds of air samplers were not used in the present study, we cannot authenticate the use of settle plate method. However, the use of settle plates has been validated as a method for OT sir sampling by other studies.[16, 18]

CONCLUSION

In conclusion, it was demonstrated that the presence of pathogenic microorganisms in the OT air in significant numbers leads to a higher risk of SSI. Patients with a longer pre-operative stay in the hospital, present with a higher rate of developing SSIs. Further studies are required to evaluate the use of sodium polyanethol sulfonate to increase culture yield from samples other than blood.

We recommend that laminar air flow with HEPA filters be used in OTs to reduce the risk of SSI. Regular monitoring of the OT environment in areas where SSI rates are high could be beneficial in identifying the source of these infections. The isolates thus obtained should be identified and their antibiotic susceptibility determined, so as to determine an effective antibiotic policy. The use of settle plates is recommended as it is an economical and technically less challenging method than active air samplers and gives a truer reflection of the bioburden in the OTs.

SUMMARY

The present study was carried out at the Department of Microbiology, JNMC, Belagavi and KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi after obtaining an approval from institutional ethics committee and written informed consent.

Two OTs at KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi were randomly selected, and surveyed on Wednesday mornings from January to December 2014. Air sampling was performed pre and post operatively using settle plates by 1/1/1 method. The isolates thus obtained were identified and their antibiotic susceptibility was determined.

Elective clean and clean-contaminated surgeries being undertaken in these OTs were studied.

The patients thus operated were followed up and three swabs were taken from the surgical incision site: moist swab and swab in modified medium (glucose broth with sodium polyanethol sulfonate) for aerobic processing, and swab in thioglycollate medium for anaerobic processing.

The isolates thus obtained were identified and their antibiotic susceptibility was determined.

59% of the 96 surgeries included were clean and 41% were clean-contaminated.

6.25% of the pre-op OT plates and 10.42% of the post-op OT plates showed significant bacterial contamination. The organism responsible was *Staphylococcus aureus*.

87.5% of these isolates of *Staph. aureus* were susceptible to Cefoxitin, 50% to Erythromycin and 62.5% to Co-trimoxazole. 87.5% of the isolates were susceptible to Ciprofloxacin and 100% to Clindamycin. None of the isolates were susceptible to Penicillin.

5.13% of patients that underwent clean-contaminated surgeries developed SSI. None of the patients that underwent clean surgeries developed SSI. SSIs developed after laparoscopic cholecystectomy and laparoscopic appendicectomy, in one case of each.

Staph. aureus was isolated from both the patients that developed SSI. These isolates were susceptible to Cefoxitin, Ciprofloxacin, Co-trimoxazole and Clindamycin. They were resistant to Penicillin and Erythromycin. The antibiotic susceptibility of the isolates matched that of the isolates obtained from their respective OTs pre-operatively.

There was no difference in the culture results obtained from the moist swab and from the swab in modified medium containing glucose broth and SPS, with two cases showing growth and 94 cases showing no growth. No anaerobes were isolated.

The patients recovered without much morbidity because they were administered the antibiotics that the infecting organism was susceptible to.

Our study showed with 95% confidence, that the presence of pathogenic microorganisms in the OT air in significant numbers leads to a higher risk of SSI.

Also, patients with a longer pre-operative stay in the hospital, present with a higher rate of developing SSIs. We could say at with 95% confidence, that the pre-op

stay of patients who got SSI was at least two days greater than that of patients who did not develop SSI.

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ANNEXURE – I - CONSENT FORM

CONSENT FOR PARTICIPATION INRESEARCH

TITLE: "Role of Operating Theatre environment in causing Surgical Site Infections -One year longitudinal study"

Study Investigator **Dr. _____**

Post Graduate Student,

Department of Microbiology,

Jawaharlal Nehru Medical College,

KLE University, Belagavi – 590 010

Guide **Dr. _____**

The purpose of this research is to monitor operating theatre (OT) air for bacteria, and study its relation with surgical site infections (SSIs) in patients operated in that particular OT; and to investigate the correlation between pre/ post operative antibiotic usage and antibiotic resistance in SSI isolates in relation to the patients' condition.

Pre- and post-operative air sampling will be carried out in the OT to detect bacterial contamination of the air. In the event of an SSI in the patient after operation, swabs will be taken from the infected site and tests to isolate and identify the bacteria will be conducted. Correlation between these isolates and those from the OT air will be studied. Also, antibiotic susceptibility of these organisms from the SSI will be determined and correlated with pre/post-operative antibiotic usage. In case of fever along with SSI, blood samples will also be taken and subjected to similar tests.

You are requested to participate in this study which will help to prevent SSIs and reduce morbidity and mortality related to the same. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with Jawaharlal Nehru Medical College. If you decide to participate you are free to withdraw at any time.

PROCEDURE INVOLVED:

Swabs will be taken from infected surgical sites after clean and clean-contaminated surgeries to isolate and identify bacteria and determine the antibiotic susceptibility of the same. In case of fever with SSI, blood samples will also be taken.

RISKS AND BENEFITS:

There are no risks/minimal risks involved and benefits are to be evaluated.

PRIVACY AND CONFIDENTIALITY:

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except in emergency to protect your rights and welfare.

AUTHORIZATION TO PUBLISH RESULTS:

When the results of research are published or discussed, in a conference no information will be disseminated that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

FINANCIAL INCENTIVES FOR PARTICIPATION:

You will not have to pay/offer any gifts for participating in the research. You will not be reimbursed for expenses.

In case you have any questions related to the study, you can contact Dr. _____ or Dr. _____

In case you have any questions about your rights as a participant, you can contact Dr._____ Chairman, J.N. Medical College Institutional Ethical Committee for Human Subjects Research, at J.N. Medical College, Belagavi.

CONSENT STATEMENT

I undersigned _____have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left hand thumb print of participant or legally authorized representative.

Participant's Name _____ signature_____

Witness Name _____ signature_____

Experimenter's Name _____signature_____

Date : Place:

CONSENT FOR OPERATING THEATRE AIR MONITORING

TITLE: "Role of Operating Theatre environment in causing Surgical Site Infections -One year longitudinal study"

Study Investigator **Dr. _____**

Post Graduate Student,

Department of Microbiology,

Jawaharlal Nehru Medical College,

KLE University, Belagavi – 590 010

Guide **Dr. _____**

The purpose of this research is to monitor operating theatre (OT) air for bacteria, and study its relation with surgical site infections (SSIs) in patients operated in that particular OT; and to investigate the correlation between pre/ post-operative antibiotic usage and antibiotic resistance in SSI isolates in relation to the patients' condition.

Pre and post-operative air sampling will be carried out by exposing nutrient agar plates in the operating theatre following the 1/1/1 scheme (for 1 hour, 1m above the floor, about 1m away from walls or any major obstacles) approximately 1m from the operating table.

You are requested to permit the same. This will help prevent SSIs and reduce morbidity and mortality related to the same.

CONSENT STATEMENT

I undersigned _____have been explained in my vernacular language about the study. I permit Dr. _____ to conduct the aforementioned study and consent to cooperate in this regard.

Signature of OT in charge or legally authorized representative.

OT in charge's Name _____ signature_____

Experimenter's Name _____signature_____

Date : Place:

ANNEXURE – II - PROFORMA

QUESTIONNAIRE (PROFORMA) USED FOR COLLECTING THE DATA

Name:

Age: Sex

I.P. No.:

DOA : Lab. No.:

Occupation:

Address :

Presenting complaints:

History of presenting illness:

Past history:

- History of similar episode in the past.
- Any treatment taken for similar complaint

Family History

- Any similar complaints in family members.

Personal history:

General Physical Examination:

Local examination :

Systemic Examination :

Laboratory Investigations:

Details of surgical procedure:-

Type of surgery:

Wound class:

Type of operation:

Duration:

Antimicrobial prophylaxis:

Use of drain:

Preoperative hospital stay:

Total hospital stay:

Follow up:

Post op day 1:

Post op day 3:

Post op day 5:

Post op day 7:

Post op day 9:

Post op day 11:

Post op day 13:

Post op day 15:

ANNEXURE – III- PROCEDURES

STAINING PROCEDURE AND BIOCHEMICAL TESTS

Gram stain (Hucker's modification):

1. Primary Stain: Crystal Violet Staining Reagent.

Solution A for crystal violet staining reagent

Crystal violet (certified 90% dye content), 2g

Ethanol, 95% (vol/vol), 20 ml

Solution B for crystal violet staining reagent

Ammonium oxalate, 0.8 g

Distilled water, 80 ml

Solutions A and B were mixed to obtain **crystal violet staining reagent.** Stored for 24 h and filtered through paper prior to use.

2. Mordant: Gram's Iodine

Iodine, 1.0 g

Potassium iodide, 2.0 g

Distilled water, 300 ml

The iodine and potassium iodide were ground in a mortar and water was added slowly with continuous grinding until the iodine was dissolved. Stored in amber colored bottles.

3. Decolorizing Agent

Acetone, 50 ml

Ethanol (95%), 50 ml

4. Counterstain: Safranin

Stock solution:

2.5g Safranin O

100 ml 95% Ethanol

Working Solution:

10 ml Stock Solution

90 ml Distilled water

Protocol :

1. Air-dried, heat-fixed smear was overlaid for 1 minute with crystal violet staining reagent.
2. Slide was washed in a gentle and indirect stream of tap water for 2 seconds.
3. The smear was overlaid with the mordant: Gram's iodine for 1 minute.
4. Slide was washed in a gentle and indirect stream of tap water for 2 seconds.
5. Slide was held tilted at an angle of 45˚ and decolorizing agent was added drop by drop until the run-off from the slide became clear.
6. The smear was overlaid with counterstain, safranin for 1 minute.
7. Slide was washed in a gentle and indirect stream of tap water until no color appeared in the effluent and then blotted dry with absorbent paper.
8. The results of the staining procedure were observed under oil immersion using a Brightfield microscope.

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Tests for anaerobic processing:

Catalase test:

Growth was removed from blood agar plate and added to a drop of 15 % hydrogen peroxide on a glass slide and observed for evolution of bubbles.

Spot indole test:

A loopful of growth from a pure culture on a blood agar plate was removed and smeared on a filter paper that had been saturated with 1 % paradimethylaminocinnamaldehyde in 10 % (V/V) concentrated hydrochloric acid. A positive reaction was indicated by the rapid development of blue colour around the growth. Negative reaction gave no color change or a pinkish color.

Nitrate test:

This test was done using nitrate discs. The disc was removed from surface of plate and placed in a clean petridish. One drop each of reagents A and B was added. Development of pink to red color indicated nitrate had been reduced to nitrite. If no colour developed in a few minutes, a small amount of zinc dust was added and waited for 5 minutes. Development of red colour indicated that nitrate was not reduced. If no colour developed it was taken as a positive test.

Nitrate reagents

Solution A

Sulfanilic acid 0.5g

Glacial acetic acid 30.0ml

Distilled water 120.0ml

Solution B

1,6-Cleve's acid (5-amino-2-naphthalenesulfonic acid) 0.2g

Glacial acetic acid 30.0ml

Distilled water 120.0ml

Reference: Sutter VL, Citron DM, Finegold SM, Bricknell KS. Wadsworth Anerobic Bacteriology Manual. 3 ed: The C.V. Mosby Company; 1980.

ANNEXURE – IV - MASTERCHART

DATE	OT NO.	PRE OP OT ISOLATES	POST OP OT ISOLATES
08/01/2014	OT 1	*Micrococcus spp. (5 col)*	NOGC
	OT 2	*Staph. aureus (>10 col)*	*Staph. aureus (>10 col)*
15/01/2014	OT 1	NOGC	*Bacillus subtilis (4 col), Micrococcus spp. (>10 col)*
29/01/2014	OT 1	*Micrococcus spp. (8 col)*	*Micrococcus spp. (10 col)*
05/02/2014	OT 1	NOGC	*Micrococcus spp. (5 col)*
	OT 2	NOGC	*Staph. aureus (5 col)*
12/02/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
26/02/2014	OT 1	*Micrococcus spp.(>10 col)*	*Micrococcus spp. (>10 col)*
05/03/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
19/03/2014	OT 1	*Micrococcus spp. (>10 col)*	*Micrococcus spp. (>10 col)*
	OT 2	*Micrococcus spp. (4 col)*	*Micrococcus spp. (4 col)*
26/03/2014	OT 1	NOGC	*Micrococcus spp. (3 col)*
	OT 2	NOGC	*Micrococcus spp. (4 col)*
02/04/2014	OT 1	*Micrococcus spp. (5 col)*	*Micrococcus spp. (5 col)*
	OT 2	*Micrococcus spp. (>10 col)*	*Micrococcus spp. (>10 col)*
09/04/2014	OT 1	NOGC	NOGC
14/05/2014	OT 1	*Bacillus subtilis (2 col), Micrococcus spp. (7 col)*	*Bacillus subtilis (1 col), Micrococcus spp. (7 col)*
	OT 2	*Bacillus subtilis (5 col), Micrococcus spp. (>10 col)*	*Bacillus subtilis (7 col), Micrococcus spp. (>10 col)*
23/05/2014	OT 1	*Staph. aureus (1 col)*	*Staph. aureus (1 col)*
	OT 2	*Bacillus subtilis (3 col), Micrococcus spp. (>10 col)*	*Bacillus subtilis (8 col), Micrococcus spp. (7 col)*
28/05/2014	OT 1	*Micrococcus spp. (2 col)*	*Micrococcus spp. (2 col)*
	OT 2	NOGC	*Micrococcus spp. (1 col)*
31/05/2014	OT 1	*Staph. aureus (>10 col), Bacillus subtilis (3 col), Micrococcus spp. (>10 col)*	*Staph. aureus (>10 col), Bacillus subtilis (1 col), Micrococcus spp. (>10 col)*
	OT 2	*Staph. aureus (>10 col)*	*Staph. aureus (>10 col)*
04/06/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
06/06/2014	OT 1	NOGC	*Bacillus subtilis (2 col), Micrococcus spp. (4 col)*
	OT 2	NOGC	*Bacillus subtilis (1 col)*
18/06/2014	OT 1	*Bacillus subtilis (1 col)*	*Bacillus subtilis (2 col)*
	OT 2	*Bacillus subtilis (>10 col)*	*Bacillus subtilis (>10 col)*

DATE	OT NO.	PRE OP OT ISOLATES	POST OP OT ISOLATES
19/06/2014	OT 1	*Bacillus subtilis (5 col), Micrococcus spp. (>10 col)*	*Bacillus subtilis (7 col), Micrococcus spp. (>10 col)*
	OT 2	*Micrococcus spp. (>10 col)*	*Micrococcus spp. (>10 col)*
20/06/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
25/06/2014	OT 1	*Bacillus subtilis (>10 col)*	*Bacillus subtilis (>10 col)*
	OT 2	*Staph. aureus (>10 col)*	*Staph. aureus (>10 col)*
27/06/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
02/07/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
04/07/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
09/07/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
11/07/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
23/07/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
26/07/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
08/08/2014	OT 1	NOGC	*Micrococcus spp. (>10 col)*
	OT 2	NOGC	NOGC
13/08/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
20/08/2014	OT 1	*Micrococcus spp. (1 col), Staph. aureus (7 col)*	*Micrococcus spp. (3 col), Staph. aureus (>10 col)*
	OT 2	*Bacillus subtilis (10 col)*	*Bacillus subtilis (4 col)*
22/08/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
08/10/2014	OT 1	*Bacillus subtilis (5 col), Staph. aureus (>10 col)*	*Bacillus subtilis (>10 col), Staph. aureus (>10 col)*
	OT 2	*Bacillus subtilis (9 col), Staph. aureus (3 col)*	*Bacillus subtilis (10 col), Staph. aureus (>10 col)*
10/10/2014	OT 1	*Bacillus subtilis (>10 col)*	*Bacillus subtilis (>10 col), Staph. aureus (>10 col)*
	OT 2	*Bacillus subtilis (>10 col)*	*Bacillus subtilis (9 col), Staph. aureus (>10 col)*

DATE	OT NO.	PRE OP OT ISOLATES	POST OP OT ISOLATES
15/10/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
17/10/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
31/10/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
05/11/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
12/11/2014	OT 1	*Micrococcus spp. (>10 col)*	*Micrococcus spp. (>10 col)*
	OT 2	*Staph. aureus (>10 col), Micrococcus spp. (5 col)*	*Staph. aureus (>10 col), Micrococcus spp. (7 col)*
14/11/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
18/11/2014	OT 1	*Bacillus subtilis (2 col)*	*Bacillus subtilis (2 col)*
	OT 2	*Bacillus subtilis (3 col)*	*Bacillus subtilis (4 col)*
20/11/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
26/11/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
28/11/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
05/12/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
10/12/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
12/12/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
17/12/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
19/12/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC
24/12/2014	OT 1	NOGC	NOGC
	OT 2	NOGC	NOGC

DATE	OT NO.	IP NO.	AGE	SEX	OPERATION	SURGERY TYPE	OUTCOME	PRE-OP STAY	POST-OP STAY	ANTIBIOTICS (PRE-OP)	ANTIBIOTICS (POST-OP)
08/01/2014	OT 1	572322	35	M	B/L mesh hernioplasty	clean	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	573331	28	M	Corn excision	clean	NO SSI	2 days	1 day	none	none
15/01/2014	OT 1	574293	14	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	3 days	2 days	ciprofloxacin	ciprofloxacin
29/01/2014	OT 1	576669	42	F	Varicose veins stripping	clean	NO SSI	2 days	4 days	cefixime	amoxicillin clavulanate
05/02/2014	OT 1	577850	50	M	Hernioplasty	clean	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	577853	23	M	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	2 days	ciprofloxacin	ciprofloxacin
12/02/2014	OT 1	579373	50	F	Varicose veins stripping	clean	NO SSI	2 days	4 days	cefixime	amoxicillin clavulanate
	OT 2	579309	25	F	Esophageal stricture dilatation	clean-contaminated	NO SSI	2 days	1 day	ciprofloxacin	none
26/02/2014	OT 1	581669	20	M	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin
05/03/2014	OT 1	582486	25	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	582289	38	M	Laparoscopic appendicectomy	clean-contaminated	NO SSI	4 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
19/03/2014	OT 1	582962	68	M	Urethral stricture dilatation	clean-contaminated	NO SSI	2 days	3 days	ciprofloxacin	ciprofloxacin
	OT 2	584364	40	M	Mesh hernioplasty	clean	NO SSI	4 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
26/03/2014	OT 1	586867	14	F	Corn excision	clean	NO SSI	2 days	1 day	cefixime	amoxicillin clavulanate
	OT 2	586783	16	F	Dermoid cyst excision	clean	NO SSI	3 days	1 day	cefixime	amoxicillin clavulanate

DATE	OT NO.	IP NO.	AGE	SEX	OPERATION	SURGERY TYPE	OUTCOME	PRE-OP STAY	POST-OP STAY	ANTIBIOTICS (PRE-OP)	ANTIBIOTICS (POST-OP)
02/04/2014	OT 1	586241	25	M	excision of submandibular gland swelling with primary open reduction of small bone with fixation	clean	NO SSI	7 days	8 days	ciprofloxacin	ciprofloxacin
	OT 2	587258	29	M	examination under anesthesia, fistulectomy and seton for fistula in ano	clean-contaminated	NO SSI	4 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
09/04/2014	OT 1	589484	14	M	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
14/05/2014	OT 1	595909	24	M	Mesh hernioplasty	clean	NO SSI	2 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	595869	41	M	Penile growth biopsy	clean-contaminated	NO SSI	6 days	2 days	ciprofloxacin	ciprofloxacin
23/05/2014	OT 1	597525	60	F	Breast lump excision	clean	NO SSI	4 days	4 days	cefixime	none
	OT 2	597571	22	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	4 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
28/05/2014	OT 1	598775	42	M	Haemorrhoidectomy	clean-contaminated	NO SSI	4 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	595518	60	F	Skin grafting- ulcer on leg	clean	NO SSI	7 days	8 days	ciprofloxacin	ciprofloxacin
31/05/2014	OT 1	599436	45	M	Umbilical hernia repair	clean	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	599031	45	M	Inguinal hernia repair	clean	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin
04/06/2014	OT 1	599121	75	F	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	3 days	2 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	600190	48	M	Hydrocoele repair	clean	NO SSI	4 days	2 days	cefixime	none

DATE	OT NO.	IP NO.	AGE	SEX	OPERATION	SURGERY TYPE	OUTCOME	PRE-OP STAY	POST-OP STAY	ANTIBIOTICS (PRE-OP)	ANTIBIOTICS (POST-OP)
06/06/2014	OT 1	600182	38	M	Upper GI endoscopy for duodenal ulcer	clean-contaminated	NO SSI	2 days	4 days	cirofloxacin	none
	OT 2	600240	14	F	Biopsy of mass over leg	clean	NO SSI	4 days	2 days	cefixime	none
18/06/2014	OT 1	603023	56	M	Inguinal hernia repair	clean	NO SSI	4 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	603076	33	F	Fibroadenoma excision	clean	NO SSI	4 days	3 days	cefixime	amoxicillin clavulanate
19/06/2014	OT 1	603356	42	F	Incisional hernia mesh repair	clean	NO SSI	2 days	5 days	Ciprofloxacin	Ciprofloxacin
	OT 2	603531	45	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
20/06/2014	OT 1	603131	55	M	Hydrocoele repair	clean	NO SSI	4 days	5 days	cefixime + metronidazole	ciprofloxacin
	OT 2	603048	26	M	Umbilical adenoma excision	clean-contaminated	NO SSI	6 days	4 days	cefixime	amoxicillin clavulanate
25/06/2014	OT 1	604462	52	M	Skin grafting- ulcer on leg	clean	NO SSI	6 days	7 days	ciprofloxacin	ciprofloxacin
	OT 2	604608	50	F	Laparoscopic cholecystectomy	clean-contaminated	Staph. aureus	6 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
27/06/2014	OT 1	605153	54	M	Sebaceous cyst excision	clean	NO SSI	2 days	2 days	cefixime	amoxicillin clavulanate
	OT 2	604094	42	F	Paraumbilical hernia repair	clean	NO SSI	6 days	4 days	cefixime	cefixime
02/07/2014	OT 1	605890	10	M	Lipoma excision	clean	NO SSI	3 days	3 days	cefixime	amoxicillin clavulanate
	OT 2	606077	48	F	Open appendicectomy	clean-contaminated	NO SSI	2 days	8 days	ciprofloxacin + metronidazole	ciprofloxacin

DATE	OT NO.	IP NO.	AGE	SEX	OPERATION	SURGERY TYPE	OUTCOME	PRE-OP STAY	POST-OP STAY	ANTIBIOTICS (PRE-OP)	ANTIBIOTICS (POST-OP)
04/07/2014	OT 1	605159	30	F	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	7 days	1 day	ciprofloxacin + metronidazole	none
	OT 2	606679	18	F	Fibroadenoma excision	clean	NO SSI	2 days	0 days	cefixime	amoxicillin clavulanate
09/07/2014	OT 1	600218	5	F	Exploratory laparotomy with closure of perforation	clean-contaminated	NO SSI	7 days	6 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	607456	60	M	Inguinal hernia repair	clean	NO SSI	2 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
11/07/2014	OT 1	607517	42	F	Hemi-thyroidectomy	clean	NO SSI	3 days	5 days	cefixime	amoxicillin clavulanate
	OT 2	606888	54	M	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
23/07/2014	OT 1	610401	50	M	Inguinal hernia repair	clean	NO SSI	2 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	605229	87	M	Skin grafting- ulcer on leg	clean	NO SSI	4 days	5 days	ciprofloxacin	ciprofloxacin
26/07/2014	OT 1	610763	25	M	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	611320	14	M	Laparoscopic appendicectomy	clean-contaminated	NO SSI	3 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
08/08/2014	OT 1	607472	40	F	Skin grafting- ulcer on leg	clean	NO SSI	4 days	3 days	ciprofloxacin	ciprofloxacin
	OT 2	612980	32	M	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin
13/08/2014	OT 1	613925	38	M	Plunging ranula excision; cervical approach	clean	NO SSI	10 days	1 day	cefixime + metronidazole	none
	OT 2	614509	22	M	Circumcision	clean	NO SSI	2 days	1 day	none	none

DATE	OT NO.	IP NO.	AGE	SEX	OPERATION	SURGERY TYPE	OUTCOME	PRE-OP STAY	POST-OP STAY	ANTIBIOTICS (PRE-OP)	ANTIBIOTICS (POST-OP)
20/08/2014	OT 1	602765	55	M	Skin grafting- ulcer on foot	clean	NO SSI	6 days	4 days	ciprofloxacin	ciprofloxacin
	OT 2	615722	37	M	open appendicectomy	clean-contaminated	NO SSI	2 days	9 days	ciprofloxacin + metronidazole	ciprofloxacin
22/08/2014	OT 1	612142	85	M	Skin grafting- ulcer on hand	clean	NO SSI	4 days	3 days	ciprofloxacin	ciprofloxacin
	OT 2	616352	45	M	Lipoma excision	clean	NO SSI	2 days	2 days	cefixime	amoxicillin clavulanate
08/10/2014	OT 1	624817	24	F	Laparoscopic appendicectomy	clean-contaminated	Staph. aureus	6 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	617903	35	M	Secondary closure-penile growth	clean	NO SSI	2 days	2 days	cefixime	amoxicillin clavulanate
10/10/2014	OT 1	625617	27	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	625522	48	M	Inguinal hernia repair	clean	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
15/10/2014	OT 1	623555	58	M	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	626086	70	M	Hydrocoele repair	clean	NO SSI	4 days	3 days	ciprofloxacin	ciprofloxacin
17/10/2014	OT 1	625125	27	M	Inguinal hernia repair	clean	NO SSI	2 days	2 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	625013	35	M	Inguinal hernia repair	clean	NO SSI	4 days	7 days	ciprofloxacin + metronidazole	ciprofloxacin
31/10/2014	OT 1	629572	27	F	Ganglion excision	clean	NO SSI	3 days	3 days	ciprofloxacin	amoxicillin clavulanate
	OT 2	629453	45	M	Inguinal hernia repair	clean	NO SSI	3 days	6 days	ciprofloxacin + metronidazole	ciprofloxacin

DATE	OT NO.	IP NO.	AGE	SEX	OPERATION	SURGERY TYPE	OUTCOME	PRE-OP STAY	POST-OP STAY	ANTIBIOTICS (PRE-OP)	ANTIBIOTICS (POST-OP)
05/11/2014	OT 1	629096	45	F	CBD stricture-exploration and excision	clean-contaminated	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	630697	62	M	Inguinal hernia repair	clean	NO SSI	3 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
12/11/2014	OT 1	632017	40	F	Lipoma excision	clean	NO SSI	3 days	2 days	cefixime	amoxicillin clavulanate
	OT 2	632024	42	M	Skin grafting- ulcer on leg	clean	NO SSI	4 days	5 days	cefixime	amoxicillin clavulanate
14/11/2014	OT 1	632417	29	F	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	631968	47	M	Inguinal hernia repair	clean	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
18/11/2014	OT 1	631597	62	M	Inguinal hernia repair	clean	NO SSI	3 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	631989	60	F	Lipoma excision	clean	NO SSI	2 days	2 days	ciprofloxacin	ciprofloxacin
20/11/2014	OT 1	633890	35	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	4 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	633935	24	M	Hydrocoele repair	clean	NO SSI	4 days	4 days	cefixime	none
26/11/2014	OT 1	634797	34	F	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	634773	45	M	Umbilical hernia repair	clean	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin
28/11/2014	OT 1	634939	26	M	Inguinal hernia repair	clean	NO SSI	2 days	7 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	624324	36	M	Skin grafting- ulcer on leg	clean	NO SSI	2 days	4 days	ciprofloxacin	ciprofloxacin
05/12/2014	OT 1	636610	38	M	Gynaecomastia excision	clean	NO SSI	4 days	3 days	cefixime	amoxicillin clavulanate
	OT 2	626086	70	M	Hydrocoele repair	clean	NO SSI	4 days	3 days	cefixime	amoxicillin clavulanate

DATE	OT NO.	IP NO.	AGE	SEX	OPERATION	SURGERY TYPE	OUTCOME	PRE-OP STAY	POST-OP STAY	ANTIBIOTICS (PRE-OP)	ANTIBIOTICS (POST-OP)
10/12/2014	OT 1	637459	44	M	Inguinal hernia repair	clean	NO SSI	2 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
	OT 2	637466	58	M	Haemorrhoidectomy	clean-contaminated	NO SSI	4 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
12/12/2014	OT 1	637964	22	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	4 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	638079	50	F	Lipoma excision	clean	NO SSI	2 days	3 days	cefixime	amoxicillin clavulanate
17/12/2014	OT 1	638936	13	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	3 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	639093	19	F	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	6 days	1 day	ciprofloxacin + metronidazole	none
19/12/2014	OT 1	639266	28	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	638945	50	F	Laparoscopic cholecystectomy	clean-contaminated	NO SSI	4 days	3 days	ciprofloxacin + metronidazole	ciprofloxacin + metronidazole
24/12/2014	OT 1	640291	15	F	Laparoscopic appendicectomy	clean-contaminated	NO SSI	2 days	5 days	ciprofloxacin + metronidazole	ciprofloxacin
	OT 2	640234	38	M	Hydrocoele repair	clean	NO SSI	4 days	4 days	ciprofloxacin	ciprofloxacin