MICROBIOLOGICAL SURVEILLANCE OF OPERATING ROOMS AT TIRUNELVELI

Dissertation Submitted

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CERTIFICATE

This is to certify that the dissertation entitled, "**Microbiological surveillance of operating rooms at Tirunelveli"** by Dr. S. Nithya Gomatheswari, Post graduate in Microbiology (2007-2010), is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. Degree Examination in Microbiology, Branch IV, to be held in March 2010.

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1.INTRODUCTION

"If I had the honour of being a surgeon, not only would I use absolutely clean instrument but after cleaning my hands with the greatest care would only use sponges previously raised to a heat of 1300-1500 Fahrenheit. I would still have to fear germs suspended in the air and surrounding the bed of the patient."

- Pasteur in his celebrated lecture to *academie de medicine* on April 30th 1873.

Until the middle of the 19th century, when Ignaz Semmelweiss and Joseph Lister became the pioneers of infection control by introducing antiseptic surgery, most wounds became infected. In cases of deep or extensive infection this resulted in a mortality rate of 70-80% *(Altemeir WA et al 1982)*.

Even today, the major concern regarding hospital care that patients and doctors alike have is the concern about infections. The infections that the common man is exposed to in daily life can be controlled by steps that are taken on their own, but infections that occur within hospitals are the responsibility of the concerned hospital or nursing home and needs to be a high priority for them. This is especially true as of today, with the rising number of new and more resistant infective agents being identified.

1.1 Microbiological air quality:-

Good hospital hygiene is an integral component for preventing Hospital Acquired Infections (HAI). HAIs constitute 10 per cent of all hospital admissions worldwide. This has made even more imperative the need for infection control in hospitals and nursing homes. The microbiological quality of theatre air is one of the significant parameter for controlling surgical wound infection. The bacterial count in operation theatre (OT) is influenced by the

1. Type of surgery,
2. Quality of air provided,

3. Rate of air exchange,
4. Number of persons present in the theatre,
5. Movement of operation room personnel,
6. Level of compliance with infection control practices,
7. Quality of cleaning process and
8. Efficiency of the sterilization agent

1.1.1 Type of surgery:-

According to *CDC Guidelines (Mangaram AJ et al 1999).*it has been classified as:

Class I/Clean: An uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is 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.

Class II/Clean-Contaminated: An operative wound 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.

Class III/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.

Class IV/Dirty-Infected: Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera.

1.1.2 Air quality & rate of air exchange:

The quality of indoor air depends on external pollutant concentrations and on internal sources such as heating and air conditioning systems, building material, cleaning products, personnel and their activity. Bacterial contamination of the air should be within acceptable limits. Microbe's level in the environment is not uniformly distributed in a given area and fluctuates with time and subject to continuous change.

Air change defined as occurring when a volume of air equivalent to the volume of the operation room has been supplied or removed from that room.The rate of air change per hour would be proportional to air borne microbial contaminants from entering the wound. The *Joint working party on ventilation in operating suites (1972)* ("The Lidwell report") advised that operating theatre should have ventilation around 20 Air change per hour.

1.1.3 Positive pressure in operation theatre (OT):

Air pressure in the operation theatre should be maintained at a higher rate i.e. positive pressure with respect to corridors and adjacent areas, except sterile store and set up rooms, which are required to have a room air pressure greater than the operation room. The positive pressure ensures there is no backflow of air from dirty areas of operating suite or other contaminated areas of the hospital to clean areas. The operating room, the surgical site and instrument table should be considered the cleanest area and infection control efforts should be directed toward providing protection through appropriate ventilation control.

Pressure management in the protective operation room environment is designed by a positive airflow out of the cleanest area of the operation room suites.

1.1.4 Type of ventilation:

The ventilation systems are essential for protecting the surgical site using particle displacement dynamics of properly directed purified air movement. The function of operation room ventilation is to prevent air borne microbial contaminants from entering surgical wounds.

A number of different types of systems are available, whereby air may be introduced in a horizontal, uni-directional fashion or vertically in an enclosed, semi-enclosed or open manner *(Howorth et al 1985)*.

Conventional ventilation maintains approximately 20 air changes per hour. It is important that displacement airflow of filtered air is delivered in such a manner that infectious particles shed by the operating team are swept away toward the return ducts and not trapped and recirculated within the vicinity of the procedure.

Laminar flow ventilation/Ultra clean ventilation systems utilising HEPA filters are recommended for ultra clean, implant surgery. Laminar flow operating theatres may operate at upwards of 300 air changes per hour *(Humphreys et al 1993, Hubble et al 1996)*. Vertical flow is preferred over horizontal airflow for space management and infection control considerations.

1.1.5 Number of persons present in the theatre:

The surgical team is a potential reservoir of infection. During an hour long surgical procedure, each individual in the operation room may shed 106 particles *(Mehta et al 2000)*. Each one of these particles may be carrying bacteria that can infect a surgical site. The microbial level in the air is directly proportional to the number of people moving about in the operation room. So movement of staff within operation room is discouraged and should be minimal. *(Scottish Quality assurance report, 2nd Edn.-1994)*

1.2 Surgical site infection (SSI):

Surgical site infection is defined as infections occurring within 30 days of operation *(Mangaram AJ et al 1999).*Surgical site infection have been responsible for the increasing cost, morbidity and mortality related to surgical operations and continues to be a major problem even in hospitals with most modern facilities and standard protocols of preoperative preparation and antibiotic prophylaxis *(Yalcin A N et al 1995)*. In spite of brief stay of patients

in the operation theatre (in majority of circumstances), the environment of operation theatre plays a great role in the onset and spread of infection because of multifactor causation of infection.

Good infrastructures do not mean a safe environment. Standard cleaning, disinfection and sterilisation procedures, good theatre practice and discipline can provide a microbiologically safe environment in the theatre. Microbiological contamination of air in the operating room is generally considered to be a major risk factor for surgical site infections in clean surgery. In order to minimize this kind of wound contamination, focusing on air bacteria released by the operating theatre personnel, Ultra clean laminar flow (LAF) ventilation system is introduced for use in operating rooms.

1.2.1 Sources of surgical site infection:

Surgical Site Infection can either be exogenous or endogenous. Although most surgical wound infections originate from the patients own flora, exogenous sources are also implicated *(Van Furth R et al 1995*). *Exogenous sources* include surgical personnel (esp. members of the surgical team), the operation room environment (including air) and all tools, instruments and materials brought to the sterile field during operation. The risk of surgical site infection is related to a number of factors. Amongst the most important are the operative procedure performed, the degree of microbiologic contamination of the operative field, the duration of the operation, and the intrinsic risk of the patient. *(Garibaldi.R.A et al 1991, Howard,J.M et al 1964)*

1.2.2 Microbes causing surgical site infection:

Endogenous organism such as aerobic Gram Positive cocci (*e.g.staphylococci)* but may include faecal flora (*anaerobic bacteria and Gram negative aerobes)* when incisions are made near perineum or groin. When a Gastrointestinal organ is opened during operation Gram negative bacilli (e.g. E.coli), Gram Positive (e.g. Enterococci) and sometimes anaerobe

(e.g. Bacteroides fragilis) may become typical surgical site infection isolates *(Parker MT et al 1978)*.

Exogenous flora are primarily aerobes especially Gram positive (Staphylococcus and streptococci). Micro organism shed by humans are the most common airborne agents in a correctly designed operation room with appropriate air filtration. Unclean floors from track dirt and accumulated debris could become an internal source for clostridium sp. or other soil micro-organism if disturbed.

1.2.3 Prevention of surgical site infection (SSI):

Maintenance of strict asepsis is essential if post operative infections and their consequences are to be minimised. Intervention to prevent surgical site infection therefore aimed at reducing or preventing microbial contamination of the patient's tissues or of sterile surgical instruments. Other interventions include preoperative antibiotic prophylaxis, careful surgical technique, adequate ventilation of the OT, etc*(Garner J.S et al 1993)*.Of the variables involved in the equation of SSI, operative characteristics such as preparation of the patients and health care workers skin, appropriate timing of antibiotic prophylaxis, and preparation of operation room is easier to control than patients risk factors such as presence of underlying diabetes, age, smoking and obesity.

1.3 Antimicrobial prophylaxis:

Antimicrobial prophylaxis is not an attempt to sterilize tissues, but a critically timed adjunct used to reduce the microbial burden of intraoperative contamination to a level that cannot overwhelm host defences *(Sanderson PJ et al 1993)*.Prolonged use of antibiotics used in prophylaxis is not appropriate, may mask the diagnosis of postoperative infection, lead to emergence of multiresistant bacteria and can be financially wasteful.

Aseptic technique and prophylactic antibiotics provide the first line of defence, but it has been shown that removing bacteria and fungi from operation room air helps to minimise

infection.

1.4 Microbiological surveillance:

It is usually necessary to study the epidemiology of infection as a multidisciplinary approach. Surveillance is an essential element of an infection control program, provides the data to identify infected patients and determine the site of infection and the factors that contributed to the infection. Surveillance data most useful for decision making, the hospital should focus on their most important and predominant problems and use surveillance methods that adhere to sound epidemiology principles.

In the developed world, the ongoing debate over the appropriate extent and frequency of microbiological surveillance of Operating Theatres (OT) still continues. We, in the "developing world", are either largely ignorant or tend to shy away from the issues and problems that persist in and around the operating theatres. We as a nation are still at a crossroad as to what is the BEST option for us in many aspects, and same is true for healthcare services.

In our areas operation rooms are not constructed according to the standard parameters, as resources are poor, standard protocols are to be designed according to the requirements. The need of the day is to study other models in our vicinity and then adopt the ones which suit our requirements and needs.

Hence this study has been attempted in the hospitals at Tirunelveli for recognising the problems and identifying the right methods of sampling. Also the extent and frequency of microbiological surveillance for instituting effective preventive measures and thereby reducing the mortality and morbidity caused by Hospital acquired infection.

2.AIM AND OBJECTIVES

This study was designed with the following objectives:

- To assess the trend and quality of air maintained between two subsequent fumigations.
- To compare the daily bacterial counts, assess the trend of variation if any and their relationship to various factors.
- To assess the results of different methods (Settle plate, air sampler, surface sampling-peptone water & Robertson cooked meat medium) and to choose the right method according to the requirement.
- To compare the different agents (40% Formaldehyde and 1%Virkon) available for theatre sterilization for their efficiency.
- To assess the air quality maintenance in operation theatres without any special filter and theatres with HEPA filter.
- To correlate surgical site infection rate and microbiological air quality in the operation room.

3.REVIEW OF LITERATURE

There is no hospital however small, airy or well ventilated, where the epidemic ulcer is not to be found at times and thus no operation dared to be performed. Every cure stands still, every wound becomes a sore and every sore is apt to run into gangrene. It has been named the hospital gangrene and such were the ravages at Hotel Dieu of Paris the great ware house of corruption and disease that the surgeons did not dare to call it by its true name. - JOHN BELL (1801) on: Hospital Infection.

3.1 Historical milestones

One of the earliest records of hospital infections are perhaps those found in an Egyptian papyrus written around 3000B.C. Needless to say, mere absence of documentation of bacterial infection does not exclude its prevalence prior to this time. The famous Hindu physician Charaka and surgeon Sushuruta (400 B.C.) have emphasized the need for prevention of infection in clinical practice.

Semmelweiss (1861) observed that puerperal sepsis was associated with morbid matter present on the hands of medical staff and students who performed autopsies, was responsible for spread of the disease. A drastic reduction in infection rates was achieved by the introduction of hand-washing practices with chlorinated lime. Florence Nightingale quoted a remark in her book “Notes on Hospitals”- It may seem a strange principle to enunciate as the very requirement in a Hospital that it should do the sick no harm. The actual mortality in hospitals, especially in those of large crowded cities, is very much higher that any calculation founded on the mortality of the same class of diseases among patients treated out of hospital…’ She established important principles of nursing, hospital design and hygiene.

3.2 Microbiological surveillance of air quality in operation theatres:-

Operating room layouts, operating room etiquette, sterilisation of instruments and

sterile surgical protocols are factors that directly affect the incidence of postoperative infections. Of all the processes, the environmental disinfection and instrument sterilisation probably need the most critical monitoring. How frequently we can do the Surveillance for air borne microbes? Yet there is no definite answer to this question. Doing too frequent surveys are expensive and may not correlate with the existing infection rate in the hospital. But can indicate the circumstances we operate which can have bearing effect if the safety standards fall.

3.3 Different methods of sampling:

Davidson A.I.G. et al 1971 assessed the importance of different bacteriological data aspects of the immediate wound environment during surgery in two different operating theatres (old and new) using Settle-plate method, Bandage plate, Deep tissue swabs, needle cultures and Finger plates method. He exposed blood agar settle plate for the duration of surgery. Both qualitative and quantitative analysis was done. The average colony count per plate was found to increase with the duration of operation. He proved that ward design with improved methods of ventilation can significantly reduce staphylococcal cross-infection. In this study, exogenous contamination was less significant.

Casewell et al 1986 used the Reuter centrifugal air sampler (RCS) to determine a range of 'expected values' for bacterial air counts in nine hospitals at 13 defined locations. Results were recorded as the number of cfu per strip per 4 min. Bacterial air counts were comparable from hospital to hospital; in operating theatres the overall median RCS counts for air-inlets and in empty operating rooms were 13 and 9·8 cfu per strip per 4 min respectively. Increased counts were readily demonstrated during surgical operations. Conversion of RCS counts to cfu m^{-3} of air usually yielded values higher than those established by other methods. He demonstrated that this instrument may replace some of the applications of the slit sampler and facilitate examination of epidemiological problems.

Chakrabarti et al 1992 did a preliminary study of fungal contamination of the environment as well as fungal colonization/invasion of 25 burn care patients to see if there was any correlation between environmental contaminants and patient isolates. He used surface sampling methods and contact plate method. The sterile cotton swab was streaked on to sabourad dextrose agar with Gentamicin and chloramphenicol and using contact plates samples were collected from patient's beds, blankets and dressing materials. The determinants of fungal counts in environmental air were carried out with slit sampler (Micro Med, Dyna micro, India).The sampler contains strips impregnated with sabourauds dextrose agar. The sampler had an intake of 40L/min of air exposed for 1 min at all sites excepting minor operating theatre, ward with restricted entry, dressing room where exposure was 2 min. The mean fungal count was expressed in CFU/16 cm^2.

Friberg B et al 1999 (May) compared airborne contamination with bacteria-carrying particles (cfu/m^3) and their sedimentation rate ($cfu/m^2/h$) in an operating room equipped with two turbulent ventilation systems. He equated that the surface counts were numerically 16-fold the air counts, i.e., the number of colonies sedimenting on four 14 cm-diameter agar plates during 1 hour will almost equal the number of airborne $cfu/m^{3.}$ He proposed that the sedimentation plates represent not only a technically easier method than air sampling but if correctly used, they are the most realistic indicator of airborne bacterial operating room contamination in areas critical for surgery.

Frieberg et al 1999 (Aug) studied the relationship between surface contamination ($cfus/m^2/h$) with particles carrying aerobic bacteria and corresponding air contamination rates ($cfus/m^3$) was evaluated in operating rooms (OR) equipped with ultra clean vertical or horizontal laminar airflow (LAF). Air contamination in the wound and instrument areas (Casella slit sampler) was related to the surface contamination rate (settle plates) in the same areas.

Kelkar et al 2003 studied environmental Bacteria Carrying Particle (BCP) load by sedimentation method and swab technique using Robertson Cooked Meat medium, was assessed weekly following high-level disinfection and prior to commencement of surgery. He reported that samples from the filters of the air-conditioning units yielded potentially pathogenic fungi on 3 (20%) occasions. On 14 (5.07%) occasions the environment in the operation room had significant risk of airborne infections. This was due to presence of unacceptably high bacterial count on 9 (3.26%) occasions and the presence of *Staphylococcus aureus* on 5 (1.81%) occasions as evidenced by examination of the settle plates.

He observed that risk of infection increases with counts in the range of 700-1800 Bacteria Carrying Particle per cubic meter, and decreases when the Bacteria Carrying Particle load is less than 180 per cubic meter. *Clostridia* were isolated only twice out of 276 evaluations.

Wellisch et al 2004 did a study on microbial monitoring in operating theatre evaluated 11 operating theatres using settle plate method and air sampler. Sampling was done before, during and after the surgical activity. They analysed that the microbial contamination increased significantly during activity. About 15.2% of samplings performed at the patient area using Surface air system and 75.8% of samplings performed using settle plates exceeded the threshold values of 180 cfu/m^3 and 25 respectively, with a significant difference of the percentages. The highest values were found in the operating theatre with inadequate structural and managerial conditions. These findings confirm that the microbiological quality of air may be considered a mirror of the hygienic conditions of the operating theatre. He concluded that Settle plates were more sensitive in detecting the increase of microbial air contamination related to conditions.

Operational circular edited by Department of Health, Western Australia Government

studied the method of air sampling .Single sample is collected from each operating room and theatre should be empty for a minimum of 15 min -1 hour. Air sampler should be placed on the middle of the theatre table and the sampling volume has to be greater than 0.25 m^3 (250 L) and optimally around 1 m^3 (1000 L). They suggested to collect air samples after all the new or refurbishment work has been completed, after completion of all engineering commissioning procedures, the ventilation system has been running continuously for 24 hours following completion of structural work (during this time the theatre surfaces and fixed equipment can be cleaned), and ducting and air diffuser plates have been cleaned.

Sri Ramachandra University, Porur, Chennai conducted 3-month pilot study of the indoor air of three different hospitals in Chennai for bioaerosols to generate baseline data using settle plate method. Commonly isolated organisms were *Staphylococci* and *Micrococci* among Gram-positive bacteria, while *Klebsiella* sp. and *Pseudomonas* sp. were among pathogenic Gram-negative bacteria. Among yeasts and molds, *Aspergillus niger* and *A. flavus* were commonly isolated.

Edmiston CE Jr et al 2005 investigated the potential sources of perioperative contamination and analysed air sample cultures from multiple points in operating room ranging from 0.5-4m from the surgical wound. He reported that failure of the traditional mask to prevent microbial shedding is likely to be associated with an increased risk of perioperative contamination of biomedical implants, especially in procedures lasting longer than 90 minutes.

He isolated Coagulase negative staphylococci from 86 % of air samples, 51% from within 0.5 m of the surgical wound, where as Staphylococcus aureus was recovered from 64% of air samples, 39% within 0.5 m from the wound.

Crimi P et al 2006 studied microbial contamination of air of wards in relation to the presence of HEPA filters. Here sampling was performed by air sampling and settle plate

method. He concluded that the wards which were not equipped with a ventilation system show an average bacterial charge 2 to 10 times higher than the other wards with ventilation system. He revealed that the presence of artificial ventilation systems can lower the bacterial and fungal load compared with a ward with natural ventilation.

Kaur et al 2007 studied air bacterial isolates in seven operation theatre in a tertiary care hospital by settle plate method. Blood agar plates were placed on the floors, operation theatre tables and other horizontal surfaces and were exposed for one hour. He reported that settle plate method is a crude measure of airborne contamination but it does provide a simple and cost effective way of enumerating the contamination rate of horizontal surfaces at multiple points.

F.O. Ekhaise et al 2008 studied the quality and quantity of airborne microflora in two major hospitals, the Faith Medical Center and Central Hospital in Benin City. Samples were collected using the settled plate techniques in nutrient agar plate and Potato dextrose agar which were exposed for 30 min. Each day, the air samples were collected three times: between 10am-11am, 12am-2pm and 5pm-6pm. The highest bacterial population was recorded in the evening between time 5pm and 6pm compared to the morning and afternoon ranging from 15cfu/m^3 to 47cfu/m^3 in the Faith Medical Hospital and 17cfu/m^3 to 52cfu/m^3 in the Central Hospital.

He isolated six bacterial and four fungal genera, among sthe bacterial isolates: *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella aerogenes and the fungal isolates include Aspergillus, Penicillum, Mucor and Fusarium*.

3.4 Effectiveness of Virkon:

Virkon is commercial product which claims as a broad spectrum high level disinfectant studied recently in different laboratories. It is based on the active ingredient Oxone, a

peroxygen compound which contains triple salt of potassium peroxymonosulfate, potassium bisulfate and potassium sulfate.

It denatures micro organism proteins and enzymes, increases virus plasmid or bacterial cell wall permeability by disrupting sulphydryl (SH) and sulphur (SS) bonds and causes lysis and exposing the nucleic acid. Virkon is available as a powder and is added to tap water to make a 1% solution.

S.C Tera Tambia et al studied in vitro short exposure test on surfaces contaminated with standard and hospital bacterial strains by Kelsey- Sykes test and phenol test. He tested the effect of Virkon with 1% or 2% solution of disinfectant on artificial contaminated surface. Seven surfaces which were previously cleaned with hot water were artificially contaminated using standard bacterial strains. Gram positive strains of *Streptococccal fecalis, Staphylococcus aureus and Gram negative included Klebsiella pneumonae, Pseudomonas aeruginosa and E.coli* were tested. He proved that 2% solution of Virkon was proven to have a complete disinfecting effect on the contaminated surface no colony growth was noticed in any bacterial strain.

Thirunarayan et al from Apollo hospital chennai challenged Virkon against organisms like *Staphylococcus aureus, Pseudomonas aeroginosa, Kluyvera ascorbata, E.coli, Salmonella typhi, Candida sp, Bacillus sp,* in the hospital and proved that 1% Virkon is effective. He tested this under simulated field conditions wherein,0.5% concentration disinfectant were applied by means of a swab to dried films of Staphylococcus aureus and Pseudomonas aeruginosa in petridishes (approx bacterial density about 2 lac cfu/ml), tested after 30 min and 60 min. The post contact swab did not yield growth on plating.

The viability of organisms was tested by Kelsey maurer in use Test at university medical school and royal infirmary, Edinburgh according to the Kelsey and Maurer protocol. The growth of bacteria was tested after exposure to Virkon disinfectant 0.25, 0.5 and 1% for

2, 5, 10 minutes. The organisms tested were *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptcoccus faecalis, Clostridium perfringens, Bacillus subtilis, E.coli, Klebsiella, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella, S.sonnei, Neisseria*. It recommended that a concentration of 1 % solution for disinfection.

Ayliffe et al 1967 reported that chemical disinfection of an operation room floor is probably unnecessary as bacteria carrying particles already on the floor are unlikely to reach an open wound in sufficient numbers to cause an infection.

Sharma et al 1996 revealed that routine culture testing of specimens from the nasopharynx or other sites of personnel, and from the operation room environment is not considered mandatory. He recommended for microbiologic evaluation of all possible samples, to track down possiblé sources in the event of an infection, and evaluation of cleaning methods to set policies. He concluded that formaldehyde is a high level disinfectant and it's decontaminating value is undeniable and also agreed upon that fogging cannot replace manual cleaning and may in fact cause a false sense of security leading to the abandonment of more effective infection control measures.

3.5 Factors influencing the bacterial contamination:

3.5.1 Number of persons:

C. R. Ford et al 1967 reported that the peak incidence of micro organisms in the air of their operating rooms was between 8:00 a.m and 11:00 a.m and there was a rise of organisms as personnel began activity and a rapid drop in afternoon hours as personnel activity diminished. Air contamination in operating rooms varied between 1.5 and 18.3 micro organisms per cubic foot, depending on the human activity present. He concluded that contamination of operating room air by bacterial organisms fluctuates in direct relationship to human activity in a given operating room.

Mehta et al 2000 reported that the bacterial count in operation theatre is influenced by the number of individuals present, ventilation and air flow.

3.5.2 Type of Ventilation:

Peter G. et al 1976 studied airborne bacteria in a horizontal laminar flow system and in a horizontal laminar flow system with a suction-hood aspiration system was compared with those in a conventional operating room. Sampling was done at the operative wound site using a slit sampler and from various locations in the operating room using settling plates. He reported that the species of bacteria isolated differed notably at the operative site where a slit sampler was used, as compared with the instrument table and the periphery of the room where settling plates were used. He concluded that the numbers of bacteria isolated were reduced by 95 percent in a horizontal laminar flow room.

Clark et al 1976 and Ferrazi et al 1986 concluded that postoperative sternal SSI rates were decreased after the opening of the new operating theatre that incorporated laminar air flow, along with many other preventive measures. They concluded that the operating theater environment may have an important role in the pathogenesis of sternal SSI and that appropriate ventilation of the operating theater could lower the rate of sternal SSI. No details of the patient mix and risk factors were provided in any of these studies.

Lidwell et al 1982 conducted a prospective, randomised-controlled, multi-centre trial, involving sites in both the United Kingdom and Sweden, with a sample number in excess of 8,000 patients undergoing hip or knee replacement surgery. Lidwell showed that the incidence of joint sepsis at reoperation was 50% less in those patients operated on in an ultraclean environment. He further concluded that vertical laminar flow ventilation performed better than horizontal ventilation.

Mehta, Geeta et al 2000 did a multicentric study of hip and knee operations by, in which it was reported that the incidence of sepsis in operations performed under laminar air

flow was significantly lower than in operations performed under conventional ventilation.

Dharan et al 2002 studied that operating theatres having conventional plenum ventilation with filtered air where particles ≥0.5 µm are removed and for orthopaedic and other implant surgery, laminar flow system with HEPA filters removes ≥0.3 µm. He reported that ultra clean air has reduced infection rates significantly in orthopaedic implant surgery. He concluded that measuring the presence of particles of varying size and numbers are better suited than bacterial sampling. He suggested that environmental bacterial sampling in operating theatres should be limited to investigation of epidemics, validation of protocols, or changes made in materials which could influence the microbial content.

Serap et al 2006 analysed the risk factors for surgical site infections mainly emphasizing the appropriate ventilation of the Operating Theatres. He studied two operating theatres the major differences were in the doors of the rooms and in the ventilation systems. His result supported that the doors of the operating theatre should be kept closed except for occasional movement through them, whatever the type of ventilation (i.e., plenum or laminar flow) and this coincides with The Hospital Infection Control Practices Advisory Committee recommends (category IB) 20. He concluded that the type of operating theater was found to be another independent risk factor for sternal SSI.

Knobben et al 2006 evaluated whether behavioral and systemic measures will decrease intra-operative contamination during total hip or knee replacements. Four swabs were taken from instruments at the beginning and end of the procedure for 207 procedures. Initial operations performed under original control conditions were included, after which the better use of the plenum (Group 1) as first behavioral measure was introduced. Group 2 followed disciplinary measures and after installation of a new laminar flow system cultures were taken. He concluded that the combination of systemic and behavioral changes in an operating room significantly decreases the incidence of intra-operative bacterial

contamination, subsequent prolonged wound discharge and superficial surgical site infection.

3.5.3 Bacterial counts:

Whyte et al 1983 suggested that for ultra clean air operating theatres the bioload should be less than 1 cfu in the centre of empty theatre and less than 10cfu during an operation and should not exceed 20 cfu at the periphery.

Arrowsmith WM 1985 recommended that for conventional operating theatres the bioload should not exceed 35cfu in an empty theatre or 180 during an operation.

Senior BW et al 1989 suggested in the surgical Operation room providing facilities for most forms of surgery, the recommended bacterial count of air should not exceed l cfu/ft^3 (35.5/m^3). Air entering the operation theatre from filters should not contain more than 0.5cfu /m^3 of bacteria-containing particles. Furthermore, the bacteria-containing particles of air within 30 cm of the operation site should not exceed 10cfu/m^3, and should not be more than 20cfu /m^3 in the rest of the operation theatre.

Kelkar et al 2003 studied Microbiological evaluation of various parameters in ophthalmic operating rooms. He suggested that the operating rooms were conducive for carrying out operative procedures only when the bacterial load was less than 180cfu per cubic meter. He concluded approximately, 180 cfus/m^3 of air correspond to 10 colonies settling on a plate.

3.5.4 Type of organism:

Jaffal et al 1997 isolated Staphylococcus aureus, Coagulase negative Staphylococci, Micrococcus sp, Alpha hemolytic Streptococci, Diphtheroid, Gram negative bacilli, Bacillus sp.and fungi from male, female, pediatric, female surgical and male surgical wards respectively.

Ajaz et al 2004 did a prospective study of nosocomial wound infection and the micro organisms responsible in post operative period among 150 patients from general surgery at a

teaching hospital of kashmir revealed wound infection rate of 11.3% Staphylococcus aureus followed by Escherichia coli 29.5%.

Lilani SP et al 2005 did a study in surgical site infections among clean and clean contaminated surgical cases. He commonly isolated Staphylococcus aureus and Pseudomonas aeruginosa and in one case E.coli was isolated preoperatively from perineum and rectum of a patient who was infected with same organism.

Endogenous sources:

Endogenous sources to be more important than exogenous sources in determining wound infection, as was similarly concluded de SA LA et al 1984 by Davidson et al 1971a, Shaw et al 1973 and Nichols RL et al 1991.

Exogenous sources:

Shrivastava et al 1969, Rao et al 1975 and Venkatraman et al 1978 found exogenous sources to be more important in the development of wound infection. Airborne bacteria may arise from desquamation of the skin from the operative team or the patient (Doig CM et al 1972, Burke et al 1963, Favero et al 1966) or from fomites and from horizontal surfaces such as the floor. Sources may be affected by the position and clothing of the operating personnel, by movement of personnel; by talking; by the type of air current, air exchange and air filtration in the operating suite (Scott et al 1971,Charnley et al 1972, Doig et al 1972). *Mehta et al 2000* proposed that most surgical wound infections originate from the patient's own microflora and exogenous sources are also implicated.

Ram J et al 2002 and Kelkar et al 2003 reported that factors associated with transmission of infective material exogenously in a hospital are use of unsterile equipment, presence of shedders of pathogenic microorganisms amongst hospital personnel, contaminated environment and contaminated surfaces.

3.5.5 Duration of surgery:

Shrivastava et al 1969, Cruse et al 1973, Davidson et al 1971b, and Rao et al 1975 found that the duration of surgery was an important factor in predicting wound sepsis.

Jepsen et al 1969, Haley et al 1985 and Gottrup et al 2005 proposed that the duration of surgery is one factor that influences the wound infection rate. Procedures that take longer than two hours are associated with higher infection rates.

Burke et al 1963 and Peter et al 1976 suggested that wound infections can be controlled by length of operation.

3.6 Infection Rate:

C. R. Ford et al 1967 concluded after a three-year study period that an overall clean wound infection rate of 3.14%.

Overall rate of wound infection by *DeSaLa* et al 1984 was 18.92%, favourably comparing with the 20% rate reported by *Agarwal* et al 1972, 25% by *Rao et al 1975*, 22.3% by *Venkatraman* et a1 1978, 16.9% by Shaw et al 1973,] and 10.19% by *Shrivastava et al 1969*. *Cruse and Foord et al 1973*, however, in a prospective study of 23,649 surgical wounds reported an infection rate of only 4.8%.

D.A.Leigh et al 1981 studied 8 years in postoperative wound infection among 29,941 patients. Overall incidence of infection was 5·4 per cent and there was a gradual fall over the period of study.

Emmerson et al 1996 did a prevalence survey surgical wound infection accounted for 12.3% of all hospital-acquired infections.

Van Griethuysen J. A. et al 1996 compared postoperative wound infection (PWI) rates before and after moving to the new site. PWI rates in clean general surgery were 28/1909 (1·5%) and 35/1891 (1·9%) before and after moving to the new site.

Mangaram et al 1999 recommends infection control practices that include improved

operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis. Despite these activities, surgical site infections remain a substantial cause of morbidity and mortality among hospitalized patients

At present, there is no international consensus on the methods (settle plate versus volumetric air sampling) frequencies of sampling and tolerable limits of bioburden in operation theatres. The environments in the operation theatre are dynamic and subject to continuous change. It is usually necessary to study the epidemiology of infection as a multidisciplinary approach.

4. MATERIALS AND METHODS

Microbiological air qualities in the operation theatre were an independent important factor in the rate of surgical site infection. Yet there were no standard protocol accepted and followed by all particularly in country like India, where the resources were limited. Protocols have to be formulated which suits our condition. So the present study was conducted at Tirunelveli Medical College Hospital, Palayamkottai and a private multispecialty Hospital at Tirunelveli, TamilNadu from October 2008 to June 2009 to assess the three different Microbiological surveillance methods (Settle plate method, Air sampling method and Surface sampling method) in an operation theatre without any special filters and in modern theatres with HEPA filters.

4.1 Inclusion criteria:

- From Tirunelveli Medical College and Hospital two operation theatres were included.

1. One emergency operation theatre (EOT).
2. Another major operation theatre (MOT) where elective cases were operated.

   Both the theatres were ventilated without any special air filters.

- Two operation theatres, from a private multi specialty Hospital

1. One operation room for routine surgeries (Private OT I).
2. Another operation room for cardiac and orthopaedic surgeries (Private OT II).

   Both the theatres were equipped with HEPA filters and designed with modern facilities.

- The efficacy of 40% Formalin and 1% Virkon (peroxygen compound) were compared in

both conventional and modern theatres using Settle plate method, Air sampling method and Surface sampling method.

- Patients who underwent surgery in these theatres were followed up post operatively for any surgical site infection.

4.2 Exclusion criteria:

- Operation theatre where septic cases were done.
- Other sterilization agents like UV radiation and other chemicals were excluded.

4.3 Methods to collect samples from operation theatre:

For one week, sampling was done daily for 6 days from Monday (Day 1) to Saturday (Day 6) following fumigation with formalin on Day 6 and another one week sampling was done for 6 days by fumigating with Virkon daily. This method was followed for each theatre. Different methods like settle plate, air sampler, surface sampling by swabs using peptone water & Robertson cooked meat medium were analysed. All these methods were compared daily before the first surgery and at the end of the day. Disinfecting agents, 40% formaldehyde and 1%Virkon (balanced and stabilized blend of per oxygen compounds, surfactant, organic acids & an inorganic buffer system - a commercially available product) were analyzed and compared for their efficiency. The relationship between the quality of air in the operating room and the rate of post operative infection was also compared in an attempt to design an effective and economical method to suit the requirements of different operating rooms.

4.3.1 Settle plate method:

In settle plate sampling Petri dishes of 90 mm in diameter with approximate internal area 64 cm^2 containing Sheep blood agar medium were used (*Guidelines on environmental monitoring 3rd Ed. 2002, BW Senior et al 1989).*This detects the growth of pathogens, commensals and saprophytic bacteria. Multiple plates were kept depending upon the size of

the theatre and results were based on overall assessment rather than on a single plate study in the room. Plates were opened and exposed for 30 min, thus allowing microbe-bearing large particles to deposit onto them *(Kelkar et al 2003, CDC guidelines 2003)*.

Blood agar plates were exposed at different areas of operating theatre (washing room, O.T. table, O.T. corner, Doctors room) for 30 min because any minor surgery takes 30 min-1 hr. Plates were incubated at 37˚C for 48 hrs. This procedure was done before commencements of surgery and repeated at the end of the day on all days.

After 48 hr, if there were any growth number of colonies were counted on the colony counter and the type of organism was identified by microscopy and biochemical tests.

Bacteria carrying particle (BCP) load in the environment was determined by a formula. Based on the colony count, area of the plate exposed and the duration of exposure.

Number of BCP on $1m^2$ medium/min = Number of particles /0.3 cubic meter of air.

Area of plate (pr^2) = $78\ cm^2$, i.e., $0.0078\ m^2$

The acceptable upper limit of bacteria is 180 BCP/m^2 = 54 BCP/$0.3m^2$

$1m^2$ medium/min= 54 colonies

$0.0078\ m^2$ /30 minutes = 54 x 30 x 0.0078 =12.6 colonies

Making the acceptable limit more stringent, 10 was fixed instead of 12 as the upper limit for certifying the operation room safe for surgery *(Kelkar et al 2003)*.

4.3.2 Air sampling:

Slit sampler and Air centrifuge equipment for bacterial counts were slowly replacing settle plate *(Scottish quality assurance guidelines 2004, BW Senior et al 1989).* With these equipments the safe level of colony counts can be calculated as per the standards created with peer reviewed studies by the manufacturers.

The sampler employed was *Reuter's centrifugal sampler* (*Manual of Himedia Air sampler system Mark II)*.The Air sampler operates on the impaction principle. The air under

examination was sucked by the impeller in a “tornado- like” spiraling conical form and the particles contained in it are centrifugally impacted against the inward facing peripheral agar medium strip as the spiraling return air escapes around the outer surface of the tornado. The impeller speed of 4100 rpm was so adjusted that 280 litres of air was sampled every minute. The sampling volume corresponds to 40 litres/minute of separation i.e. 1/7 of the sampling volume based on the mathematical computation in the design principle. The separation volume per unit of time forms the basis of calculating the number of organisms per volume of air being sampled. Blood agar strip was used in this method. The viable microbes which grow into colonies in the strip media (Blood agar) was counted and calculated for m^3.

Samples were obtained from different areas of operation theatre (A/C, 6ft height and 1 feet height above the O.T table). The sampling time was 8 min, as recommended by the manufacturer. The exposed Blood agar strips were incubated at 37°C for 48 hrs and counted for colony forming units. The air counts were then compared before surgery and repeated at the end of the session on all days.

Colony count:

The Colony forming units (CFUs) could be visually counted from the strip. The organism detected per unit volume of air can be calculated as CFU/m^3 (*WHO Guidelines 1978, Kelkar et al 2003*)

CFU/m^3 = No. of colonies on agar strip×25/Sampling time (in min)

No. of BCP on 1m^2 medium/min = no. of particles /0.3 cubic meter of air.

According to *WHO publication in guidelines to laboratory methods*, 10 cfu was taken as the upper limit for certifying the operation theatre safe for surgery. The operating rooms were said to be conducive for carrying out operative procedures only when the bacterial load was less than 180 per cubic meter. Approximately 180 bacteria /cubic meter of air = 10 colonies settling on a plate.

If cfu were not discrete (coincidence) entities or were Too Numerous to Count (TNTC - usually greater than 300 cfu per sample), the result were recorded as TNTC. If one type of cfu tends to grow in a spreading manner, this is counted as "one spreading colony" and recorded it as such. All samples (contaminated or not) were disposed of according to biomedical waste management directions *(Guidelines on environmental monitoring 3rd Ed. 2002)*.

The critical value of CFU/ m^3, in case of air sampler was decided considering many factors like sampling area, volume of air, type of organism, type of theatre (number of air exchange/hr, with or without HEPA) meant for CNS, transplant and critical type of surgery etc.

4.3.3 Surface Swab collection:

Surfaces become contaminated in a number of ways e.g. micro organisms settling out from the environment or from the direct touch by an operator. The routine sampling of the inanimate environment particularly surfaces of culture studies does not contribute to the prevention of nosocomial infections or predict when post operative infections were most likely to occur. Despite these issues, latest studies indicate a consistent relationship between surface contamination and air counts, as the settling of bacteria or fungal spores on surfaces inside the operating room, represents their air contamination level over a longer period. One of the objectives of surface sampling was to determine the efficiency of routine cleaning procedures in removing contamination. Therefore, sampling was performed before the first surgery and at the end of the surgery.

The surface swabs were collected from different areas (washing room(WR), operation theatre table(OTT), light, A/C, Boyles' apparatus, drug rack, floor, Wall) using peptone and Robertson Cooked meat medium *(Meyer's and Koshi's CMC Manual 2001, Senior BW et al 1989, Kelkar et al 2003)***.** Peptone water swabs were streaked on Maconkey and Nutrient agar plates. The plates were incubated at 37˚C fo4 hrs and the colonies were subjected to

biochemical testing and to identify the prevalent organism in the operation theatre. Isolate was subjected to antibiotic sensitivity testing.

Robertson cooked meat medium was used after being placed in a water bath of boiling water (80˚C-15 min*) (Meyer's and Koshi's CMC Manual 2001, Forbes B.A et al 1995, Murray P R et al 2003).*The medium was allowed to cool to room temperature. Then inoculation was done and tube was incubated at 37˚C for 72 hours. Cooked meat media were looked for evidence of growth. Smears were made from all the tubes and Gram stain was done. Smears were examined microscopically for organism morphologically suggestive of *Clostridium tetani*. Tubes showing spores were subcultured to blood agar and the plates were incubated in Gaspak Jar at 37˚C for 48 hours along with *Pseudomonas aeruginosa* as a biological control. Culture showing thin swarming growth was subjected to aerotolerence testing. In the absence of detection of bacilli suggestive of *Clostridium tetani* from the anaerobically incubated plates. Cooked meat media were held for 7 days and again smears were made and the procedure was repeated.

4.4 To Test the efficacy of disinfectant (VIRKON):

The efficacy of disinfectant was tested by a simplified bactericidal activity (*Laboratory evaluation by Apollo hospitals, Chennai*).Aliquots of disinfectant solution were prepared by adding 250 micro liters of a 4% and 2%solution of Virkon in distilled water to 750 micro liters of peptone water to obtain a final concentration of 1% and 0.5%.About 200 microlitres of each prepared culture was added to each of the 1%and 0.5% aliquots of disinfectant and incubated. Samples were plated after 10 minutes, 30 minutes and 60 minutes. Aliquots of peptone water and distilled water mixed in the same proportion (750 microlitres and 250 microlitres respectively) were inoculated with cultures to serve as controls. This was tested for microbicidal activity against common isolates *like Staphylococcus aureus, Escherchia coli, Klebsiella pneumoniae, Proteus vulgaris, Acinetobacter sp, Enterobacter sp, Citrobacter*

koseri, Salmonella typhi and candida albicans. ATCC control strains of E.coli 25922, Klebsiella pneumoniae 700603, Staphylococcus aureus 25923, Pseudomonas aeruginosa 27853 & Acinetobacter baumanii 17978 were obtained from CMC, Vellore.

4.5 Follow up of patients*:*

During the period of surveillance of air quality in the operating room, the patients operated in the theatre were followed for signs and symptoms of post operative infection. Appropriate specimen was collected and culture & sensitivity was done. Patients were followed up postoperatively till discharge and observed for signs and symptoms of surgical site infection. Any wound discharge, pus was collected and processed for further analysis.

As bacterial counts of the operation theatre was a suitable index in predicting when post operative infections were likely to occur. So it was decided to monitor the levels of bacterial contamination of air in the operation theatre during the period between fumigations both pre operatively and post operatively with special emphasis on type of surgery, duration of operation, position of patient on operation list, number of persons in operation theatre.

4.6 Statistical analysis:

Two chemicals formalin and Virkon were compared in different operation theatres. By colony counts obtained before and after surgery were calculated by statistics of mean and Standard deviation. They were interpreted by the test of significance. The above statistical analysis and interpretation were performed by the statistical package S.P.S.S (13.0) at 5% level of significance (0.05).

Surveillance data most useful for decision making, the hospital should focus on their most important and predominant problems and use surveillance methods that adhere to sound epidemiology principles. Settle plate method, Air sampling method and Surface sampling method were analyzed to adopt a suitable method in a conventional and modern type of operation theatres using formaldehyde and virkon.

5. RESULTS

Two Operating Theatres in Govt. hospital without any special filters (EOT & Major) and two operating theatres (with HEPA filters) in a private multi specialty hospital were taken for study. All these theatres were assessed for the microbiological air quality maintenance by different methods (Settle plate, air sampler, surface sampling in peptone water & Robertson cooked meat medium). The disinfectants (Formalin & Virkon) used were also compared for their efficiency. This study was an attempt to adopt a simple, practical and cost effective method to suit our conditions. To correlate with surgical site infection the microbes isolated from operation theatre environment were identified to species level by standard microbiological methods *(Forbes BA et al 1998)*.

5.1 Settle plate method:

The preoperative & postoperative colony forming units ranges were compared for formalin and virkon fogging in all theatres with special attention to the immediate environment of surgical site

(Operating table (OTT)) and the results are tabulated in the Table-1.

Formalin Fumigation:

EOT: The preoperative count ranges from 13 to 17 cfu in OTT where as the post operative values ranges from 9 to 35 cfu in OTT.

MOT: The preoperative count ranges from 16 to 23 cfu in OTT and of the post operative values ranges from 20 to 57 cfu in OTT.

PRIVATE OT I: The preoperative count ranges from 1to 9 cfu in OTT & post operative count ranges from 4 to 41 cfu in OTT.

PRIVATE OT II: The preoperative count ranges from 0 to 9 cfu in OTT & post operative count ranges from 9 to 32 cfus in OTT.

The above result reveals that the preoperative and post operative counts in OTT

exceeds the acceptable limit in Govt. hospital where as in private hospital the preoperative counts were within the acceptable limits but the post operative count exceeds the acceptable limit.

Virkon fogging:

EOT: Bacterial count ranges from 2 to 9 cfus in OTT before the surgery where as the counts at the end of the day ranges from

11 to 64 cfus in OTT.

MOT: Bacterial count ranges from 8 to15 cfus in OTT before the surgery where as the counts at the end of the day ranges from

14 to 40 cfus in OTT.

PRIVATE OT I: Bacterial count ranges from 0 to 4 cfus in OTT, before the surgery where as the counts at the end of the day ranges from 2 to 43 cfus in OTT.

PRIVATE OT II: Bacterial count ranges from 0 to 3 cfus in OTT, before the surgery where as the counts at the end of the day ranges from 2 to 42 cfus in OTT.

The above result shows that preoperative counts were within acceptable limit in both Govt. and private hospital. Post operatively the count exceeds the acceptable limit.

5.2 Air sampling method:

The bacterial counts before and after surgery were compared after formalin and virkon fogging in all theatres at 1 feet ht above the operating table and the results are enumerated in the Table -2.

Formalin Fumigation:

EOT: The preoperative count ranges from 84 to 178 cfus/ m^3 at

1 feet ht and the post operative bacterial count ranges from

112 to 170 cfus/ m^3 at 1 feet ht.

MOT: The preoperative count ranges from 71 to156 cfus/ m^3 at 1 feet ht and the post operative bacterial count ranges from 146 to 177 cfus/ m^3 at 1 feet ht.

PRIVATE OT I: The preoperative count ranges from 12 to 43 cfus/ m^3 at 1 feet ht & post operative count ranges from 22 to 75 cfus/ m^3 at 1 feet ht.

PRIVATE OT II: The preoperative counts ranges from 0 to 37 cfus/ m^3 at 1 feet ht & post operative counts ranges from 22 to 65 cfus/ m^3 at 1 feet ht.

The above result explains that preoperative and post operative counts were within acceptable limit in both Govt. and private hospital.

Virkon fogging:

EOT: Bacterial counts ranges from 11 to 37 cfus/ m^3 at 1 feet ht before the surgery and the counts after completion of all surgeries ranges from 59 to 162 cfus/ m^3 at 1 feet ht.

MOT: Bacterial count ranges from 18 to 65 cfus/ m^3 at 1 feet ht before the surgery where as counts after the end of all surgeries ranges from 56 to 146 cfus/ m^3 at 1 feet ht.

PRIVATE OT I: Bacterial count ranges from 0 to 28 cfus/ m^3 at 1 feet ht before the surgery where as counts after the end of all surgeries ranges from 12 to78 cfus/ m^3 at 1 feet ht.

PRIVATE OT II: Bacterial count ranges from 0 to 34 cfus/ m^3 at 1 feet ht before the surgery where as counts after the end of all surgeries ranges from 20 to 91 cfus/ m^3 at1 feet ht.

The above results conclude that bacterial counts before the surgery and after the end of all surgeries were within the acceptable limit in both Govt. and private hospital theatres.

Since operation theatres were fumigated with formalin on Day 6 and Virkon was sprayed on all days in the morning, to get an unbiased data Day 1 was considered very apt for comparison.

SETTLE PLATE: The results of Day 1 after formalin & virkon application are tabulated in Table-3, 4, 5, 6 and Fig-1, 2, 3, 4.

Formalin Fumigation

EOT: The preoperative count was 13 cfus and post operative counts were 18 cfus.

MOT: The preoperative counts were 16 cfus and post operative counts were 57 cfus.

PRIVATE OTI: The preoperative counts were 1 cfus and post operative counts were 4 cfus.

PRIVATE OTII: The Preoperative counts were 0 cfus and post operative counts were 14 cfus.

The above result reveals that the preoperative and post operative counts in OTT exceeds the acceptable limit in Govt. hospital where as in private hospital the preoperative counts were within the acceptable limits but the post operative count varies from well below the acceptable level to just above the critical level.

Virkon fogging:

EOT: Bacterial Counts preoperatively and postoperatively were 7 cfus and 11 cfus.

MOT: Bacterial Counts preoperatively and postoperatively were 10 cfus and 14 cfus.

PRIVATE OTI: Bacterial Counts preoperatively and postoperatively were 0 cfus and 2 cfus.

PRIVATE OTII: Bacterial Counts preoperatively and postoperatively were 0 cfus and 9 cfus.

The above result shows that preoperative counts were within acceptable limit in both Govt. and private hospital. Post operatively the counts were just around the acceptable limit.

AIR SAMPLING: The results are posted in the Table 7, 8, 9 and 10 & Fig 5, 6, 7 and 8.

Formalin:

EOT: Bacterial Counts preoperatively and postoperatively were 84 and 112 cfus/m^3.

MOT: Bacterial Counts preoperatively and postoperatively were 71 and 162 cfus/m^3.

PRIVATEOT I: Bacterial Counts preoperatively and postoperatively were 12 and 22

cfus/m^3.

PRIVATEOT II: Bacterial Counts preoperatively and postoperatively were 0 and 22 cfus/m^3.

The above result concludes that bacterial counts before the surgery and after the end of all surgeries were within the acceptable limit in both Govt. and private hospital theatres.

Virkon fogging:

EOT: Bacterial Counts preoperatively and postoperatively were 28 and 137 cfus/m^3.

MOT: Bacterial Counts preoperatively and postoperatively were 18 and 93 cfus/m^3.

PRIVATE OTI: Bacterial Counts preoperatively and postoperatively were 0 and 22 cfus/m^3.

PRIVATE OTII: Bacterial Counts preoperatively and postoperatively were 0 and 20 cfus/m^3.

The above result shows that bacterial counts before the surgery and after the end of all surgeries were within the acceptable limit in both Govt. and private hospital theatres.

5.3 Organism isolated in operating rooms:

5.3.1 SETTLE PLATE METHOD:

Formalin Fumigation:

EOT and MOT Organism isolated in operating table (OTT), operation theatre corner (OTC) and Doctors room (DR) were *Acinetobacter baumanii, Micrococci, Methicillin sensitive Staphylococcus aureus*. In addition to these *Klebsiella pneumoniae and Pseudomonas aeruginosa* were isolated from washing room (WR). *Coagulase negative Staphylococcus*

(CONS) was also isolated in MOT.

Antibiogram:

Acinetobacter baumanii isolated were sensitive to Amikacin and Imipenem and intermediate sensitive to ciprofloxacin & ceftazidime, resistant to piperacillin-tazobactum and third generation Cephalosporins.

Pseudomonas aeruginosa was sensitive to Gentamicin, Amikacin, ceftazidime and Imipenem and resistant to piperacillin-tazobactum and third generation Cephalosporins.

Klebsiella pneumoniae was sensitive to Amikacin, Amoxclav, Cefoxitin and resistant to third generation cephalosporin and ciproflox. It was found to be ESBL positive.

Staphylococcus aureus isolated were sensitive to cloxacillin, Amikacin, Vancomycin and cefoxitin and resistant to cephalexin, erythromycin.

PRIVATE OT I: *Aerobic spore forming bacilli and Micrococci* were isolated in OTT & WR.

PRIVATE OT II: No growth was obtained from OTT. Aerobic spore forming bacilli & Micrococci were isolated in WR.

It was observed that Acinetobacter baumanii, Pseudomonas aeruginosa which were resistant to third generation cephalosporins and ESBL producing Klebsiella pneumoniae were isolated in Govt. hospital where as in private hospital only aerobic spore forming bacilli and Micrococci were isolated.

Virkon Fogging*:*

EOT and MOT: From OTT, OTC, WR, DR-*Micrococci and Aerobic spore forming bacilli* were detected. In addition *Methicillin sensitive Staphylococcus aureus* (MSSA) were isolated from OTC and DR.

Antibiogram:

Staphylococcus aureus isolated were all sensitive to Amikacin, cloxacillin, Vancomycin

and cefoxitin and resistant to cephalexin, erythromycin.

PRIVATE OT I: *Aerobic spore forming bacilli and Micrococci* were isolated in OTT & WR.

PRIVATE OT II: No growth was obtained from OTT. *Aerobic spore forming bacilli & Micrococci* were isolated in WR.

It was found that Micrococci, Aerobic spore forming bacilli and MSSA were isolated from Govt hospital where as in private hospital Micrococci, Aerobic spore forming bacilli were detected.

5.3.2 AIR SAMPLING METHOD:

Formalin Fumigation:

EOT, MOT: Acinetobacter baumanii, Micrococci, Staphylococcus aureus, CONS were isolated from OTT and A/C.

Antibiogram:

Acinetobacter baumanii were sensitive to Amikacin and Imipenem, intermediate sensitive to ceftazidime and resistant to piperacillin-tazobactum and third generation Cephalosporins.

*Staphylococcus aureus i*solated were all sensitive to Cloxacillin, Amikacin, Vancomycin and cefoxitin and resistant to cephalexin, erythromycin.

CONS was sensitive to Amikacin, Vancomycin, cefoxitin, intermediate sensitive to cloxacillin and resistant to erythromycin, ampicillin.

PRIVATE OT I and II: *Staphylococcus aureus, Aerobic spore forming bacilli, Micrococci* were isolated from OTT and A/C.

Antibiogram:

Staphylococcus aureus isolated were all sensitive to Vancomycin, cefoxitin & intermediate sensitive to Amikacin, cloxacillin and resistant to ciprofloxacin, cephalexin.

It was inferred that same pattern of organisms were isolated in settle plate and air sampler in Govt. hospital following formalin fumigation. In private hospital, the organisms isolated in settle plate were also isolated in air sampler in addition Staphylococcus aureus also detected by air sampler.

Virkon Fogging:

EOT and MOT: Micrococci, aerobic spore forming bacilli, Staphylococcus aureus were detected in OTT and A/C. In addition to this Enterobacter aerogenes, Citrobacter koseri were also detected in EOT.

Antibiogram:

Enterobacter was sensitive to Amikacin, third generation cephalosporins, Ciprofloxacin, resistant to Gentamicin and Ampicillin.

Citrobacter was sensitive to Amikacin, cefotaxime, Ciprofloxacin, resistant to Gentamicin and Ampicillin.

Staphylococcus aureus isolated were all sensitive to Amikacin , Vancomycin and cefoxitin, intermediate sensitive to Cloxacillin, and resistant to cephalexin, erythromycin.

PRIVATE OT 1 and II: No growth was observed in OTT. *Aerobic spore forming bacilli, Micrococci* isolated from A/C.

The above results reveal that in Govt. hospital same pattern of organisms were isolated in OTT by both settle plate and air sampler. In private hospital no growth was observed in OTT by both settle plate and air sampler.

5.3.3 SURFACE SAMPLING METHOD:

The organism isolated from peptone water & Robertson cooked meat medium are

enumerated in the Table -11, 12, 13, 14.

Peptone Water:

Formalin Fumigation:

EOT AND MOT:

Common isolates were *Acinetobacter sp., Klebsiella pneumoniae, Pseudomonas aeruginosa, CONS, Micrococci & Aerobic spore forming bacilli* from different sites. (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, walls, WR)

PRIVATE OT I and II:

Common isolates were *Pseudomonas aeruginosa, Acinetobacter baumanii, Micrococci and aerobic spore forming bacilli* from different areas. (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, wall, WR).

In Govt. hospital, same types of organisms were isolated by all the three methods in the operating rooms. In private hospital, same organisms were detected by all the three methods and additionally Acinetobacter was isolated by surface sampling.

Virkon Fogging:

EOT AND MOT:

Micrococci and Aerobic spore forming bacilli were isolated from different sites. (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, wall, WR).

PRIVATE OT I and II:

Micrococci and Aerobic spore forming bacilli were isolated from different sites. (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, wall, WR).

The above result shows that same types of organisms were isolated by all the three

methods in both Govt. and Private hospital. Methicillin sensitive Staphylococcus aureus was detected by settle plate and air sampler but not in surface sampling.

Robertson Cooked Meat Medium:

Formalin:

Gram stain:

EOT and MOT: Different areas (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, wall, WR) were negative for Clostridium tetani. Spores were detected

Anaerobic culture**:** No growth was observed.

PRIVATE OT I and II: Different areas (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, wall, WR) were negative for Clostridium tetani and other spores.

Anaerobic culture**:** No growth was observed.

Virkon fogging:

EOT, MOT: Different areas (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, wall, WR) were negative for Clostridium tetani and other spores.

Anaerobic culture**:** No growth was observed.

PRIVATE OT I and II: Different areas (OTT, Boyle's apparatus, Lamp, Drug rack, Floor, wall, WR) were negative for Clostridium tetani and other spores.

Anaerobic culture**:** No growth was observed.

The above result explains that surface sampling by Robertson cooked meat medium was negative for clostridium tetani in both Govt. and private hospital. Spores were detected by Gram stain in Govt. hospital but not in private hospital.

5.4 Effectiveness of formalin & Virkon by settle plate and air sampling method: (Table-15)

Statistically, the effectiveness of formalin and virkon by settle plate method were assessed from operating table colony counts in different theatres. The mean air counts by

formalin were 7.5 ± 8.2 and by virkon were 4.25 ± 5.05. The difference between them was not statistically significant (P<0.05).

By air sampling method, effectiveness of formalin and virkon assessed at 1 feet in all the theatres were 41.75 ± 41.9 & 11.5 ± 13.8. The mean difference between them was not statistically significant (P<0.05). *Both disinfectants were equally effective in all the theatres.*

5.5 Surgical Site Infection:

The Surgical Site infection rates after formalin & virkon fogging are posted in the Table-16, Fig (9, 10). Of the total 81 cases operated in Govt. hospital, 11 developed surgical site infection and the overall infection rate was 13.6%. In EOT, after fumigating with formalin and Virkon the infection rate was observed as 25% and 17.4% respectively. In MOT, the infection rate was 6.7% after formalin fumigation where as 4.3% with virkon. The infection rate were nil in the private operation theatres.

During the study period, total of 17.1 % & 10.9 % of infections were reported among the formalin & virkon fumigation respectively. The percentage difference was 6.2%, the same was not statistically significant (P>0.05).

The above results conclude that in Govt. hospital the infection rate was higher in EOT as compared to MOT and after virkon fogging, the infection rate was less with respect to formalin. In private OT I and II, there was no surgical site infection. Statistically with respect to infection, the effectiveness of disinfection by both chemicals was same.

5.5.1 Factors influencing the SSI:

The factors which affect the surgical site infection are tabulated in Table-17.

EOT:

Formalin Fumigation:

Of the total, 4 dirty wounds 3 were infective. (75%)

Of the total, 3 contaminated wounds 2 were infective (66.7%).

Virkon Fogging:

Of the total, 6 contaminated wound there was

1 infective case (16.7%).

Of the total, 4 clean contaminated wound,

3 infective cases (75%).

MOT:

Formalin Fumigation:

There was one case which was infected in contaminated

wound (100%)

Virkon Fogging:

There was 1 infective case among total 6 contaminated

wounds (33.3%).

It was inferred that infection occurred in contaminated, clean contaminated, dirty cases. None of the clean cases were infected. It was also observed that duration of surgery in most cases were more than 60 min.

5.5.2 Correlation of daily bacterial counts with post operative infection:

Average of pre and post operative bacterial counts from the operation theatre table in settle plate method and 1 foot over the theatre table in air sampler method were considered. The bacterial counts were correlated with the number of infective cases.

Settle plate method:

The results are tabulated in Table 18, 19, 20, 21.

Formalin:

EOT: The lowest bacterial count was 12 cfus and highest was 25 cfus. There were nil infective cases in both the limits.

MOT: The lowest bacterial count was 22 cfus and highest was 37 cfus. There was one

infective case with highest limit.

PRIVATE OTI: The lowest bacterial count was 3 cfus and highest was 25 cfus. There were no infective cases in both limits.

PRIVATE OTII: The lowest bacterial count was 7 cfus and highest was 19 cfus. There were no infective cases in both limits.

The above result reveals that infection rate was nil even in counts above the acceptable limit. Further it shows that bacterial counts did not correlate with the surgical site infection in both Govt. and private hospital theatres.

Virkon fogging:

The results are tabulated in Table- 22, 23, 24, 25.

EOT: The lowest bacterial count was 9 cfus and highest was 34 cfus. There were two infective cases with highest limit.

MOT: The lowest bacterial count was 12 cfus and highest was 24 cfus. There were no infective cases in both limits.

PRIVATE OTI: The lowest bacterial count was 2 cfus and highest was 13 cfus. There were no infective cases in both limits.

PRIVATE OTII: The lowest bacterial count was 2 cfus and highest was 22 cfus. There were no infective cases in both limits.

The result shows that infection rate was nil even in counts above the acceptable limit. Further it reveals that bacterial counts did not correlate with the surgical site infection in both Govt. and private hospital theatres.

Air Sampling:

The results are tabulated in Table 18, 19, 20, 21.

Formalin:

EOT: The lowest bacterial count was 98 cfus and highest was 192 cfus. There were 2

infective cases in lowest limit and one infective case with highest limit.

MOT: The lowest bacterial count was 128 cfus and highest was 164 cfus. There was one infective case with lowest limit.

PRIVATE OTI: The lowest bacterial count was 17 cfus and highest was 55 cfus. There were no infective cases in both limits.

PRIVATE OTII: The lowest bacterial count was 11 cfus and highest was 51 cfus. There were no infective cases in both limits.

The result shows that infections were present even in counts within the acceptable limit. Further it reveals that bacterial counts did not correlate with the surgical site infection in both Govt. and private hospital theatres.

Virkon fogging:

The results are tabulated in Table- 22, 23, 24, 25.

EOT: The lowest bacterial count was 50 cfus and highest was 97 cfus. There was one infective case in lowest limit.

MOT: The lowest bacterial count was 42 cfus and highest was 90 cfus. There was no infective case in both limits.

PRIVATE OTI: The lowest bacterial count was 11cfus and highest was 50 cfus. There was no infective case in both limits.

PRIVATE OTII: The lowest bacterial count was 10 cfus and highest was 55 cfus. There was no infective case in both limits.

The result shows that infections were present even in counts within the acceptable limit. Further it reveals that bacterial counts did not correlate with the surgical site infection in both Govt. and private hospital theatres.

5.6 Patients Follow Up:

Patients were followed up till discharge and looked for any signs and symptoms of infection. Among the total 11 infective cases, organism was isolated from 8 cases (72.7%). Organisms such as *E.coli* were isolated from 6 cases (54.5%), *Klebsiella pneumonia* from 1 case (9.1%) & *Staphylococcus aureus* from 1 case (9.1%). Three pus cutures showed no growth aerobically (27.3%). (Table-26, Fig-11)

E.coli was sensitive to ciprofloxacin, Amikacin, intermediate sensitive to ceftriaxone, ceftazidime and resistant ampicillin, Gentamicin.

Klebsiella peumoniae isolated from wound swabs of patients were sensitive to Amikacin, Amoxclav and cefoxitin, intermediate sensitive to ciproflox, Gentamicin and were resistant to third generation cephalosporins. Klebsiella pneumonia isolated was an ESBL,

Staphylococcus aureus was sensitive to cloxacillin, amikacin, Vancomycin, cefoxitin and amikacin and intermediate sensitive to ciproflox. It was *Methicillin sensitive Staphylococcus aureus*.

5.7 Results by testing the efficacy of disinfectant (VIRKON):

Virkon is tested for bactericidal activity against common isolates like *Staphylococcus aureus, Escherchia coli, Klebsiella pneumoniae, Proteus vulgaris, Acinetobacter baumanii, Enterobacter aerogenes, Citrobacter koseri, Salmonella typhi, Pseudomonas aeruginosa* and the results are posted in Table-27. It was observed that the microbicidal activity of Proteus vulgaris, Acinetobacter baumanii was seen in 30 min and 60 min at 0.5% but its action was good at 1% in 10 min, other organisms like *Staphylococcus aureus, Escherchia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Citrobacter koseri, Salmonella typhi, Pseudomonas aeruginosa* microbicidal activity was seen in 10 min in both 1% and 0.5%. There was growth in all the control cultures at 1% and 0.5% concentration.

It was inferred from that the bactericidal activity of 1% concentration of virkon was effective at 10 minutes against the commonly isolated organisms.

6. DISCUSSION

Operating room layouts, operating room etiquette, sterilisation of instruments and sterile surgical protocols are factors that directly affect the incidence of postoperative infections. Of all the processes, the environmental disinfection and instrument sterilisation probably need the most critical monitoring. How frequently we can do the surveillance for air borne microbes? Yet there is no definite answer to this question. Doing too frequent surveys are expensive and may not correlate with the existing infection rate in the hospital. But can indicate the circumstances we operate which can have bearing effect if the safety standards fall.

In this study the microbial contamination of air was assessed with various parameters, different methods of surveillance, types of ventilation, disinfectants and was correlated with surgical site infections at Tirunelveli Medical College and a Private hospital, Tirunelveli, in an attempt to make a theatre protocol to be followed in our set up.

As mentioned earlier to compare formalin (applied on day 6 only) and virkon (applied on all days) without any bias the values of day 1 was taken into consideration.

6.1 Settle plate method:

Formalin fumigation:

In the Govt. hospital the preoperative values in operating table of EOT & MOT were 13 cfus & 16 cfus respectively and post operative counts were 18 cfus and 57 cfus whereas in private OT I and II the preoperative values were 1 cfus & 0 cfus and the post operative counts were 4 cfus and 14 cfus.

Virkon Fogging:

In Govt. hospital, the preoperative values on the operating tables of EOT, MOT were 7 & 10 cfus and the post operative counts were 11 & 14 cfus respectively. In private hospital the

preoperative counts were 0 cfus in both OTs and post operative values were 2 cfus & 9 cfus.

By the settle plate method the preoperative colony forming units over the OTT in both Govt. theatres were above the acceptable limits where as in private operation theatre, the preoperative counts were within acceptable limits. This may be due to the presence of HEPA filters and strict adherence to theatre protocol in private hospital. This finding correlates with *Peter G. et al 1976 and Crimi P et al 2006* who found that by settle plate method, bacterial counts were reduced in laminar flow theatres as compared with conventional air flow.

The post operative cfus were higher than the preoperative values in both Govt. and private operating theatres after formalin fumigation as well as virkon fogging. The findings of *C. R. Ford et al 1967, Casewell et al 1986, Mehta et al 2000, Wellisch et al 2004 and F .O. Ekhaise et al 2008* that human activity significantly increases the microbial contamination and reaches the peak at the end of the day in operation theatres was established in this study also.

6.2 Air sampling method:

Formalin fumigation:

The cfus/m^3 over the operating tables before surgery in EOT & MOT of Govt. hospital was 84 & 71 respectively whereas the post operative counts were 112 & 162 cfus/m^3. In case of private hospital, the pre operative values were 12 & 0 cfus /m^3 respectively for OT I & OT II and the post operative counts in both OTs were each 22cfus/m^3.

Virkon Fogging:

The preoperative counts over operative tables in EOT, MOT of Govt. hospitals were 28 & 18 cfus/m^3 respectively and the post operative counts were 137 & 93 cfus/m^3 Govt. whereas in private OTs I & II the cfus were 0 cfu/m^3 over theatre tables and the post operative

values were 20 & 22 cfus/m^3 in both theatres.

The acceptable limit of cfus in settle plate method is 10 cfus/m^2 and air sampler is 180 cfus /m^3 (WHO Guidelines 1978, Kelkar et al 2003). By air sampling method, at 1 feet ht from the operating tables in both Govt. and private hospitals, the pre operative as well post operative cfus/m^3 were within acceptable limits. Mehta et al 2000, Kelkar et al 2003 and Wellisch et al 2004 applied these WHO guidelines in their studies. In private hospital the bacterial counts were not as much as of Govt. hospital operating theatres. This may be attributed to the laminar air flow arrangement made in private hospital operating theatres as established by *Peter G et al 1976 and Crimi P et al 2006* studies by air sampling methods.

In settle plate method the cfus were above the acceptable limits whereas in air sampling the colony counts were within acceptable limits in all operating theatres after the application of either formalin or virkon. There was a big difference between the number of cfus in air sampler and settle plate method. This is explained by the fact that the air sampler detects all the bacteria carrying particles (BCP) which were suspended in the air and also the cfu/m^3 was derived from this value by applying a formula where as in settle plate the number of cfus represents only the heavy particles carrying bacteria which settled over the medium.

The average preoperative cfus by settle plate as well air sampling method after virkon fogging were less when compared to the same after formalin fumigation. This may be attributed to the application of virkon on every day where as formalin fumigation was done on only one day (Day 6)

Taken the post operative values by both the settle plate & air sampling methods with either formalin or virkon application the number of cfu was increased. This was explained by the theatre activities. The high number of cfu after virkon usage when compared with formalin fumigation may be attributed to the differences in other factors like type of surgeries, human activities which were variable.

6.3 Organism isolated after formalin & virkon application:

Peptone water:

In the Govt. hospital operation theatre complex, organisms like *Acinetobacter baumanii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Aerobic Spore forming Bacilli & Micrococci* were isolated by all the three methods. In private hospital, organisms like *Acinetobacter baumanii, Pseudomonas aeruginosa, Aerobic Spore forming bacilli, Micrococci* were detected by all the three methods. After virkon application also organisms like *Methicillin sensitive Staphylococcus aureus, Aerobic Spore forming bacilli & Micrococci* were isolated by all the three methods in both Govt. and Private hospital. The types of organisms isolated were decided by different variable factors like the immediate environment, endogenous flora of the patients and the team inside the theatre. So the above results were attributable to those variable factors.

Anaerobic culture:-

Robertson cooked meat medium was negative for clostridium tetani in both Govt. and private hospital. Spores were detected in direct Gram stain in Govt. hospital but not in private hospital. Further in anaerobic culture there was no growth. *Kelkar et al 2003* did surveillance of operation theatre where he isolated *Clostridia* only twice out of 276 evaluations and concluded that this could be a tool for good sentinel surveillance.

The utility of the bacteriological assessment of the surfaces of the operation theatre for the presence of *Clostridia* is questionable. The age old traditions of detection of anaerobic spores of clostridium tetani are losing ground with onset of more awareness on theatre sterilization. Routine testing for the anaerobes may not be essential except when there were suspected cases of Tetanus or Gas gangrene attributed to operating in a particular operation theatre. But it may be ideal to survey the operation theatres for anaerobes when newly constructed or any remodelling or structural alterations were done. In such situations which

will have trust worthy safety of the theatre.

6.4 Efficiency of disinfectants:

To compare the efficiency of formalin and virkon as disinfectants, the pre operative values by settle plate and air sampling methods on day one were taken to circumvent the influence of other factors. The results proved the efficiency of both the agents were within or around the acceptable limits in all type of theatres. In vitro testing of virkon revealed that 1% solution was effective against most of the pathogens and the results are tabulated in Table 27. Statistically, also it was proved that both disinfectants were equally effective in different operating theatres (Table-15).

As suggested by *Sharma et al 1996*, formaldehyde is a high level disinfectant and its decontaminating value is undeniable but it was also agreed upon that fogging cannot replace manual cleaning and may in fact cause a false sense of security leading to the abandonment of more effective infection control measures. *Ayliffe et al 1967* reported that chemical disinfection of an operation room floor is probably unnecessary as bacteria carrying particles already on the floor are unlikely to reach an open wound in sufficient numbers to cause an infection. So proper cleaning and maintenance of strict theatre protocol are more important. More over formalin is irritating, corrosive and toxic chemical. It has the potential to cause cancers of the lung, nasopharynx and oropharynx, and nasal passages (*Guidelines for formalin -1910.1048 AppA.)*. Most hospitals in the developed countries do not use formalin fogging for any purpose because of its toxic (carcinogenic) nature and the fact that other methods of cleaning and disinfection can be as good if not better. So the controversy whether formalin fogging can be given up in Indian situation may be concluded to make a protocol to use any alternative high level disinfectant, the efficiency of which was proved beyond doubt, in the place of formalin.

6.5 Infection rate:

In Govt. operation theatres where there were no HEPA filters, overall infection rate was 13.6% and comparable with other reported rates by De Sa La et al 1984-18.92%, 20% rate reported by Agarwal et al 1972, 25% by Rao et al 1975, 22.3% by Venkatraman et a1 1978, 16.9% by Shaw et al 1973 and 10.19% by Shrivastava et al 1969 and only 4.8% by Cruse and Foord 1973. In Govt. OT, the infection rate after formalin and virkon fumigation was 17.1% and 10.9% respectively. In EOT & MOT, the infection rate was high after formalin as compared to virkon application. This difference was not statistically significant (P >0.05) & the results were enumerated in the Table 16. One of the limitations was follow up of all the patients was not possible till 30 days.

In this study, post operative surgical site infection rate was nil in private hospital where the operating theatres were equipped with HEPA filters. *Clark et al 1976, Ferrazi et al 1986 & Mehta et al 2000* also observed that in operation theatre with HEPA filters there is less infection. *Dharan et al 2002* reported that ultra clean air has reduced infection rates significantly. *Serap et al 2006* emphasized that the appropriate ventilation of the operating theatres were an independent risk factor for sternal SSI. *Knobben et al 2006* revealed that laminar flow system will decrease intra-operative contamination during total hip or knee replacements.

Among surgical site infection, 54.5% of *E.coli* and 9.1% of *Klebsiella pneumoniae and Staphylococcus aureus* was isolated and 27.3% of pus cultures were negative for growth aerobically. These organisms could be endogenous or exogenous. Lilani SP et al 2005 found *Staphylococcus aureus and Pseudomonas aeruginosa* were commonly isolated and in one case *E.coli* was isolated preoperatively from perineum and rectum of a patient who was infected with same organism. Nichols RL et al 1991 also found that the normal endogenous microflora of the surgically resected organ were the most frequently isolated pathogens.

Shrivastava et al 1969, Rao et al 1975 and Venkatraman et al 1978 found exogenous sources to be more important in the development of wound infection. *Doig CM et al 1972, Burke et al 1963, Favero et al 1966* suggested that source could be either operating team or patient. *Mehta et al 2000* proposed that most surgical wound infections originate from the patient's own microflora and exogenous sources are also implicated. *De SA LA et al 1984 by Davidson et al 1971a and Shaw et al 1973* found that endogenous sources to be more important. Screening of patients and health care personnel's for presence of Staphylococcus aureus was not assessed during the study period. Periodic screening and treatment of shedders would ensure that the risk of transmission of infections can be minimised.

This study revealed that wound infection rate is influenced by duration of surgery. *Burke et al 1963* and Peter et al suggested that wound infections can be controlled by length of operation. *Cruse et al 1973, Davidson et al 1971b, Rao et al 1975 and Shrivastava et al 1969* also found that increase in duration of surgery, the rate of infection increased in direct proportion. It was noted in the study that procedures that take longer than one hour was associated with infection. *Haley et al 1985, Jepsen et al 1969 and Gottrup et al 2005* proposed that procedures that take longer than two hours are associated with higher infection rates.

The environments in the operation theatre are dynamic and subject to continuous change. Good infrastructures do not mean a safe environment as human make a greater difference in making the environment unsafe. The present study was carried out for few weeks in operation theatres in the presence and absence of HEPA filters and there is need to conduct similar prospective studies of sufficient duration before a set of national guidelines that can be established.

This study proposes settle plate method can be an alternative to check bacteriological contamination in a hospital where air sampler cannot be afforded. Though settle plates are insensitive unless a long exposure period were adopted in order to detect the low levels of airborne micro organism. This study also emphasises the use of special air flow arrangement in operation theatres.

7. SUMMARY

- Study period October 2008 – June 2009
- Two conventional operation theatres with no special arrangement for air flow and another two with laminar air flow facilities were analysed for bacterial air quality with three different methods (Settle plate method, Air sampling method and Surface sampling method).
- Sampling was done every day in each operation theatre after formaldehyde fumigation for a period of one week which is followed by taking samples for a week after virkon application.
- Bacterial colony forming units were analysed by the settle plate and air sampler method on all days before the start of surgeries and after the end of all surgeries.
- Surface contamination was assessed by taking swabs in peptone water and Robertson cooked meat medium.
- The post operative bacterial counts were more than the preoperative counts in all the theatres by settle plate and as well as air sampling irrespective of the disinfectant (whether 1% virkon or 40% formaldehyde)
- The number of colony forming units in air sampler and settle plate varies much because in the former it indicates the total number of suspended bacteria carrying particles derived by a formula where as in settle plate the large bacteria carrying particles settling were directly enumerated.
- The bacterial colony forming units in Govt. operation theatres without any special filter for air flow were higher than the private operating theatres with HEPA filters.

- Surface sampling was a qualitative analysis to correlate the organisms obtained by surface contamination with air sampler and settle plate method.
- Organisms isolated by aerobic culture were *Acinetobacter baumanii, Methicillin sensitive Staphylococcus aureus, ESBL producing Klebsiella pneumonia, Pseudomonas aeruginosa and Micrococci*.
- Robertson cooked meat medium was negative for *clostridium tetani*.
- The overall surgical site infection rate was 13.6% in patients operated in OT without any special air flow arrangement.
- The infection rate was 0 % in patients operated in theatres equipped with laminar air flow.
- E.coli was isolated from 6 cases (54.5%), Klebsiella pneumonia from 1 case (9.1%) & Staphylococcus aureus from 1 case (9.1%). Three pus cultures were negative for any growth (27.3%).
- 1% Virkon was challenged against common isolates *like Staphylococcus aureus, Escherchia coli, Klebsiella pneumoniae, Proteus vulgaris, Acinetobacter baumanii, Enterobacter aerogenes, Citrobacter koseri, Salmonella typhi and candida albicans tested* for its efficiency as disinfectant and proved to be effective within 10 minute.

8. CONCLUSION

1. The maintenance of quality of air in the operating theatre equipped with special arrangement for air flow (plenum- positive pressure, horizontal or vertical laminar flow) was better than the counterparts with no special air filters.
2. Fogging cannot replace manual cleaning and may in fact cause a false sense of security leading to the abandonment of more effective infection control measures.
3. Use of formalin, an irritant, toxic, corrosive, and carcinogenic chemical should be discarded and any other agent or method like virkon with proven disinfectant efficiency may be used.
4. Routine testing for the anaerobes may not be essential except when there were suspected cases of Tetanus or Gas gangrene or whenever any construction or structural alterations were done.
5. The overall surgical site infection rate was 13.6% in patients operated in theatres without any special air flow arrangement.
6. The infection rate was 0 % in patients operated in theatres equipped with laminar air flow.
7. Virkon's bactericidal activity of 1% concentration was effective at 10 minutes against the commonly isolated organisms.
8. Using air sampler, requires trained personnel's apart from its cost which may inhibit its use in small centers. Proper cleaning and maintenance of strict theatre protocol are more important. which doesn't require technical expertise and a cost effective approach.

9. SUGGESTIONS

GENERAL INSTRUCTIONS

- Entry into the operation theatre should be restricted to authorized persons and to be kept within the minimal required number only. (Label must be pasted on the main door).
- Theatre attire (includes head cap, mask, apron, chappel etc.) should be made available for all persons who are entering into the operation theatre (surgeons, anesthetist, microbiologist team, nurses, theatre assistants & helper).
- All apparatus such as Boyle's, suction, table, cautery, focus lights, A/C units, should be cleaned completely as per the manufacturer instructions.

At the Beginning of the Day

Remove dust with cloth wetted with clean water.

Between the procedures

Clean operation tables or contaminated surfaces with disinfectant solutions. Do not discard soiled linen and gowns in the operation theatre floor.

At the end of the day

Clean all the table tops, sinks, door handles and floor with detergent followed by low level disinfectant. Use vacuum cleaners instead of brooming the floor.

Cleaning alone followed by drying will considerably reduce bacterial population.

Considering the toxic nature and inhalation hazard, formalin fumigation may be replaced with any other safe agent with proven disinfectant efficiency

Cleaning of operation theatre is the foremost thing followed by using suitable disinfectant.

Monitoring of operation theatre for Bacteria carrying particle (BCP):

This can be done weekly in case of theatres which don't have an air handling unit with

adequate filters & physical parameters were not strictly adhered. Bacteria carrying load should be less than 180 cfu/m3 or 10 colonies when done by sedimentation method using 10 cm plate. Settle plate is a suitable, simple & cost effective method.

Assessment of surface sampling for presence of Clostridium spores:

Suggested when there are suspected cases of Tetanus or Gas gangrene attributed to operating in a particular operation theatre and at the time of new construction or any remodeling or structural alterations are done. In such situations which will have trust worthy safety of the theatre. Clostridium spores should be absent.

Evaluation of operation theatre staff for Staphylococcus aureus:

Evaluation should be done at least twice a year. Carriers and shedders should be treated to prevent transmission of organisms.

There is no single definite answer for the frequency of microbiological surveillance, testing method, disinfectant choice because it is influenced by various factors. So it is suggested that each centre to follow a protocol depending upon the type of theatres, with or without special air flow arrangement, type of surgeries, manpower and resources, which suits their needs and guides them to maintain the air quality in the surgical environment.

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ANNEXURE II

DATA SHEET FOR COLLECTION OF CLINICAL AND LABORATORY DATA FOR P.G DISSERTATION WORK ON "MICROBIOLOGICAL SURVEILLANCE OF OPERATING ROOMS" AT TIRUNELVELI.

Name:
Age / Sex:
Reg.no:
Unit:
Ward:
DOA:
DOS:
DOD:
Probable Diagnosis:
Procedure done:
Complaints of:
Past history:
DM/HT/CAD/Smoking/malnutrition/nicotine use
Body Mass Index (BMI):

Preoperative event:

Preoperative stay:
Linen used:
Preoperative skin preparation:
Antimicrobial prophylaxis:

Investigations
Hb
TLC
DLC
P/C
ESR
LFT
Blood sugar
Intra operative event:
Duration of surgical scrub:
Duration of operation:
Position of patient on operative list-
No. of persons in OT:
GA/RA:
Any anaesthetic complication:
Post operative event:
Post op ward/general ward:
When shifted to general ward:
Antimicrobial drugs:
Post operative stay:
The following details were recorded in the proforma for all the samples:

1. Sample location.
2. Date, sample taken, duration of plate exposed, if appropriate, for settle plates.
3. Number of colony forming units (cfu) per sample.
4. Batch number and expiry of media.
5. Surgeries being undertaken in the operation theatre
6. Result and date read.
7. Person reviewing /Quality Controller.

ANNEXURE I

Blood agar preparation:

Sterile defibrinated blood	5 ml
Nutrient agar	100ml

Melt the Nutrient agar. Add sterile defibrinated blood to Nutrient agar, the latter should be cooled to about 45 to 50 ° C before blood is added. Mix well and pour about 15 ml into plates with sterile precautions in each petri dish.

Air sampler strip:

Blood agar is poured into the wells of air sampler strip using sterile syringe.

Robertson's cooked meat medium *(Meyer & Koshi's CMC MANUAL 2001):*

Glucose broth

Minced and dried meat

Use lean meat. Remove fat and connective tissue before grinding. Mix meat (one part) and water (two parts). Cool, refrigerate overnight and skim off any remaining fat. Boil for 30 minutes. Filter the mixture through two layers of gauze and spread the meat particles out to dry. Adjust the pH to 7.4 to 7.6.

The dried meat particles are distributed in 15 x 150 mm test tubes to a height of 1.5 to 2.5 cm (1 part). The pH adjusted filtrate is then added to get 3 to 4 parts (v/v) liquid per tube. The tubes are plugged and sterilized by autoclaving.

Biochemical tests:

To identify micro organisms various biochemical reactions were performed. Ready made media such as Indole, citrate, urease and TSI, MR VP and other tests like Oxidation fermentation test, Lysine, Arginine and ornithine tests was obtained from Hi Media.