Sudan University of Science and Technology

College of Graduate Studies

Isolation of Bacterial Contaminants from Operating Theatres in private Hospitals in Khartoum State.

عزل الملوثات البكتيرية من غرف العمليات في المستشفيات الخاصة في ولاية الخرطوم

A dissertation submitted in partial fulfillment of the requirements of M.Sc degree in Medical Laboratory Science (Microbiology)

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2019

الآية

بسم الله الرحمن الرحيم

قال تعالى:
{قَالَ يَا قَوْمِ أَرَأَيْتُمْ إِن كُنتُ عَلَىَ بَيِّنَةٍ مِّن رَّبِّي وَرَزَقَنِي مِنْهُ رِزْقًا حَسَنًا وَمَا أُرِيدُ أَنْ أُخَالِفَكُمْ إِلَى مَا أَنْهَاكُمْ عَنْهُ إِنْ أُرِيدُ إِلاَّ الإِصْلاَحَ مَا اسْتَطَعْتُ وَمَا تَوْفِيقِي إِلاَّ بِاللّه عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ }

سورة هود الآية : (88)

Dedication

To my mother

my father

my brother and sisters

my friends

and everyone who supported me

Acknowledgements

All thanks to ALLAH for giving me the knowledge and support me to complete this work. After that I would like to express my appreciation to my supervisor Prof. **Yousif Fadlalla HamedElneil** for his kind help and support.

I would like to thank the staff of Microbiology laboratory in the College of Medical Laboratory Science at Sudan University of Science and Technology for kind help and support. Also thanks extended to everyone who support me throughout this work by providing samples and to everyone who help me to complete this work.

Abstract

Microbial contamination of hospital environment, especially in an operating theatre and other specialized units had continued to increase prevalence of nosocomial infections. However, the contamination of operating theatres is considered among the most common life- threatening sources of nosocomial infections.

The objective of this study was to isolate and identify bacterial contaminants in operating theatres in private hospitals in Khartoum State. Between March and April 2018, 518 samples (From 50 hospitals) were collected from different sites of the operating theatres. By employing standard microbiological techniques, all isolated bacteria were identified. During this study 6 types of bacteria were isolated from the 26(5%) positive specimens in 21 hospitals. The ratio of contaminated hospitals from total of Khartoum hospitals was 10(45.5%), and 6(40%), 5(38.5%) in Omdurman and Bahri hospitals respectively.

The result showed that the common bacterial contaminants isolated from operating theatres were Pseudomonas *aeruginosa* 11(42.3%), *Bacillus subtilis* 8(30.7%), *Micrococcus spp* 3(11.5%), *Acinetobacter baumannii* 2(7.7%), *Citrobacter freundii* and *Klebsiella pneumonia*e 1(3.8%). The highest contaminants were isolated from the sucker 13(50%), and the lowest contaminants were isolated from Focusing lamp and anesthesia mask 1(3.8%).

The result of antimicrobial susceptibility testing showed that all pathogenic organisms isolated from operating theatres are highly resistant to ceftazidime and ceftrixone and highly sensitive to rest of antimicrobial agents. Except *Klebsiella pneumonia*e which was resistant to ciprofloxacin.

This study may point to fact that there was a bacterial contaminants at operating theatre in private hospitals in Khartoum State that can cause surgical site infection (SSI). The reason of contamination may be due to an excessive presence, movement of staff, un-effective sterilization and disinfection procedure.

مستخلص البحث

التلوث الميكروبي في بيئة المستشفي خاصة غرف العمليات والوحدات الخاصة الأخرى زاد من استمرارية انتشار الإصابة في بيئة المستشفي. عليه، يعتبر التلوث في غرف العمليات الأكثر شيوعا من مصادر الإصابة في بيئة المستشفي المهددة للحياة. الهدف من هذه الدراسة هو عزل وتحديد الملوثات البكتيرية في غرف العمليات في المستشفيات الخاصة في ولاية الخرطوم خلال الفترة من مارس إلي ابريل 2018.

تم جمع 518عينة (من 50 مستشفي) من مواقع مختلفة من غرف العمليات . عن طريق استخدام معيار التقنيات الحيوية، تم تحديد البكتيريا التي عزلت . أثناء هذه الدراسة تم عزل 6 أنواع من البكتيريا من 26 (5%)عينة إيجابية من مجموع العينات في 21مستشفي. معدل التلوث في مستشفيات الخرطوم ، ام درمان وبحري كان (45.5%)10، (40%)6، (38.5%)5.

أظهرت النتائج أن أكثر بكتيريا ملوثة لغرف العمليات هي الزائفة الزنجارية (42.3%) 11، العصوية الرقيقة(30.7%)8 المكورة الدقيقة (11.5%)3، الراكدة البومانية (7.6%) 2، سيتروباكتر فروندي والكلبسيلا الرئوية (3.8%) 1.

أعلي نسبة تلوث عزلت من الشفاط (50%) 13، واقل نسبة تلوث عزلت من الكشاف وكمامة التخدير (3.8%)1. أظهرت نتائج إختبارات الحساسية أن كل أنواع البكتيريا الممرضة التي عزلت من غرف العمليات أكثر مقاومة للسفتازيديم والسفترايكسون وأكثر حساسية لباقي المضادات الحيوية المستخدمة، عدا الكلبسيلا الرئوية المقاومة للسبروفلوكساسين.

أوجدت هذه الدراسة حقائق ، أن هناك تلوث بكتيري بغرف العمليات الخاصة في ولاية الخرطوم يمكن إن ينتج عنه التهابات بالمواقع الجراحية . المسبب للتلوث ربما يرجع إلي التواجد والحركة الزائدة للكادر الطبي المتواجد داخل غرف العمليات وعمليات التعقيم والتطهير الغير مؤثرة.

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CHAPTER ONE

INTRODUCTION

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INTRODUCTION

1.1 Introduction

Microbial contamination of hospital environment, especially in an operating theatre and other specialized units had continued to increase prevalence of nosocomial infections (Kiranmai and Madhavi, 2016). The contamination of operating theatres is considered among the most common life- threatening sources of nosocomial infections (Ensayef *et al*., 2009).

The operating room specifically used for the anesthesia and surgical teams and should not be used for other purposes; however, it is considered to be one of the most hazardous environments in the health care system. Therefore, microbial contamination in the operating room is a major risk factor for surgical site infections (SSIs) during clean surgery (Nwankwo and Azeez, 2015).

SSIs, one of the most common causes of nosocomial infections are a common complication associated with surgery. Despite the technical advances in infection control and surgical practices, SSIs still continue to a major problem, even in hospitals with most modern facilities. These infections are usually caused by exogenous and/ or endogenous microorganisms that enter the operative wound either during the surgery (primary infection) or after surgery (second infection) (Vikrant *et al*., 2015). Post cesarean surgical site infection (SSI) is one of the common complications diagnosed in 2.5%-16% of the cases and is associated with significant increase in maternal morbidity, hospital stay, costs, and psychological stress to the new parents (Chhetry *et al*., 2016).

Sources of microbial contamination in the operating theatre are diverse these include frequent movement of surgical and medical team, movement within the operating theatre, high presence of human population, especially the theatre staff and medical students, theatre gown, foot wares, drainage of the wounds, and transportation of patients . All these factors play a role in contaminating the operating theatre and subsequently cause post-operative infection (Obi *et al*., 2015).

Hospitals provide a reservoir of microorganisms, many of which are multi- resistant to antibiotics. The emergency of resistance to antimicrobial agents is a global public problem particularly in pathogens causing nosocomial infections which contributed to the morbidity, mortality, increasing health care costs resulting from treatment failure and longer hospital stay (Fithamlak *et al*., 2017).

The nosocomial infection rate in patients in a facility is an indicator of quality and safety of care. The development of a surveillance process to monitor this rate is an essential first step to identify local problems and priorities, and evaluate the effectiveness of infection control activity. Surveillance, by itself, is an effective process to decrease the frequency of hospital acquired infections (Ducel *et al*., 2002).

Lack of microbiologically safe environment in the theatre results delay of recovery and they are associated with SSIs (Alicia *et al*., 1999). Regular cleaning following institutionalized guidelines of infection control policies can minimize the possibility of contamination and prevent the hospital acquired infections (HAI) so that the morbidity and mortality related to HAI will be reduced (Pradhan and shrestha, 2012).

1.2. Rationale

Surgical site infections (SSIs), is one of the most causes of nosocomial infections are a common complication associated with surgery, with a reported incidence rates of 2-20%. Despite the technical advances in infection control and surgical practices, SSIs still continue as a major problem, even in hospitals with most modern facilities (Vikrant *et al*., 2015).

The result of study carried out in Sudan which showed that the incidence of positive cultures was (39%). identification of isolated bacteria from operating theatres, which revealed that *Staphylococcus aureus* was the most common isolate (30.8%), followed by coagulase negative Staphylococci (25.6%), *Escherichia coli*(20.5%), *Pseudomonas aeruginosa*(15.4%), *Proteus mirabilis* (5.1%), and *Enterobacter cloacae* (2.6%) (Mohammed, 2010). In other study carried out in Nigeria which showed that the isolates of clinical importance observed were Coagulase Negative *Staphylococcus* (COANS) spp. (34.5%), *P. aeruginosa* (26.2%), Non-hemolytic *Streptococcus* spp. (14.5%), *Proteus mirabilis* (10.3%), *E.coli* (8.3%), and *Staphylococcus aureus* (2.1%) (Nwankwo and Azeez, 2015).

T0 protect the patient and health care authorities from hospital acquired infection originated from operating theatres and reduce post-operative infection, continuous microbiological surveillance of operating theatres must be implemented.

Hospitals provide a reservoir of microorganisms, many of which are multi-resistant to antibiotics. Emergence of multi-drug resistant strains in a hospital environment, particularly in developing countries is an increasing problem to infection treatment (Fithamlak *et al*., 2017). Therefore, periodic detection of organism's level and elimination is crucial prevention of infections acquired from hospital.

1.3 Objectives

1.3.1 General objective

To isolate bacterial contaminants from operating theatres in private hospitals in Khartoum State during period from march to April 2018 using standard Microbiological techniques.

1.3.2 Specific objectives

-To isolate and identify bacterial contaminants from operating theatre.

-To detect the common bacterial contaminants in the operating theatres.

-To determine the most common sources of theatre contaminants.

-To examine the antimicrobial susceptibility pattern of the isolated bacteria.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1 Definitions of nosocomial infection

Nosocomial infection also called “hospital acquired infection” can be defined as: An infection acquired in hospital by a patient who was admitted for a reason other than that infection (Ducel *et al*.,2002). Nosocomial infections are those infections that were neither present nor incubating at the time the patient was admitted to the health care facility. The patients with infectious diseases are frequently admitted to hospital. Some of these patients are able to spread their organisms to other patients and they provide one source of infection in the patients admitted in the hospital for other causes (Pradhan and Shrestha, 2012).

Nosocomial infections may also be considered either endemic or epidemic. Endemic infections are most common. Epidemic infections occur during outbreaks and are defined as an unusual increase above the baseline of a specific infection or infecting organism (Ducel *et al*., 2002). The sources and transmission mechanisms of infection agents in the hospital setting including operating theatres are variable and in some cases remain unnoticed. Although majority of nosocomial infections (30 – 40%) reported were associated with urinary tract, nevertheless, post-surgical wound infections (equally with lower respiratory tract) constitute 15 to 20% of all nosocomial infections reporte (Nwankwo and Azeez, 2015). The infecting microorganisms are variable, depending on the type and location of surgery and antimicrobials received by the patient. The main risk factor is the extent of contamination during the procedure (clean, clean contaminated, contaminated, dirty), which is, to a large part, dependent on the length of the operation, and the patient’s general condition (NNIS System, 1991).

2.2 Most common nosocomial pathogens

2.2.1 Commensal bacteria

Found in normal flora of healthy humans. These have significant protective role by preventing colonization by pathogenic microorganisms. Some commensal bacteria may cause infection if the natural host is compromised. For example, cutaneous coagulase negative staphylococci cause intravascular line infection and intestinal *E.coli* are the most common cause of urinary infection (Ducel *et al*., 2002).

2.2.2 Pathogenic bacteria

Have greater virulence, and cause infections (sporadic or epidemic) regardless of host status. For example:

Anaerobic Gram-positive rods (e.g. *Clostridium*) cause gangrene, *S. aureus* (cutaneous bacteria that colonize the skin and nose of both hospital staff and patients) cause a wide variety of lung, bone, heart and bloodstream infections and are frequently resistant to antibiotics; beta haemolytic streptococci are also important, Gram-negative Enterobacteriacae (e.g. *E. coli*, *Proteus*, *Klebsiella*, *Enterobacter*, *Serratia marcescens*), may colonize sites when the host defenses are compromised (catheter insertion, bladder catheter, cannula insertion) and cause serious infections (surgical site lung, bacteriaemia, peritoneum infection). They may also be highly resistant, Gram-negative organisms such as *Pseudomonas* species are often isolated in water and damp areas. They may colonize the digestive tract of hospitalized patients. Other bacteria are a unique risk in hospitals. For instance, *Legionella* species may cause pneumonia (sporadic or endemic) through inhalation of aerosols containing contaminated water (air conditioning, showers, therapeutic aerosol) (Ducel *et al.,* 2002).

Gram-negative bacilli predominate over Gram-positive bacilli in nosocomial infection in most studies. In 2017 study carried out in India in

a private hospital showed the vascular line infection rates being 9%. UTI was 11.7%. Commonest nosocomial isolates were:

Pseudomonas - 30%, MRSA - 15%, *E. coli* - 13%, *Klebsiella* - 10%, *Enterobacter* - 9%, *Acinetobacter* -6% (Varsha*et al*., 2017).

2.3 Surgical Site Infection (SSI)

SSIs is the infections of the tissues, organs, or spaces exposed by surgeons during performance of an invasive procedure. CDC definition states that only infections occurring within 30 days of surgery (or within a year in the case of implants) should be classified as SSIs. SSIs are characterized by a breach of mechanical/anatomic defense mechanisms (barriers) and are associated with greater morbidity, mortality, and increased cost of care. SSI can increase the length of time a patient stays in hospital and thereby increase the costs of health care, not only the patient but his family also suffers. The additional costs may be related to re-operation, extra nursing care and interventions, and antibiotics. The indirect costs may be due to loss of productivity, patient dissatisfaction and litigation, and reduced quality of life (Hemant and Rahul, 2015).

Surgical Site Infections are the third most frequently reported nosocomial infection, accounting for 14% - 16% of all nosocomial infections among hospitalized patients. Among surgical patients, SSIs were the most common nosocomial infections, accounting for 38% of all such infections. Of these SSIs, two thirds were confined to the incision, and one third involved organs or spaces accessed during the operation. When surgical patients with nosocomial SSI died, 77% of the deaths were reported to be related to the infection, and the majorities (93%) were serious infections involving organs or spaces accessed during the operation. In 1980, Cruse estimated that an SSI increased a patient's hospital stay by approximately 10 days and cost (Cruse and Foord, 1980). The definition of SSI is mainly clinical: purulent discharge around the

wound or the insertion site of the drain, or spreading cellulitis from the wound. Infections of the surgical wound (whether above or below the Apo neurosis), and deep infections of organs or organ spaces are identified separately (Ducel *et al.*, 2002).

Advances in infection control practices include improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis. Despite these activities, SSIs remain a substantial cause of morbidity and mortality among hospitalized patients. This may be partially explained by the emergence of antimicrobial-resistant pathogens and the increased numbers of surgical patients who are elderly and or have a wide variety of chronic, debilitating, or immune compromising underlying diseases. There also are increased numbers of prosthetic implant and organ transplant operations performed. To reduce the risk of Surgical Site Infections a systematic, but realistic approach must be applied with the awareness that this risk is influenced by characteristics of the patient, operation, personnel, and hospital (Alicia *et al*., 1999). Of Surgical Site Infection, the organism is usually patient's endogenous flora. In abdominal surgeries the opening of the gastrointestinal tract increases the chances of infections by coli forms, Gram negative bacilli. This group of organisms tends to be endemic in hospital environment by being easily transferred from object to object, they also tend to be resistant to common antibiotics and are difficult to eradicate in the long term. This group of organisms play a greater role in the many hospital acquired infections. We found that *E.coli*, *Pseudomonas aeruginosa, S. aureus*, *Citrobacter*, *Klebsiella*, and MRSA(Methicillin resistante *S. aureus*)are most commonly organisms found in SSIs (Hemant and Rahul, 2015).

2.4 The sources of contamination

2.4.1 Air

2.4.1.1 Air borne contamination and transmission

The viable and the non-viable airborne contaminants present in the operating theatre air can affect the health and safety of both the personnel and the patient (Francesco *et al*., 2015). Infection may be transmitted over short distances by large droplets and at longer distances by droplet nuclei generated by coughing and sneezing. Droplet nuclei remain airborne for long periods, may disseminate widely in an environment such as a hospital ward or an operating room, and can be acquired by (and infect) patients directly, or indirectly through contaminated medical devices. The number of organisms present in room air will depend on the number of people occupying the room, the amount of activity, and the rate of air exchange. Bacteria recovered from air samples usually consist of Gram-positive cocci originating from the skin. They can reach large numbers if dispersed from an infected lesion, particularly an infected exfoliates skin lesion. However, since the contaminated skin scales are relatively heavy, they do not remain suspended in the air for long. Gram negative bacteria usually found in the air only when associated with aerosols from contaminated fluids, and tend to die on drying (Ducel *et al*., 2002).

Controlling airborne pathogens in health facilities is not only important for the safety of the patient, but it is also important for hospital. Evaluation of the quality of air in operating theatres can be performed routinely by microbiological sampling and particle counting. Total numbers of bacteria in an empty operating theatre should be <35 cfu with less than one colony of *Clostridium perfringens* or *S. aureus.* During an operation, total air counts should be < 180 cfu (Kiranmaiand Madhavi, 2016). Kaur and Hans (2007) reported that common bacterial

contaminants in operating theatre from air *S. epidemidis* was 39 %, *S. aureus* was17.4%, *P. aeruginos* was 30% and coliform was 13%.

2.4.1.2 Factors influencing airborne contamination in operating theatres

Type of surgery, quality of air provided, rate of air exchange, number of persons present in operating theatreand Movement of operating room personnel: Movement can shed up to 10,000 skin scales per minute, of which 10% carry clusters of microorganisms. These will contain *S. aureus* and coagulase-negative staphylococci which are frequent causes of infection of joint replacement. In a well-run and organized theatre, movement is kept to a minimum and dispersal of skin organisms are reduced, also the factor include the level of compliance with infection control practices, quality of staff clothing and quality of cleaning process (Ducel *et al*., 2002).

2.4.2 Environmental surfaces

More than 100 years, the inanimate environment in the operating theatre (e. g ., walls, tables, floors, and equipment surfaces) has been considered a potential source for pathogens that may cause SSIs, few studies have evaluated the importance of Operating Room(OR) surface contamination. Thus, the role of the environment in the patient acquisition process within this setting is still debated. Here, we review accumulating evidence that the inanimate environment in the OR can become contaminated and contribute to the transmission of certain nosocomial pathogens to the hands of health care workers in OR (Yezil *et al*., 2014).

There are no data to support routine disinfection of environmental surfaces or equipment between operations in the absence of contamination or visible soiling. When visible soiling of surfaces or equipment occurs during an operation, an Environmental Protection Agency (EPA)-approved hospital disinfectant should be used to

decontaminate the affected areas before the next operation (Nichols, 1992). Environmental monitoring by the microbiological testing of surfaces and equipments is useful to detect changing trends of types and counts of microbial flora (Kiranmai and Madhavi, 2016).

2.4.3 Floor

Contamination of the floors could be important because OR floors can transmit organisms to patients through the generation of airborne contamination or inadvertent contamination of surfaces during routine care. Additionally, some patients equipment can come into contact with the floor in ORs. For example, IV tubing frequently contacts the floor as it drapes between the patient and the pump (Yezil *et al*., 2014).

2.4.4 Operation lamps

The operating lamps were contaminated as the floor which indicates the contamination due to transfer of bacteria via shoes or wheel was kept at source low level and the main source of contamination was due to sedimentation of airborne bacteria-carrying particles. The contamination of the wall was about half that of the floor and the lamp. The light handles are attached to an unsterile light which, by its size, probably disturbs the laminar flow and creates eddies of air around it. The handle may also be inadvertently touched by the unclean heads of scrubbed personnel (Ducel *et al*., 2002)**.**

2.4.5 Shoes

Pathogenic bacteria were found on all shoes, with outdoor shoes the most heavily contaminated. Floor bacteria may contribute up to 15% of airborne bacteria (CFU) in operation room; dedicated operation room shoes and strict floor washing protocols may control the level of shoes contamination, especially coagulase-negative staphylococci (Amirfeyz *et al*., 2007).

2.4.6 Suckers

The tips of suckers have rates of contamination of 16% - 55%, but they are still used by most surgeons (Grabe *et al*., 1985).

2.4.7 Gloves

Gloves become contaminated during preparation of the skin of the patient and double glove is a device (McCue *et al*.,1981). Currently, many surgeons wear two pairs of gloves and change the outer ones frequently during the operation because of the high incidence of perforation common to orthopedic surgery. (Sean and Andersons, 1999).

2.4.8 Equipments

The contamination of some equipment commonly in use during arthroplasty, including the collection bag used during hip replacement and the 'sterile' handles attached to the theatre lights, has not been explored. The bags are often used as a resting place for instruments and swabs during surgery, a habit which may facilitate transfer of bacteria into the wound. Ensayef *etal* (2009) reported common bacterial contaminants in operating theatres during 2001 was *S. epidermidis,* followed by *P. aeruginosa*, and whereas in 2002 coliform bacteria were the highest followed by *P. aeruginosa*. The percentage of positive culture bacteria in 2001 were 39.1% for *S. epidermidis, P.aeruginosa* was 30.4%, *S. aureus*was17.4% and coliform was 13.0%. In 2002 *S.epidermidis* was 8.3%, *S. aureus*was 4.2%, *P. aeruginosa* was 25.0% and coliform was 62.5%.

2.5 Prevention and control of infection in operating theatres

2.5.1 Ventilation

Ventilation systems should be designed and maintained to minimize microbial contamination. The air conditioning filters should be cleaned periodically and fans that can spread airborne pathogens should be avoided in high-risk areas. High-risk areas such as operating rooms,

critical care units and transplant units require special ventilation systems. Filtration systems (air handling units) designed to provide clean air should have high efficiency particulate air (HEPA) filters in high-risk areas. Unidirectional laminar airflow systems should be available in appropriate areas in the hospital construction. Ultra clean air is valuable in some types of cardiac surgery/neurosurgery/implant surgery theatres and transplant units (WHO, 2004).

Regular inspection and maintenance of filters, humidifiers, and grills in the ventilation system must be performed and documented. Cooling towers and humidifiers should be regularly inspected and cleaned to prevent aerosolization of Legionella species (Ducel *et al*., 2002). For the operating room, the critical parameters for air quality include:

Frequent maintenance/validation of efficacy of filters (in accordance with manufacturer's requirements), pressure gradient across the filter bed and in the operation theatre, air changes per hour (minimum 15 air changes per hour), temperature should be maintained between 20°C and 22°C and humidity between 30% and 60% to inhibit bacterial multiplication and general areas should be well ventilated if they are not air-conditioned. (Report of WHO, 2004).

2.5.2 Ultra-clean air

For minimizing airborne particles, air must be circulated into the room with a velocity of at least 0.25 m/sec through (HEPA) filter, which excludes particulate matter of defined size. If particles 0.3 microns in diameter and larger are removed, the air entering the room will be essentially clean and free of bacterial contaminants. This principle has been applied to microbiology laboratories, pharmacies, special intensive care units, and operating rooms for operating theatres, a unidirectional clean airflow system with a minimum size of 9 mm (Ducel *et al*., 2002).

2.5.3 Sterilization

2.5.3.1 Conventional sterilization of surgical instruments

Inadequate sterilization of surgical instruments has resulted in SSI outbreaks (Sessler *et al.,* 1992). Surgical instruments can be sterilized by steam under pressure, dry heat, ethyloxide, or other approved methods. Microbial monitoring of steam autoclave performanceis necessary and can be accomplished by use of a biological indicator.

2.5.3.2 Flash sterilization of surgical instruments

During any operation, the need for emergency sterilization of equipment may arise e.g., to reprocess an inadvertently dropped instrument. However, flash sterilization is not intended to be used for either reasons of convenience or as an alternative to purchasing additional instrument sets or to save time. Also, flash sterilization is not recommended for implantable devices because of the potential for serious infections (Favero and Bond, 1991). Flash sterilization is not recommended as a routine sterilization method because of the lack of timely biologic indicators to monitor performance, absence of protective packaging following sterilization, possibility for contamination of processed items during transportation to operating rooms, and use of minimal sterilization cycle parameters (i.e., time, temperature, pressure) (Favero and Manian, 1993). Many hospitals have placed equipment for flash sterilization in close proximity to operating rooms and new biologic indicators that provide results in 1 to 3 hours are now available for flash-sterilized items (Rutala *et al*., 1993).

2.5.4 Disinfection of patient equipment

Disinfection removes microorganisms without complete sterilization to prevent transmission of organisms between patients. It meets criteria for killing of organisms, have a deterrent effect, act independently of the number of bacteria present, the degree of hardness of the water, or the

presence of soap and proteins (that inhibit some disinfectants). To be acceptable in the hospital environment, they must also be easy to use, non-volatile, not harmful to equipment, staff or patient, free from unpleasant smells, and effective within a relatively short time. Different products or processes achieve different levels of disinfection. These are classified as high, intermediate or low-level disinfection (Ducel *et al*., 2002).

High-level disinfection (critical)-this will destroy all microorganisms, with the exception of heavy contamination by bacterial spores.

Intermediate disinfection (semi-critical) this inactivates *Mycobacterium tuberculosis,* vegetative bacteria, most viruses and most fungi, but does not necessarily kill bacterial spores.

Low-level disinfection (non-critical) - this can kill most bacteria, some viruses and some fungi, but cannot be relied on for killing more resistant bacteria such as *M.tuberculosis* or bacterial spores. These levels of disinfection are attained by using the appropriate chemical product in the manner appropriate for the desired level of disinfection (Ducel *et al.*,2002).

2.6 Antimicrobial prophylaxis

Surgical antimicrobial prophylaxis (AMP) refers to a very brief course of an antimicrobial agent that initiates just before an operation begins (Nichols and Holmes, 1995). , antibiotics must be initiated intravenously within one hour prior to the intervention. It is often most efficient to order therapy given at call to the operating room or at the time of induction of a anesthesia. In most cases, prophylaxis with a single preoperative dose is sufficient. The regimen selected depends on the prevailing pathogen(s), the pattern of resistance in the surgical service, the type of surgery, the serum half- life of the antibiotic, and the cost of the drugs. Administration of prophylactic antibiotics for a longer period prior to the operation is

counterproductive, as there will be a risk of infection by a resistant pathogen (Ducel *et al.*, 2002).

2.7 Resistance to antibiotics

Hospitals provide a reservoir of microorganisms, many of which are multi- resistant to antibiotics. The emergency of resistance to antimicrobial agents is a global public problem particularly in pathogens causing nosocomial infections which contributed to the morbidity, mortality, increasing health care costs resulting from treatment failure and longer hospital stay (Fithamlak *et al.*, 2017).

2.8 Multidrug-Resistant Organisms (MDROs)

In general, MDROs are defined as microorganisms-predominantly bacteria- that are resistant to one or more classes of antimicrobial agents. MDROs are transmitted by the same routes as antimicrobial susceptible infectious agents. Patient- to patient transmission in healthcare settings, usually via Health Care Workers (HCWs), has been major factor accounting for the increase in MDRO incidence and prevalence. Preventing the emergence and transmission of these pathogens requires a comprehensive approach that includes administrative involvement and measure (e.g nurse staffing, communication systems, performance improvement processes to ensure adherence to recommended infection control measures), education and training of medical and other health care personnel , judicious antibiotic use, comprehensive surveillance for targeted MDROs, application of infection control precaution during patient care, environmental measures and decolonization therapy when appropriate (Balaji, 2017).

2.9 Surgical attire

The term surgical attire refers to scrub suits, caps, hoods, shoe covers, masks, gloves, and gowns. Although experimental data show that live microorganisms are shed from hair, exposed skin, and mucous

membranes of operating room personnel, few controlled clinical studies have evaluated the relationship between the use of surgical attire and SSI risk (Hardin and Nichols, 1995).

2.9.1 Face masks

Face masks are used as deflectors of bacteria expelled from the respiratory tract of the wearer. Normally, very few bacteria are dispersed when the person is silent. Hence the main purpose of a mask is to protect the wound from direct contamination while the surgeon is talking during the operation. It is reasonable for all members of the surgical team to wear a mask when operating. It should be changed after each operation since it easily becomes contaminated. There is no evidence to suggest that there is any need for non-scrubbed staff to wear a mask if they are not in the operating area (Sean and Anderson, 1999).

2.9.2 Surgical caps, hoods and shoe covers

In aseptic units, operating rooms, or performing selected invasive procedures, staff must wear caps or hoods which completely cover the hair (Ducel *et al.,* 2002). Surgical caps, hoods are inexpensive and reduce contamination of the surgical field by organisms shed from the hair and scalp. SSI outbreaks have occasionally been traced to organisms isolated from the hair or scalp e.g *S. aureus* and group A *Streptococcus* even when caps were worn by personnel during the operation and in the operating suites (Mastro *et al*., 1990). The use of shoe covers has never been shown to decrease SSI risk or to decrease bacteria counts on the operating room floor (Humphreys *et al.,*1991). Shoe covers may, however, protect surgical team members from exposure to blood and other body fluids during an operation.

2.9.3 Sterile gloves

Sterile gloves are put on after donning sterile gowns. A strong theoretical rationale supports the wearing of sterile gloves by all scrubbed members

of the surgical team. Sterile gloves are worn to minimize transmission of microorganisms from the hands of team members to patients and to prevent contamination of team members 'hands with patients' blood and body fluids. If the integrity of a glove is compromised (e.g punctured), should be changed as promptly as safety permits (Dodds *et al.*, 1988). Wearing two pairs of gloves (double-gloving) has been shown to reduce hand contact with patients' blood and body fluids when compared to wearing only a single pair (Short and Bell, 1993).

2.9.4 Gowns and drapes

Sterile surgical gowns and drapes are used to create a barrier between the surgical field and potential sources of bacteria. Gowns are worn by all scrubbed surgical team members and drapes are placed over There are limited data that can be used to understand the relationship of gown or drape characteristics with SSI risk .Gowns and drapes are classified as disposable (single use) or reusable (multiple uses). Regardless of the material used to manufacture gowns and drapes, these items should be impermeable to liquids and viruses. In general, only gowns reinforced with films, coatings, or membranes appear to meet standards developed by the American Society for Testing and Material. However, such "liquid-proof" gowns may be uncomfortable because they also inhibit heat loss and the evaporation of sweat from the wearer's body. These factors should be considered when selecting gowns (Lewis and Brown, 1998).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE
MATERIALS AND METHODS

3.1 study design

This is descriptive cross sectional study carried out to isolate bacterial contaminants in operating theatres in private hospitals in Khartoum State.

3.2 Study area

Private Hospitals in Khartoum State (Omdurman, Khartoum and Khartoum north)

3.3 Study duration

This study was carried out during the period from March 2018 to May 2018.

3.4 Data collection

Data was collected using check list.

3.5 Study sample

Swab samples from operating theatres in private hospitals in Khartoum state (General surgery, Ear, Nose and Throat, Delivery) were collected.

3.6 Sample size

Five hundred and eighteen Swabs were collected from 82 operating rooms in 50 hospitals.

3.7 Inclusion criteria

Sample from decontaminated operating theatres.

3.8 Exclusion criteria

Sample from contaminated operating theatres.

3.9 Ethical consideration

Permission to carry out this study was obtained from College of Medical Laboratory Science, Sudan University of Science and Technology and Public Health Laboratories in the Laboratories Administration.

3.10 Methods

3.10.1 Sample collection

Samples were collected from target operating theatre after sterilization process is done. The selection of sites for culture depends upon the known epidemiology and survival of the characteristics organisms. Different samples from certain sites in operating theatre (operating tables, sucker, anesthesia machine, focusing lamp, trolley, and floor) were collected by dipping sterile cotton swabs in normal saline and then equipments of interest were swabbed gently and then the samples were transferred to the laboratory immediately for bacteriological examination.

3.10.2 Culture of specimens

Under aseptic condition (near Bunsen burner), all collected specimens were inoculated on blood agar and MacConkey agar and the inoculated plate were incubated aerobically at 37^0C for 24 hours.

3.10.3 Examination of the growth

Discrete colonies from primary cultures that showed significant growth were tested at the end of the incubation period for their fermentative and non-fermentative character on MacConkey agar, haemolysis on blood agar and proceeded for further investigations.

3.10.4 Identification

3.10.4.1 Gram's stain

A smear was prepared from each suspected isolate by dipping a portion of a colony in a slide containing normal saline and well mixed. The suspension was spread over the slide and left to dry. Then each dried smear was fixed by passing the slide through the flame of Bunsen burner 2-3 times. Fixed smears were stained with Grams stains according to the well-known conventional method. Smears were prepared from significant bacterial growth (colonies), stained with Gram stain method and examined under the microscope to detect the appearance of bacteria and

stain reaction. Gram negative bacteria appear as red color and Gram positive bacteria appear as violet color (Cheesbrough, 2006).

3.10.4.2 Biochemical tests

Oxidase test

This test was used to detect the ability of the organism to produce oxidase enzyme. Oxidase disk was placed inside the petri dish, small inoculums were taken by using wooden stick, and then smeared on the disk by opening the petri dish partially. Appearance of deep blue - purple color within 20 second indicate positive result (Cheesbrough, 2006).

Citrate utilization test

This test was used to detect the ability of organism use citrate as a sole source of carbon and ammonia as sole source of nitrogen. Under aseptic condition the tested organisms (colony) were streaked by using sterile straight wire in Simmon's citrate agar and incubated for 18-24 hours at 37OC. Appearance of blue color indicates positive result, no change in color indicates negative result(Cheesbrough, 2006).

Indole test

This test was used to detect the ability of organism to break down tryptophan and indole production was detected by Kovac's reagent. Under aseptic condition the tested organisms were inoculated in the test tube containing 3ml of sterile peptone water then incubated at $37^{O}C$ for overnight. The positive result was indicated by presence of red ring, negative result showed no change in color (Cheesbrough, 2006).

Urease test

This test use to identify some bacteria that produce urease enzyme which break down the urea into ammonia and carbon dioxide. The test was done by inoculating urea agar with tested organism and incubated at $37^{O}C$ for 24hours. The change in color to pink color indicates positive test (Cheesbrough, 2006).

Sugar fermentation, gas and hydrogen sulphide production test

The tested organisms were inoculated on the KIA agar media by using sterile straight loop, by stabbing on the butt, then streaked the slope of the media and incubated for 24 hours at $37^{O}C$. The result depends on the ability of the organism to ferment the glucose in the butt and lactose in the slope, gas production appears as gap and cracking of the media, H2S production as blacking in the media (Cheesbrough, 2006).

Methyl Red (MR**) test**

This test is employed to detect the production of sufficient acid during fermentation of glucose and maintenance of conditions such that pH of an old culture is sustained below a value of about 4.5, as shown change in the color of the methyl red indicator which is added at the end period of incubation. Peptone water was inoculated with the test organism using wire loop and incubated at $37^{o}C$ for 48 hours. After the incubation period, methyl red was added and well mixed. The test is considered positive when bright red color was produced and negative when yellow color was produced (Cheesbrough, 2006).

Voges - Proskauer (VP) **test**

VP test detects butylene glycol producers. Acetyl-methyl carbinol (a cetoin) is an intermediate in the production of butylene glycol. In this test two reagents, 40% KOH and alpha-naphthol are added to the test broth after incubation and exposed to atmospheric oxygen. Ifacetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alpha naphthol to produce red color. Role of alpha-naphthol is that of a catalyst and a color intensifier. Tested organism is inoculated into glucose phosphate broth and incubated for at least 48 hours. 0.6 ml of alpha-naphthol is added to the test broth and shaken. 0.2 ml of 40% KOH is added to the broth and shaken. The tube was allowed to stand for 15

minutes. Appearance of red color is taken as a positive test. The negative tubes must be held for one hour, since maximum color development occurs within one hour after addition of reagents (Cheesbrough, 2006).

Motility test

This test detects motile organisms. The concentration of agar in a test medium is 0.4% or less to allow free spread of organisms. The test organisms were inoculated in a test medium by using a sterile straight loop, and then incubated at 37^{o}C for 24 hours. Movement from stab line or hazy appearance throughout the medium indicates motile organism (Arora and Arora, 2007).

Catalase test

This test was used to differentiate catalase producing bacteria such as staphylococci, from non-catalase producing bacteria such as streptococci. This test was performed by using the tube method .The breakdown of hydrogen peroxide (H_2O_2), into oxygen and water is mediated by the enzyme catalase. When a small amount of an organism that produces catalase is introduced into H_2O_2, rapid elaboration of bubbles of oxygen, the gaseous product of the enzyme's activity is produced (Cheesbrough, 2006).

3.9.5 Antimicrobial susceptibility test

Was performed using the Modifie KirbyBauer agar disk diffusion method described by Bauer *et al* (1966). Use disks of blotting paper impregnated with a known volume and appropriate concentration of antimicrobial, these include Amikacin (30mcg), Gentamicin (30mcg), Meropenem (10mcg), Ciprofloxacin (5mcg), Ceftazidim (30mcg), Ceftrixone (30mcg)**.**

3.9.5.1 Preparation of inoculums

Suspensions were prepared by using sterile normal saline, 3–5 colonies were emulsified from each isolate in separate tube and compared with o.5

McFarland standard for adjustment until match occurred (Cheesbrough, 2006).

3.9.5.2. Seeding on Muller Hinton agar

Seeding on Muller Hinton agar was under aseptic condition by using sterile swabs. Swabs were immersed in suspension and pressed and rotated against the side of the tubes above the level of suspension to remove the excess. Mueller Hinton surface was inoculated by swabbing (Cheesbrough, 2006).

3.9.5.3. Application of antimicrobial discs

By using sterile forceps, gentamicin, amikacin, meropenem, ceftrixone, ciprofloxacin and ceftazidime discs were placed at about 24 mm from disc to disc on the inoculated plates and 15mm from the edges of the plate (Cheesbrough, 2006).

3.9.5.4. Incubation

Inoculated plates were incubated at 37^{O}C for overnight (Cheesbrough, 2006).

3.9.5.5. Measuring of zone of inhibition

By using ruler zone of inhibition was measured in mm and the end point of inhibition is where growth start (Cheesbrough, 2006).

3.9.5.6. Quality control

Standard strain of *P. aeruginosa*ATCC (American Type culture Collection) No (27853) was brought from National Public Health Laboratory and sensitivity testing was performed on Muller Hinton agar in similar way (disc diffusion method) and condition to our isolates to determine the validity of the selected antibiotics, and the result of antimicrobial susceptibility of control strain showed that it was sensitive to all selected antibiotics.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

The study was conducted during the period of March to May 2018 in Khartoum State private hospitals (Khartoum, Bahri, and Omdurman). In this study a total of 518 samples from 50 private hospitals and centers were collected. The processes of practical were done in the Public Health Laboratories in the laboratories administration and Sudan University of Science and Technology – College of Medical Laboratory Science.

The sites of sample collection in operating theatre were sucker, operation room bed, trolly, floor, focusing lamp and anesthesia mask. The selection of sites were based upon the known epidemiology and survival characteristics of the organisms. During this study 6 types of bacteria were isolated and identified from the 26(5%) positive specimens in 21(42%) hospitals.

The most frequently isolated bacteria were *Pseudomonas aeruginosa* 11(42.3%), *Bacillus subtilis* 8(30.7%), *Micrococcus spp* 3(11.5%), *Acinetobacter baumannii* 2(7.6%), *Citrobacter freundii* 1(3.8%), and *Klebsiella pneumonia*e 1(3.8%) as shown in table (4.1). The total number of contaminated hospitals from the total of hospitals in Khartoum was 10(45.5%), Omdurman 6(40%), and Bahri 5(38.5%) as shown in table (4.2).

The most contaminated site was sucker 13(50%), followed by operation room bed 5(19.2%), Trolly 4(15.4%), Floor 2(7.7%), Focusing lamp and anesthesia mask had one contaminant for each (3.8%) as shown in table (4.3).

Antimicrobial susceptibility testing was performed on pathogenic isolated bacteria and the result showed that all pathogenic organisms isolated from

operating theatres are highly resistant to ceftazidime and ceftrixone and highly sensitive to meropenem, amikacin, gentamicin and ciprofloxacin respectively except *Klebsiella pneumonia* which was resistant to ciprofloxacin as shown in table (4.4).

Table (4.1): Type and frequency of isolated bacteria from operating theatres:

No	Type of isolated bacteria	Frequency	%
1	*Pseudomonas aeruginosa*	11	42.3%
2	*Bacillus subtilis*	8	30.8%
3	*Micrococcus spp*	3	11.5%
4	*Acinetobacterbaumannii*	2	7.7%
5	*Citrobacterfreundii*	1	3.8%
6	*Klebsiellapneumonia*e	1	3.8%
	Total	26	100%

Table (4.2):The Number of surveyed and percentage of contaminated hospitals:

Place of hospitals	Total No	Contaminated hospitals
Khartoum	22(44%)	10(45.5%)
Omdurman	15(30%)	6(40%)
Bahri	13(26%)	5(38.5%)
Total	50(100%)	21(100%)

Table(4.3): Frequency of isolated bacteria according to the site of collection:

Site of sample collection	No. isolated bacteria	Type of isolated bacteria
Sucker	13(50%)	*Pseudomonas aeruginosa*6(46.2%), *Bacillus subtilis* 4(30.8%), *Klebsiella pneumonia*e 1(7.7%), *Acinetobacter baumannii* 1(7.7%), *Citrobacter freundii* 1(7.7%)
Operation room bed	5(19.2%)	*Pseudomonas aeruginosa*2(40%), *Bacillus subtilis* 2(40%),*Acinetobacter baumannii* 1(20%)
Trolly	4(15.4%)	*Pseudomonas aeruginosa*2(50%), *Micrococcus spp* 2(50%)
Floor	2(7.7%)	*Bacillus subtilis* 1(50%),*Micrococcus spp* 1(50%)
Anesthesia mask	1(3.8%)	*Pseudomonas aeruginosa*1(100%),
Focusing lamb	1(3.8%)	*Bacillus subtilis* 1(100%)
Total	26(100%)	

Table (4.4) : Antimicrobial susceptibility test for bacteria isolate from operating theater:

Isolate	Susceptibility of antimicrobial agents					
	GEN	**MRP**	**AK**	**CAZ**	**CIP**	**CTR**
Pseudomonas aeruginosa	72%	100%	72.7%	0%	90.9%	27.3%
Acinetobacter baumannii	100%	100%	100%	0%	100%	50%
Citrobacter freundii	100%	100%	100%	0%	100%	0%
*Klebsiella pneumonia*e	100%	100%	100%	0%	0%	0%

Key:

0: means that all isolates were resistant

(Number)%: means that (Number)% from isolates were sensitive

GEN (Gentamicin), CAZ (Ceftazidime), MRP (Meropenem), CIP (Ciprofloxacin), AK (Amikacin), CTR (Ceftrixone)

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

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DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1. Discussion

The operating theatres in any hospital should be situated in a strictly sterile zone. Therefore, and obviously, bacterial contamination of these surgical theatres is highly dangerous to the patients and performed huge hazard to the healthcare authorities. The associated structures of the operation theatres are uniquely predisposed to contamination by various microorganisms mainly bacteria, viruses, fungi and parasites. In our study we tried to isolate bacterial contaminants from operating theatre. In this study the rate of contaminated specimens with bacteria from the total of specimens were 5% . The highest contaminated area in operation rooms site were the sucker (50%), followed by operation room bed (19.2%), trolly (15.4%), floor (7.7%), focusing lamp and anesthesia mask had one contaminant for each (3.8%). All this sites are more contact with patient and the staff who work inside the operation theatre and dealing with patients. In study carried out in Royal Free Hospital in London by (Greenough, 1986) about contamination of suckers used during total hip replacement, reported that the bacterial contamination of the sucker tip was(37%) and reported the organism such as *Staphylococcus epidermidis, Micrococci* and *Pseudomonas aeruginosa.*

In this study the most frequently isolated bacteria were *Pseudomonas aeruginosa* (42.3%), followed by *Bacillus subtilis* (30.7%), *Micrococcus spp* (11.5%), *Acinetobacter baumannii* (7.6%), *Citrobacter freundii* (3.8%), and *Klebsiella pneumonia*e (3.8%). In case of *Pseudomonas aeruginosa* the result agreed with study carried out in Baghdad by

(Ensayef *et al.*, 2009) who reported that common bacterial contaminants in operating theatres was *Pseudomonas aeruginosa* as Gram negative bacilli, and the prevalence was (30.4%). Also the result was agreed with result of study carried out in Sudan by (Hassan, 2013) who reported that the predominant isolated organism was *Pseudomonas aeruginosa* (32.5%), and disagreed with the result of study carried out in Sudan by (Mohammed, 2010) who reported that the predominant isolated organism was *Staphylococcus aureus* (30.8%). The possible source of contamination of Pseudomonas *aeruginosa* was antiseptic solutions, especially this bacterium is an opportunistic pathogen that can found in most moist environments. In addition, it has a combination of properties such as its ability to survive and spread in hospital environments, acquisition of multiple virulence determinants and intrinsic resistance to commonly used antibiotics and disinfectants. This makes *Pseudomonas aeruginosa*a major nosocomials pathogen that is responsible for many outbreaks in operating theatres (Bellido and Hancock, 1993).

In this study *Bacillus subtilis* were isolated (30.7%) which considered as contaminant and *Micrococcus spp* (11.5%) which considered as normal flora, the possible contamination source was usually endogenous from normal skin flora of patients or exogenous from surgical staff. In recent study carried out by (Kiranmani and Madhavi, 2016) in operating theatres and intensive care units of a teaching hospital in Telangana in india which reported that *Bacillus subtilis* 45% (contaminants) and *Micrococci* 33% (normal flora) were most common isolates followed by *Klebsiella* 9%. In the present *Acinetobacter baumannii* and *Klebsiella pneumonia*e were common isolated from operating theatre but by low percent, this was agreement with study carried out in India by Kaur and Hans (2007) which reported that common bacterial contamination in operating theatre were *Staphylococcus aureus* (16%), Coagulase Negative *Staphylococcus*

(26.07%), *cinetobacter spp* (2.03%), and *Klebsiella spp* (0.3%). In case of *Citrobacter freundii* which was not reported by any of the previous studies.

The variations in the results were unknown, but could be due to sample size, used by the previous investigators, personal hygiene, the safety, cleaning methods, social level of patients, operating room ventilation, sterilization methods, surgical technique, and availability of antimicrobial prophylaxis, could make the observed differences. The antimicrobial susceptibility testing showed that all pathogenic organisms isolated from operating theatres are more resistant to ceftazidime and ceftrixone and more sensitive to meropenem, amikacin, gentamicin and ciprofloxacin respectively except *Klebsiella pneumonia* which was resistant to ciprofloxacin, Antibiotics resistant rate could be due to the wide spread antibiotics that routinely used in hospitals. Furthermore, bacterial strains in hospitals are often resistant to multiple antibiotics.

5.2. Conclusion

The rate of bacterial contamination of target operating theatre in this study was 5%.

The highest bacterial contamination was found in sucker, bed and trolly.

The predominant isolated organism from theatre environment was *Pseudomonas aeruginosa* (42.3%), These organism act as a dangerous source of nosocomial infection and life threading to patients and hospital staff, This might indicate that the sterilization methods are not efficient enough, which can put the patients at risk of post-operative infections.

All pathogenic organisms are highly resistant to ceftazidime and ceftrixone and highly sensitive to the rest of antibiotics.

5.3. Recommendations

- Continuous monitoring programs using advanced techniques (e.g. RT-PCR) for isolation and identification of bacterial contaminants in operating theatres are highly important to solve this problem.
- Implementation of comprehensive infection control programs and surveillance of infections, in hospitals by infection control committee. Health education of hospital staff, in order to protect themselves and the patients from the contaminating bacteria, as well as from spreading pathogenic bacteria themselves.
- The healthcare authorities should draw attention to this high prevalence of resistant of bacterial contaminants in different sites of operating theatres.

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Appendices

Appendices

Appendix 1

Materials

MacConkey(HiMedia Laboratory Pvt. Ltd. Mumbai, India).

Ingredients	**grams/Litre**
Peptic digest of animl tissue	20.00
Lactose	10.00
Sodium taurocholate	5.00
Neutral red	0.04
Agar	20.00

Preparation

Suspend 55.04 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121oC) for 15 minutes. Avoid overheating. Cool to 45oC -50oC Mix well and pour.

Blood agar (HiMedia Laboratory Pvt. Ltd. Mumbai, India).

Ingredients

Nutritious agar	500 ml
Sterile defibrinated blood	25 ml

Preparation

Prepare the agar medium. Sterile by autoclaving at 121 for 15oC minutes. Transfer to 50oC, add aseptically the sterile blood mix gently but well. Avoid forming air bubbles.

Gram's stain

1. Crystal violet

To make 1 litre

Crystal violet	20.00 gram

Ammonium oxalate	9 .00 gram
Ethanol or methanol, absolute	95 ml
Distilled water	1 litre

2. Lugol's iodine solution:

To make 1 litre

Potassium iodide	20.00 gram
Iodine	10.00 gram
Distilled water	1 litre

3. Acetone- alcohol decolorizer

To make 1 litre

Acetone	500 ml
Ethanol or methanol, absolute	475 ml
Distilled water	25 ml

4. Saffranin

Saffranin	0.54 gram
Distilled water	100 ml

Peptone water (HiMedia Laboratory Pvt. Ltd. Mumbai, India).

Ingredients	**Grams /litre**
Peptic digest of animal tissue	10.00
Sodium chloride	5.00

Preparation

Suspend 15.0 grams in 1000 ml distilled water. Sterilize by autoclaving at 15 IBS pressure (121°C) for 15 minutes. Mix well and dispense into tubes.

Urea agar base (HiMedia Laboratory Pvt. Ltd. Mumbai, India).

Ingredients	**Grams/Litre**
Peptic digest of animal tissue	1.00
Dextrose	1.00
Sodium chloride	5.00

Disodium Phosphate	1.20
Phenol red	0.012
Agar	15.00
Monopotassium Phosphate	0.80
Urea	2.00

Preparation

Suspend 24 grams in 950 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving. Cool to 50oC and aseptically add 2 grams of urea to each 100 ml of urea agar base. Dispense into sterile tubes and allow to set in the slanting position.

Simmon's citrate medium (HiMedia Laboratory Pvt. Ltd. Mumbai, India).

Ingredients	**Gram/Liter**
Magnesium Sulphate	0.20
Ammonium Dihydrogen Phosphate	1.00
Dipotassium Phosphate	1.00
Sodium Citrate	2.00
Sodium Chloride	5.00
Bromothymole blue	0.08
Agar	15.00

Preparation:

Suspend 24.28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired in tubes or flasks**.**

Kligler iron agar (HiMedia Laboratory Pvt. Ltd. Mumbai, India).

Ingredient Grams/litre	
Peptic digest of animal tissue	15.00
Beef extract	3.00
Yeast extract	3.00

Protease peptone	5.00
Lactose	10.00
Glucose (dextrose)	1.00
Ferrous sulfate	0.20
Sodium chloride	5.00
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	15 .00

Preparation

57.5 of the powder dissolve in 1 litre of distilled water and sterilized by autoclave at 121 foC or 15 minutes. Allow the medium to solidify in a sloped position to give a butt 25-30mm deep and a slope 20-25mm long.

Mueller's Hinton agar (HiMedia Laboratory Pvt. Ltd. Mumbai, India).

Ingredients	**grams/litre**
Beef, infusion form	300.00
Casein acid hydrolysate	17.500
Starch	1.500
Agar	17.000

Final pH (at 25oC) 7.3±0.1

Preparation

Suspend 38.0 grams in 1000 ml D.W. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 121°C for 15 minutes. Mix well before pouring.

Instruments

-Refrigerator	Cold air	Sudan
-Autoclave	Express Equipment	England
-Water bath	Memmert	Germany
-Microscope	Olympus optical	Japan
-Incubator	Tone picanardi	Italy
-Sensitive balance	KERN &Sohn GmbH	Germany
-Hot air oven	Memmert	Germany
-Dry cabinet	GARANTITO-Cheshi Engineering	UK

Reagents and stains

Kovac's reagent	HiMedia Laboratories Pvt. Ltd	India
Normal saline	Industry Ltd	Saudi Arabia
methyl red	HiMedia Laboratories Pvt. Ltd	India
40% KOH	HiMedia Laboratories Pvt. Ltd	India
Alpha-naphthol	HiMedia Laboratories Pvt. Ltd	India
H2O2,	HiMedia Laboratories Pvt. Ltd	India
Crystal violet	Sod-fine-CHEM-Ltd	China
Lugol's iodine	Sod-fine-CHEM-Ltd	China
70% alcohol	Sod-fine-CHEM-Ltd	China
Saffranin	Sod-fine-CHEM-Ltd	China
McFarland	Kimball Ltd	Sudan

Glass wares

Slides	Bomix	China
Petri dishes	Pyrex	USA
Test tubes	Pyrex	USA
Bottles	Pyrex	USA

Other materials

Benzene burner	Italy
Forceps	England
Wire loops	India
Cotton –wool swab	England
Wooden stick	India
Oil immersion	India
Filter paper	England

Standard strain

*P. aeruginosa*ATCC NO (27853), (National Public Health Laboratory).

Antimicrobial discs

Amikacin……(30mcg)(HIMEDIA)

Gentamicin …(30mcg)(HIMEDIA)

Meropenem… (10mcg)(HIMEDIA)

,Ciprofloxacin.. (5mcg)(HIMEDIA)

Ceftazidim…… (30mcg) (HIMEDIA)

Ceftrixone…….(30mcg)(HIMEDIA)

Table (1): Biochemical characteristics of isolated bacteria

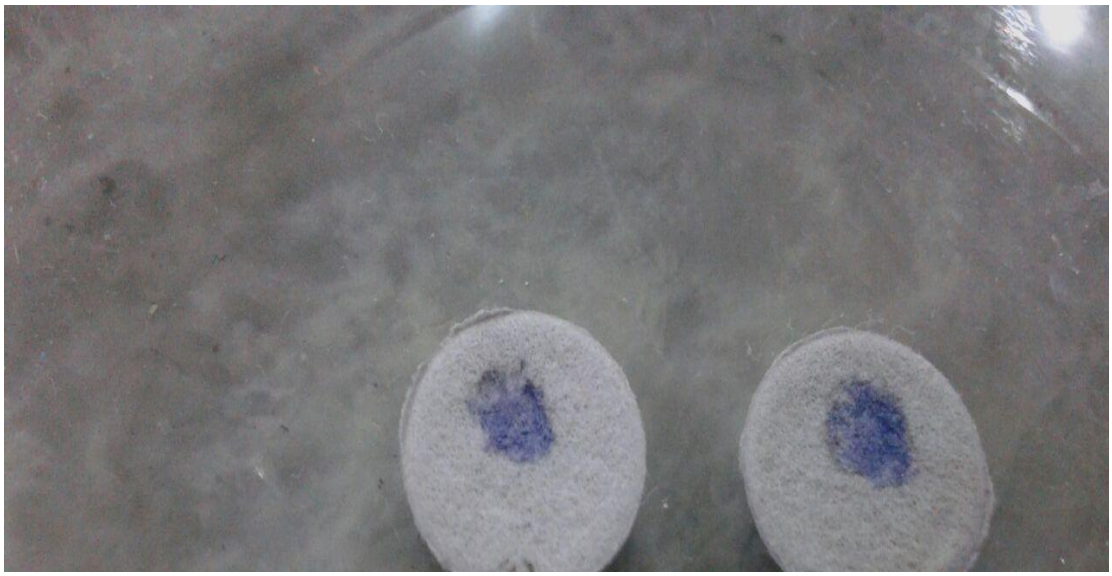
Biochemical properaties											Identified bacteria
Kligler Iron Agar				Oxidase	Indol	Motility	M.R	V.P	Urease	Citrate	
Slop	Butt	H2S	Gas								
R	R	-	-	+	-	+	+	-	V	+	*Pseudomonas aeruginosa*
Y	Y	-	+	-	-	-	-	+	+	+	*Klebsiella pneumonia*e
Y	Y	+	+	-	-	+	+	-	-	+	*Citrobacter freundii*
R	Y	-	-	-	-	-	-	-	-	+	*Acinetobacter baumannii*
R	Y	-	-	+	-	+	-	+	-	+	*Bacillus subtilis*

Key: R: red, Y: yellow, V: variable

Table (2): Biochemical characteristics of *Micrococcusspp*

Gram stain	**Catalase**	**Oxidase**	**Glucose fermentation**	**Lactose fermentation**
Gram positive cocci arranged in tetrad	**+**	**+**	**+**	**-**

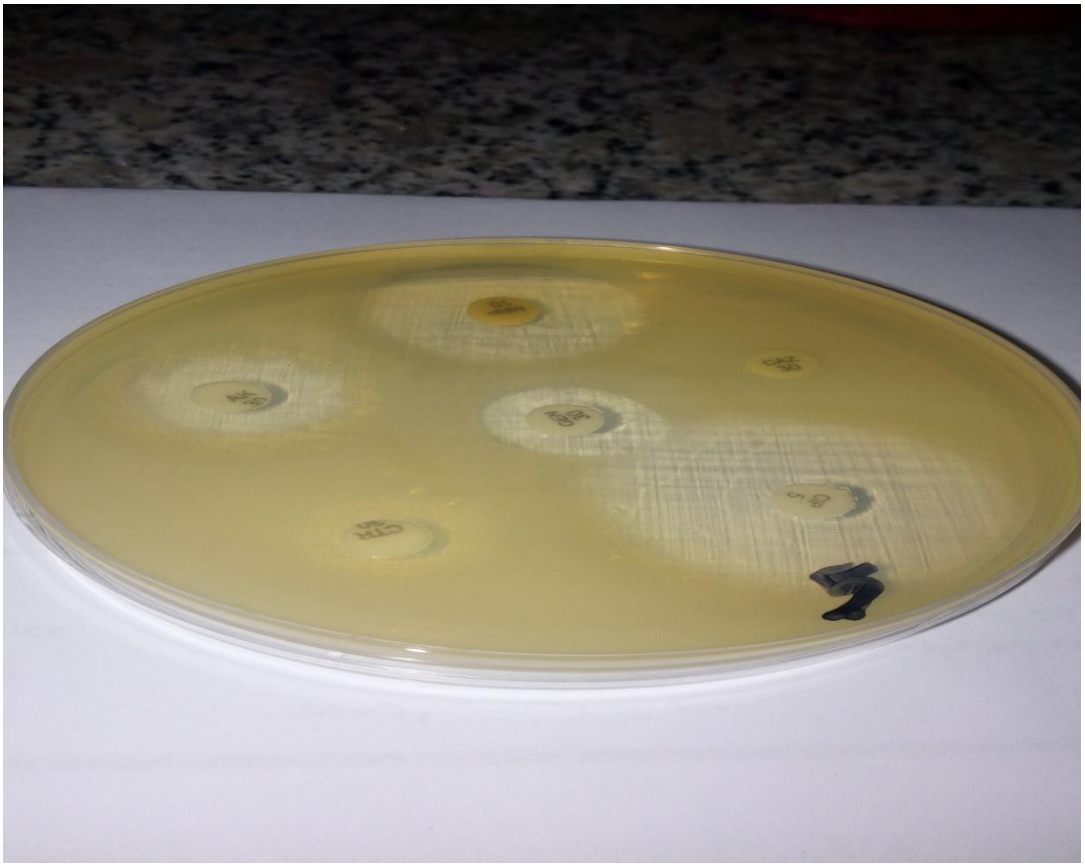
Appendix 2



Color plate (1) Oxidase test for *P. aeruginosa*

Color plate (2)Biochemical set of *Acinetobacter baumannii*

Color plate (3) Biochemical set of *Citrobacter freundii*



Color plate (4) Susceptibility testing of *Pseudomonas aeruginosa*on Mueller Hinton agar