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GRADUATE INSTITUTE OF HEALTH SCIENCES

ISOLATION OF PYOGENIC MICROOGNISMS FROM INFECTED WOUND IN THE GENERAL SURGERY AND ORTHOPEADIC DEPARTMENT OF NEAR EAST HOSPITAL: A RETROSPECTIVE STUDY

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Hope Oyindmola Alaje: **ISOLATION OF PYOGENIC MICROOGNISMS FROM INFECTED WOUND IN THE GENERAL SURGERY AND ORTHOPEADIC DEPARTMENT OF NEAR EAST HOSPITAL: A RETROSPECTIVE STUDY**

Approval of Director of Graduate School of Health science

NEAR EAST UNIVERSITY

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THESIS APPROVAL CERTIFICATE

The thesis study of Medical microbiology and Clinical Microbiology graduate student **HOPE OYINDAMOLA ALAJE** with student number 20177192 titled **"ISOLATION OF PYOGENIC MICROOGNISMS FROM INFECTED WOUND IN THE GENERAL SURGERY AND ORTHOPEADIC DEPARTMENT OF NEAR EAST HOSPITAL: A RETROSPECTIVE STUDY"** in Department of Medical Microbiology and Clinical Microbiology " it is accepted as Master's Thesis".

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APPROVAL

This thesis has been approved by the above jury in accordance with the relevant articles of the Near East University Postgraduate Education, Teaching and Examination Directive and has been accepted by the decision of the Institute Board.

Prof. Dr. Hüsnü Can Başer

Director of the Institute of Health Sciences

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Hope Oyindamola Alaje

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To my family…

ABSTRACT

Postsurgical wound infections are one of the important causes of morbidity and mortality worldwide. Therefore a retrospective study was conducted to evaluate microorganisms responsible for wound infections and their patterns of antibiotic sensitivity and resistance. In imparting a summarized analysis of wound microbiology, together with current opinion and controversies respecting wound evaluation and treatment, this study has attempted to acquire and approach microbiological aspects that are influential to the management of microorganisms in wounds. Study was designed on the hospital data of past four years from general surgery and orthopedic department of the Near East University hospital in which 185 samples were collected. Identification, isolation and antimicrobial susceptibility checking of isolates were done by using standard microbiological techniques. The most common pathogens isolated from wound were *Escherichia coli and Pseudomonas aeruginosa.*

The swabs were plated on blood agar and Eosin methylene blue agar for 24 hours at 37°C. Biochemical tests were carried out on the representative isolate on each plate, and antibacterial sensitivity pattern was determined using the BD Phoenix System. The study revealed that the orthopedic department had a percentage of 123 (66.5%) and general surgery 62 (33.5%). Out of the 123 samples cultured from the orthopedic department, 56 (45.5%) were found to be positive for microorganisms, while 67 (54.5%) showed no bacterial growth. In total, 13 different isolates were identified, with the predominant bacteria being *Escherichia coli.* (11.3%) which was followed by *Pseudomonas species* (8.1%), *Staphylococcus aureus* (8.1%), *Coagulase negative staphylococcus* (CoNS) (4.8%), *Enterococcus faecium* (2.4%), *Enterococcus faecalis* (2.4%), *Candida species* (1.6%), *Citrobacter species* (1.6%), *Acinetobacter species* (1.6%), *Proteus species* (1.6%), *Enterobacter species* (0.8%), *Klebsiella species* (1.1%) and *Burkholderia cepacia* (0.8%). While in the general surgery department there are a total of 62 patient samples were considered for this study and a total of 41(66.1%) showed growth and a total of 21(33.9%) samples with no growth. In total, 11 different isolates were identified from the general surgery department, with the predominant bacteria being *Escherichia coli.* (22.6%) which is followed by *Pseudomonas species* (9.7%), *Coagulase negative staphylococcus* (CoNS) (9.7%), *Proteus species* (4.8%), *Staphylococcus aureus* (4.8%), *Citrobacter species* (4.8%), *Klebsiella pneumoniae* (3.2%), *Enterococcus faecium* (1.6%), *Candida species* (1.6%), *Acinetobacter baumannii* (1.6%), and *Enterobacter cloacae* (1.6%). Although *Pseudomonas species* showed varying resistance levels to gentamicin and ciprofloxacin, all the *Acinetobacter species* were resistant to most of the tested antibiotics used. Resistant gram negative bacteria are the most common isolates associated with wounds infection. Hence a careful selection of antibiotics to control the wound infection is required for proper management of wounds infections in order to help reduce morbidity and mortality.

ÖZET

Cerrahi sonrası yara enfeksiyonları dünya çapında morbidite ve mortalitenin önemli nedenlerinden biridir. Bu nedenle, yara enfeksiyonlarından sorumlu mikroorganizmaları ve antibiyotik duyarlılık ve direnç modellerini değerlendirmek için geriye dönük bir çalışma yapılmıştır. Yara mikrobiyolojisinin özet bir analizini yaparken, yara değerlendirmesi ve tedavisine ilişkin mevcut görüş ve tartışmalarla birlikte, bu çalışma, yaralardaki mikroorganizmaların yönetiminde etkili olan mikrobiyolojik yönleri edinmeye ve bunlara yaklaşmaya çalışmıştır. Çalışma, son dört yıllık hastane verileri üzerine, 185 numunenin toplandığı Yakın Doğu Üniversitesi hastanesinin genel cerrahi ve ortopedi bölümünden tasarlandı. İzolatların tanımlanması, izolasyonu ve antimikrobiyal duyarlılık kontrolü standart mikrobiyolojik teknikler kullanılarak yapılmıştır. Yaradan izole edilen en yaygın patojenler Escherichia coli ve Pseudomonas aeruginosa idi.

Bezler 37 ° C'de 24 saat boyunca kanlı agar ve Eosin metilen mavisi agar üzerine kaplandı. Her plakadaki temsili izolat üzerinde biyokimyasal testler yapıldı ve antibakteriyel duyarlılık paterni BD Phoenix Sistemi kullanılarak belirlendi. Çalışma ortopedik bölümün yüzdesi 123 (% 66.5) ve genel cerrahi 62 (% 33.5) olduğunu ortaya koydu. Ortopedik bölümden kültürlenen 123 örnekten 56'sının (% 45.5) mikroorganizmalar için pozitif olduğu görülürken, 67'sinde (% 54.5) bakteri üremesi görülmedi. Toplamda 13 farklı izolat tanımlandı, baskın bakteriler Escherichia coli idi. (% 11.3) ardından Pseudomonas türleri (% 8.1), Staphylococcus aureus (% 8.1), Koagülaz negatif stafilokok (CoNS) (% 4.8), Enterococcus faecium (% 2.4), Enterococcus faecalis (% 2.4), Candida türleri (% 1.6), CNS (% 10.1), Citrobacter türleri (% 1.6), Acinetobacter türleri (% 1.6), Proteus türleri (% 1.6), Enterobacter türleri (% 0.8), Klebsiella türleri (% 1.1) ve Burkholderia cepacia (% 0.8)). Genel cerrahi bölümünde bu çalışma için toplam 62 hasta örneği düşünülmüş ve toplam 41 (% 66.1) büyüme ve toplam 21 (% 33.9) büyüme göstermemiştir. Toplamda, genel cerrahi bölümünden 11 farklı izolat tanımlanmıştır, baskın bakteriler Escherichia coli'dir. (% 22.6) bunu Pseudomonas türleri (% 9.7), Koagülaz negatif stafilokok CoNS (% 9.7), Proteus türleri (% 4.8), Staphylococcus aureus (% 4.8), Citrobacter türleri (% 4.8), Klebsiella pneumoniae (% 3.2)), Enterococcus faecium (% 1.6), Candida türleri (% 1.6), Acinetobacter baumannii (% 1.6) ve Enterobacter cloacae (% 1.6). Her ne kadar Pseudomonas türleri gentamisin ve siprofloksasine karşı değişen direnç seviyeleri gösterse de, tüm Acinetobacter türleri kullanılan test edilmiş antibiyotiklerin çoğuna dirençliydi. Dirençli gram negatif bakteriler, yara enfeksiyonu ile ilişkili en yaygın izolatlardır. Bu nedenle, morbidite ve mortaliteyi azaltmaya yardımcı olmak amacıyla yara enfeksiyonlarının uygun yönetimi için yara enfeksiyonunu kontrol etmek için dikkatli bir antibiyotik seçimi gereklidir.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

EMB	Eosin Methylene Blue
MIC	Minimum Inhibitory Concentration
ICU	Intensive Care Unit
TSI	Triple Sugar Iron
MIU	Motility Indole, Urease
TRNC	Turkish Republic Of Northern Cyprus
NCCL	National Committee for Clinical Laboratory Standards
WHO	World Health Organization
MRSA	Methicillin Resistant Staphylococcus Aureus
MSSA	Methicillin-Sensitive Staphylococcus Aureus
HA- MRSA	Hospital-Acquired Methicillin Resistant Staphylococcus Aureus
VRE	Vancomycin-Resistant Enterococcus
IV	Intravenous
TSS	Toxic Shock Syndrome
STI	Soft Tissue Infections
CDC	Center for Disease Control
VRSA	Vancomycin Resistance S. Aureus
NI	Nosocomial Infection
SPSS	Statistical Package for the Social Sciences
BSI	Bloodstream Infection
SSI	Surgical Site Infection
UTI	Urinary Tract Infection

CHAPTER ONE

Wound infection is defined as the presence of a wound environment characterized by microorganisms in sufficiently large numbers, or of sufficient virulence to provoke an immune response locally, systemically or both. The break-in skin integrity can cause bacteria to enter the body and begin multiplying and proliferating. Without the protective barrier of the skin, sensitive tissues in the wound bed are vulnerable to microbial colonization. Proliferation of microorganisms in a wound can cause, local tissue damage and wound healing are disrupted (Siddiqui and Bernstein, 2010).

Nosocomial Infections are complications that result from frequent hospitalization. (Inan et al, 2005). This has been recognized as a problem that affects the quality of health care service and has a principal source of adverse outcomes. Today, nosocomial infections are said to affect about 2 million patients annually. (Apostolopoulou and Veldekis 2005)

Intensive Care Units' (ICUs) patients are more vulnerable to these infections compared to other patients in the hospital. Studies show that an ICU patient has up to seven-fold higher risk of nosocomial infections, which contributes to about 25% of all nosocomial infections in hospitals. (Barai et al, 2010; Gunserena et al, 1999). The increased use of invasive devices, immunosuppressive drugs, and immunocompromised status as well as the irrational use of antibiotic therapy in ICUs are all factors that contributes to this cause. (Shehabi and Baadran 1996).

A large percentage of nosocomial infections are related to devise utilized for patients' life support but are also responsible for such complications as ventilator-associated Bloodstream infection (BSI), Surgical site infection (SSI), and Urinary tract infection (UTI).(Corona and Raimondi 2004)

The pattern of organisms causing infections differs widely from one hospital to another, even ICUs within a hospital and also from one country to another. Local surveillance data should play an integral role in developing effective intervention studies. Therefore, the present study is aimed to know the prevalence of commonly isolated organisms in patients admitted in ICUs of Near East University Hospital.

1.1 Back Ground Of Study

1.1.1 What Is Wound?

The skin forms an effective barrier that keeps the underlying organ and tissue intact which serves as a primary defense organ. (Ndip et al, 2007) therefore a wound can represent a simple or a severe disorder to an organ such as the skin or a tissue and can spread to other tissues and anatomical sites like the subcutaneous tissue, muscles, tendons, nerves, vessels, and even to the bone. The skin is without doubt the most exposed to impairment and injury, scratches, and burns. Damaging the human skin epithelium and connective structures, the human body (HB) capability to provide protection from the outer environment is weakened.(Koppen and Hartmann 2015) It is therefore imperative to refabricate a functional epidermis or even other layers of skin. This happens by a cascade of intersecting phases, known as wound healing or wound repair.

The repair is reached by the HB's capacity to substitute lost skin structure with a viable one, and by the formation of a scar. (Bowler et al, 2001)

1.1.2 What Is Wound Infection or Colonization?

The subcutaneous or underlying tissue provides a moist, nutritious environment, which facilitates microbial colonization and proliferation. Wound colonization is poly microbial which means potentially pathogenic microorganisms are present, thus any wound is at some risk of getting infected (Dai et al, 2010).

This wound infection or skin and soft tissue infections occur mainly due to a break in the surface of the skin, wound infections can also occur as complications of ;

1. Surgery,
2. Trauma,
3. Bites or,
4. Diseases that disrupt the skin.

The cutaneous barrier is disrupted by every incision on the skin therefore microbial contamination is, thus, unavoidable, despite the best preparation made on the skin before an incision (Cochranet al, 2002). Furthermore, even though a burn site after a burn trauma is relatively believed to be sterile for the first 24 hours, wound colonization by Gram-negative bacteria is common afterward and almost inevitable (Howard 1980). Infections such as these are major obstacles to wound healing, which negatively impact the patient's quality of life and wound healing rate. Infected wounds are;

i. Odorous,
ii. Hypersensitive and,
iii. Painful.

Which results to the patient discomfort (Pruitt et al, 1998)

1.1.3 Types of wounds

The two types of wounds can be open or closed. Open wounds occurs when the wound have exposed body tissue in the base of the wound or can also be when there is a break in the skin and the underlying tissues exposed. Closed wounds have damage that occurs without exposing the underlying body tissue. External causes such as penetrating objects or blunt trauma, or internal causes such as immune, metabolic, and neurologic etiologies are responsible for these type of wounds (Garner et al 2001)

Open wound types

Penetrating wounds:

- Puncture wounds: caused by an object that punctures and penetrates the skin (e.g. knife, needle)
- Surgical wounds and Incisions: wounds caused by clean, sharp objects such as a surgical knife, and razor.
- Thermal, chemical, or electrical burns

- Bites and stings
- Gunshot wounds or other high velocity projectile which penetrates the body (which has one wound at site of entry and another at site of exit)

Blunt trauma wounds:

- Abrasions: superficial wounds due to the top layer of skin being traumatically removed (e.g. fall or slide on a rough surface).
- Lacerations: wounds that are linear and regular in shape from sharp cuts, to irregularly shaped tears from trauma.
- Skin tears: can be chronic like a wound in the base of a skin fissure, or acute due to trauma and friction.

Closed Wound Types

- Contusions: blunt trauma causing pressure damage to the skin and / or underlying tissues (includes bruises)
- Blisters: fluid filled pockets under the skin
- Seroma: a fluid filled area that develops under the skin or body tissue (commonly occur after blunt trauma or surgery)
- Hematoma: a blood filled area that develops under the skin or body tissue (occur due to internal blood vessel damage to an artery or vein)
- Crush injuries: can be caused by extreme forces, or lesser forces over a long period of time.

Ulcers

Ulcers are lesions that wear down the skin or mucous membrane that can have various causes depending on their location. Cells require blood, oxygen, and nutrients and anything that reduces the supply of these requirements can lead to ulcer formation. (Garner et al 2001; Cochranet al, 2002)

Skin ulcer types:

- Pressure ulcer: injury that causes breakdown of the skin and often the underlying tissue as well. The underlying bone or muscle. Pressure ulcers can range in severity from discolored skin areas to large open wounds that expose Diabetic Foot Ulcer (DFU): a major complication of diabetes that occurs when neuropathic (nerve) and vascular (blood vessel) complications of the disease cause altered or complete loss of feeling in the foot and/or leg
- Venous ulcer (VLU): an open sore that develops when the skin is broken and air or bacteria gets into the underlying tissues. VLUs are caused by venous disease; a disease of the veins of the leg. (Cochranet al, 2002)
- Ulcerative dermatitis: an ulcer due to a dermatological condition
- Genital ulcer: painful, non-sexually acquired genital ulceration.

1.2 Microorganisms Associated With Wound Infections

Staphylococcus aureus (*S. aureus*) and *Pseudomonas aeruginosa (P. aeruginosa)* are the most common pyogenic bacteria associated with wound infection. These two bacteria together account for up to 20 - 40% of all nosocomial, post-surgery and burn infections. Enterococci and Enterobacter are other microorganisms that also been associated with wound infections, especially after abdominal surgery in immunocompromised patients (Kotz et al, 2009).

The mentioned risks associated and antibiotic resistance, makes wound infections a global problem. The antimicrobial resistance factors include changes in microbial ecology, genetics and the non-selective use of antimicrobial agents. *Methicillin-Resistant S. aureus* (*MRSA*), *Vancomycin-resistant Enterococci* (*VRE*) are the medically relevant examples (Al-Waili et al, 2011)

Given the need for effectively treating wound infections, this current study is aimed to identify pathogenic bacteria present in wound infections and determine their antimicrobial susceptibility pattern.

Wound diseases represent a 70-80% death rate (Wilson et al, 2204). Wound may be countered in clinically either postoperatively, taking after injury, or could principally be of infective birthplace (Sule et al, 2002). Despite the starting point, all wounds may be debased by microorganisms both externally and internally (Bellchambers et al, 1999).

Wound tainting sources are from:

(I) Nature (exogenous microorganisms noticeable all around),

(ii) The skin (including those from ordinary skin microflora for example, *Staphylococcus epidermidis (S. epidermidis)*, *Micrococci*, skin diphtherias, and *Propionibacterium*),

(iii) Endogenous sources including mucous layers (fundamentally the gastrointestinal, oropharyngeal, and genitourinary mucosae). (Duerden 1994)

The role and essentialness of microorganisms in wound healing has been debated for a long time. A few studies consider the microbial numbers to be basic in anticipating wound recuperation and infection, while others consider the sorts of microorganisms to be of more prominent hugeness. Albeit wound contaminations are brought on by microorganisms, broad debate still exists concerning the system by which they cause disease. (Bowler 1998)

Pyogenic infection is characterized by local inflammations and usually presents with pus formation. (Koneman et al, 2005) Pyogenic wound infections may be from either endogenous or exogenous sources. The wound infection and soft tissue infections (SSTIs) are caused by microbial pathogens during or after trauma, burn injuries and surgical procedures. (Cogenet al, 2008). Which result in the production of pus. Both aerobic and anaerobic bacteria can cause these pyogenic wound infections, which commonly occur under the hospital environment resulting in;

1. Significant morbidity,
2. Prolonged hospitalization and,
3. Huge economic burden (Dryden 2010; Scalise et al, 2015).

CoNS is a more dominant organism in pus (Bowler et al, 2001; Chopra et al, 1994).

According to Courvalin (Courvalin 2005) antibiotic resistance among bacteria is becoming a more serious problem throughout the world. Courvalin said that the evolution of bacteria towards resistance to antimicrobial drugs, including multidrug resistance, is actually unavoidable because it represents a particular aspect of the general evolution of bacteria that is unstoppable.

The empiric antimicrobial drugs used to treat patients caused the emergence of Antimicrobial resistance. It is important to monitor the of resistance patterns in the hospital, this is needed to overcome these difficulties and to improve the outcome of serious wound infections in hospital settings (El-Azizi et al, 2005)The emergence of antibiotic-resistant pathogenic bacteria are considered as grave threats to the public health worldwide (Rice 2006).

During the last few decades, multidrug-resistant Gram-negative bacterial strains such as;

1. *Acinetobacter baumannii (A. baumannii),*
2. *Escherichia coli (E. coli),*
3. *Klebsiella pneumonia (K. pneumonia),*
4. *P. aeruginosa and,*
5. *MRSA*

Were increasingly associated with pyogenic wound infections under hospital settings due to extensive overuse and inadequate dose regimen of antibiotics. (Rice 2006; Misic et al, 2014; Iredell 2016).

Rapid emergence of multidrug-resistant bacteria poses a serious threat to public health globally due to the limited treatment options and the discovery of new classes of antibiotics. (Iredell 2016; Cerceo et al, 2016). Therefore, this present study was undertaken to see bacteriological profiles of pus with their resistant pattern.

1.3 Aim and Objective

The aim of this work is to determine the microorganisms that cause pus formation in wound infection.

Given the need for rapidly and effectively treating wound infections, the study aimed to identify pathogenic bacteria present in wound infections and determine their antimicrobial susceptibility pattern.

CHAPTER TWO

Review of Literature

At the point when microorganisms or different organisms present in the wound move further into wound tissue and multiply in a manner that overpowers the immune system of the individual. (Collier, 2004; Siddiqui and Bernstein, 2010).

Local wound infection is always in a subtle manner that may not be readily detected without conscientious wound assessment and a strong understanding of the early indicators that microorganisms may have proliferated in large quantities that as a poses threat to the individual. (Sibbald et al, 2006; 2007). Wound breakdown, delayed healing, a pocket of pus and epithelial bridging are the early signs that the wound tissue is not properly or not healing along the optimal trajectory (Cutting and Harding, 1994; Gardner et al, 2001; Collier, 2004; Gardner and Frantz, 2008).

Detection of the earliest signs and symptoms of wound infection is essential and should not be overlooked at any point. Prompt intervention with appropriate infection management strategies is important for preventing extensive local tissue damage, avoiding further microbial proliferation, delays to wound healing and several potential severe sequelae. In the 2016 update of the Wound Infection in Clinical Practice consensus document (IWII, 2016) these early indicators of local wound infection gradually emerge (particularly in chronic wounds) are, therefore, referred to as covert signs of infection.

Further along, the wound infection, when the host immune system response to microbial invasion increases, the signs of wound infection becomes more evident. Pus discharge, erythema, and swelling increase in body temperature increase in malodor and increase in pain are the most common signs of pyogenic infection in any organ of the body (Sibbald et al, 2003; IWII, 2016)

In wounds infections, these signs of infection are also followed by delays in wound healing beyond expectations. When these pyogenic bacteria inhibit the development of new and healthy granulation tissue, undermining can occur. When these pyogenic bacteria interfere with the formation of new healthy collagen, friable granulation tissue can occur due to the bacterial burden (Sibbald et al, 2003).

2.1 Biofilm formation.

As microorganisms increase in number and virulence, biofilm is bound to form, mature and continue to disperse (Cutting and McGuire, 2015; Nouraldin et al, 2016; Uppuluri and Lopez-Ribot, 2016). Biofilm is more likely to form and be present in chronic wounds because they have a sufficient amount of bacterial virulence and or numbers to evoke an inflammatory and immune response from the host.

Clinical signs and symptoms that biofilm may be present are the same as those of early local infection, which include, increasing exudate (pus) and moisture, inflammation and erythema, poor granulation or friable hyper granulation. Biofilm is most likely to be present, especially in chronic wounds that have delayed healing, insensitivity to antimicrobial therapy and failure of using appropriate antibiotics to resolve symptoms. (IWII, 2016). The clinical signs and symptoms listed above are confirmed present, the biofilm-based wound care, which includes appropriate cleansing, wound bed debridement and topical antiseptics, these are appropriate course of wound management (Rhoads et al, 2008; Leaper et al,2012; Wolcott, 2015; Bianchi et al, 2016).

2.2 Surgical site wound infection

S.aureus is the most important pathogen after surgical procedures and also an important microorganisms that form biofilm in wound infection. The nasal carriage is the main risk factor for Surgical Site Infection (SSI) caused by *S. aureus* because carriers are two to nine times likely to acquire Staphylococcus aureus SSIs than non -carriers. Which in turn means this carriage rate in the general population is about 37.2%. Several studies have conducted shown that nasal decolonization in patients undergoing orthopedic surgery by using perioperative intranasal mupirocin, which is a prevention strategy, a safe method to eradicate nasal colonization. This decolonization of the Preoperative patient significantly decreases the incidence of S. aureus Surgical Site Infections." [Barbos et al. (2009)].

MRSA raises particular concern, recent studies and reviews suggest the rates of per prosthetic joint infection with MRSA are becoming more increased. About 50% of SSIs in hip adjustment and replacement surgeries are caused by *S. aureus*, and up to 50-59% of the isolates are methicillin-resistant.

Surgical Site Infection in an orthopedic patient is a devastating complication of total joint surgery. Treatment is available but it is complicated by the overly frequent presence of multi-resistant organisms. The recent review of per prosthetic joint infections in a community hospital for a period in 2005, shows that up to 92% of the pyogenic wound infections were reported to be caused by gram-positive organisms and up to 30% of those infections were caused by MRSA, and a rate that is higher than the 13% rate of MRSA infection were observed per prosthetic joints in 1999. [Eiselt (2009)].

Reports shows that the nasal colonization with MRSA increases the risk of SSI with MRSA for patients treated in surgical intensive care units or general surgery department (Mest et al. 1994), patients who are on admission in the hospital (Davis et al. 2004), and those who have undergone continuous ambulatory peritoneal dialysis (CAPD) (Lye et al. 1993).

A particular study has identified the nasal carriage of *S. aureus* as the only important risk factor for the development of SSI following orthopedic surgery or implant surgery [Kalmeijer et al. (2000)].

In the early stages, when microbial colonization of pyogenic microorganisms has not interfered with the host's wound healing process, being alert to the risks of local infection is important. These (and other) stages on the wound infection should, implement methods to prevent cross-infection, facilitate wound drainage, using antimicrobial dressings and optimizing the wound bed environment, all these mentioned optimize healing and reduce the risk of the wound infection by bacterial proliferation.

Optimizing the health of the individual or patient is important, including optimization of nutritional status, is important to strengthening the potential of the immune response to respond rapidly and effectively (WUWHS, 2007; Australian Wound Management Association and New Zealand Wound Care Society,

2012; National Pressure Ulcer Advisory Panel et al, 2014; Lipsky et al, 2016).

Emergence of signs of local wound infection and rapid response with appropriate management methods and strategies is imperative. This point having the clinical skills to be able to identify covert signs of pyogenic infection is an advantage but, when the earliest indicators of local infection are evident, more aggressive treatment should be initiated. Which generally includes the use of topical antimicrobial therapies, including antibacterial wound dressings and topical antiseptics (Leaper et al, 2012).

Antiseptics are non-selective solutions that disrupt the ability of microorganisms to proliferate. However, these antiseptics can have potential adverse effects because some are cytotoxic (may kill healthy wound tissues). However the cytotoxicity is thought to be dependent on the concentration used (Siddiqui et al, 2010; Leaper et al, 2012), antiseptic use is, therefore, advised to be used at the lowest concentration, and some preparations that cause more excessive tissue damage are no longer recommended or used. (E.g. hydrogen peroxide and sodium hypochlorite) (IWII, 2016).

The increasing antibiotic resistance which has become a global crisis (World Health Organization, 2014; Centers for Disease Control and Prevention, 2016) requires all antibiotics use to be reserved or used only when local interventions are insufficient to control infection.

Therefore use of antibiotic therapy is not recommended for use or routine procedure in promoting wound healing, however, there is the use of antibiotics when there is overt signs of wound infection present and causative agent are detected, confirmed and its sensitivities are known (Gürgen, 2014).

CHAPTER THREE

Materials and Methods

3.1 Study Areas

Northern Cyprus, officially the Turkish Republic of Northern Cyprus (TRNC) is the state that comprises of the northeastern part of the eastern Mediterranean island of Cyprus, divided between Turkey and Greece since the late-20th century.

The total population of North Cyprus is estimated to be 326,000 (2017). The present study was conducted in North Cyprus from the month of January 2019 to the month of December 2019.

3.2 Sampling

185 samples for this study considered from the period of September 2015 to August 2019 and were collected from Near East Hospital Cyprus. These samples included the samples collected from two different departments (General surgery department and orthopedic department). These samples only included wound and Pus culture test. The demographic information (age, sex) were obtained from the patient's medical record. The sensitivity pattern of the pyogenic organisms was determined against commonly used antibiotics using BD-Phoenix instrument. The samples were labeled accordingly and were subjected for screening in Microbiology Laboratory at Near East University Hospital, Nicosia North Cyprus.

3.3 PURE CULTURE ISOLATION

All the samples were first processed to get pure culture by sub culturing using the Blood agar and EMB agar.

3.3.1 Eosin Methylene blue (EMB) media.

Eosin methylene blue agar from OXIDE private limited was used. The media was prepared according to the protocol provided by the company and autoclaved at 121°C for 20 minutes. After autoclave 25 ml of the media were poured into sterilized Petri plates (99 mm in diameter) under aseptic condition. After the media get solidified samples were inoculated under aseptic conditions using a sterile inoculating loop. After inoculation, the plates were incubated at 37°C overnight

3.3.2 Blood Agar

Blood agar consists of a base containing a protein source (e.g. Tryptones), soybean protein digest, sodium chloride (Nacl), agar and 5% sheep blood. The blood agar base was prepared as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes and transfer the prepared blood agar base to a 50°C water bath. When the agar base is cooled to 50°C, sterile blood was added aseptically and mix well gently to avoid the formation of air bubbles. Afterwards dispense to sterile petri plates aseptically.

3.3.3 Gram Staining

Using sterile techniques, a smear of each isolate was prepared, dried and heat-fixed on glass slides. The smear was flooded with crystal violet and allowed for one minute. It was then washed with distilled water and flooded with Gram's Iodine and allowed for one minute. Then it was washed with distilled water, decolorized with 95% ethyl alcohol and again washed with distilled water. After that, it was counterstained with safranin for 45 seconds and washed with distilled water. The slide was dried and examined under a compound microscope at 100 X using oil emulsion.

3.4 BIOCHEMICAL TESTS

3.4.1 Preparation of cell suspension

Cell suspension was prepared for running biochemical tests. Cell suspension was prepared in saline water (0.85% NaCl) and compared with McFarland turbidity standard solution (Gomes et at, 2001).

3.4.2 Catalase Test

Catalase test was used for the detection of catalase enzyme. This test was performed by taking 2-3 ml of hydrogen peroxide. Then take a colony of bacterial culture from the nutrient agar plate by using glass or wood sticks and put on hydrogen peroxide. Production of bubbles was considered a positive result.

3.4.3 Oxidase Test

This test was performed by soaking a piece of filter paper using oxidase reagents. Pick some fresh growth from the culture plate with a disposable loop or stick and rub onto the filter paper. Examine for blue color within 10 seconds for positive test.

3.4.4 Indole production test

Using sterile techniques experimental organism was inoculated into its appropriately labeled deep tube containing motility indole urea (MIU) media with the help of a wire loop. The tubes were incubated for 24 hours at 37°C. After that add Kovac's reagent and observe red color within 10 min.

3.4.5 Citrate utilization test

Using sterile techniques, organisms were inoculated into Simmons citrate agar by mean of streak inoculation. Cultures were incubated for 24 hours at 37°C. Observed the change in the color of media from green to blue color.

3.4.6 Motility Test

Inoculated tubes contain semisolid nutrient agar with a pure culture by stabbing the center of the column of medium to greater than half the depth. Tubes were incubated for 24-48 hours at 35°C in the anaerobic atmosphere.

3.4.7 Preparation of the inoculums

Bacterial suspension was prepared in 5 ml normal saline (0.85%NaCl solution). For this purpose fresh culture of 24 hours old was used. 2 to 3 well-isolated colonies were taken with the help of a platinum wire loop. After shacking the bacterial suspension was compared to 0.5% Mc Farland standard.

3.5 The BD Phoenix System.

The BD Phoenix System consists of an instrument, software, disposable panels, broths for ID and AST, and an AST indicator. The ID method employs modified conventional, fluorogenic, and chromogenic substrates. The AST method is a broth-based micro dilution test that utilizes a redox indicator to enhance the detection of organism growth. The NMIC/ID-26 panels were used in this study. A 0.5 McFarland suspension of the test organism was made in the ID broth. The crystal Spec Nephelometer was used to verify the density of the suspension and 25µL of this suspension was added to the AST broth. One drop of the AST indicator was previously added to the AST broth. The suspension in the ID broth was used to inoculate the ID wells of the panel and the suspension in the AST broth was used to inoculate the AST wells. After loading the panels into the instrument, the panels are read at 20minute intervals by the instrument. IDs, minimal inhibitory concentrations (MICs), and category interpretations are generated. Organism identification is used in the interpretation of the MIC values of each antimicrobial agent producing Susceptible, Intermediate, or Resistant (SIR) result classifications. Final results are available in 2-12 hours for ID and 4-16 hours for AST, however, the majority of IDs were completed in 2-3 hours and MICs in 6-8 hours. The Phoenix system also includes the BD Xpert system software which analyzes ID and AST results against pre-defined rules and notifies the user of atypical results and patient conditions that may require further action. Nadarajah R et al., (2004).

3.6 Statistical Analysis:

After collecting the data were successfully analyzed through SPSS version 22 Statistical consults and the result will be compared to literature.

CHAPTER FOUR

RESULTS

Approximately185 samples were considered for this study to determine the sensitivity and resistance pattern of different pathogens causing wound infections, in the general surgery and orthopedic unit. Various samples are collected for isolation of certain pathogens includes: pus c/s, pus swab, wound c/s were among the major source of isolation of these infectious pathogens in this study. Table I down below shows that, the orthopedic unit had a total of 123 patient samples that were considered with a percentage of 66.5% while the general surgery unit had a total of 62 patient samples considered with a percentage of 33.5%

Table I- Percentage of patient from the two different departments

	Frequency	Percent (%)
Orthopedic	123	66.5
General surgery	62	33.5
Total	185	100.0

4.1 Orthopedic Department Results.

Out of the 123 samples cultured from the orthopedic department, 56 (45.5%) were found to be positive for microorganisms, while 67 (54.5%) showed no bacterial growth. Out of the 123 patients from the orthopedic unit, the minimum age considered is 21 while the maximum is 87 and the Mean age in the orthopedic department is 65 shown in (Table II) Both male and female were considered for the study. The males have a total number of 40 (32.5%) and females with 83 (67.5%) shown in the (Figure I). However Gender had no significance to growth as the P value is 0.640. The total number of outpatient is 15 (12.2%) and inpatient is 108 (87.8%) in the orthopedic Department shown in (Figure II). However inpatient and outpatient has no significance to growth in this study as the P value is 0.517.

Table II; The mean Age of patients in the Orthopedic Department.

	Number	Minimum	Maximum	Mean	Standard deviation
	123	21	87	65	18,504
	123				

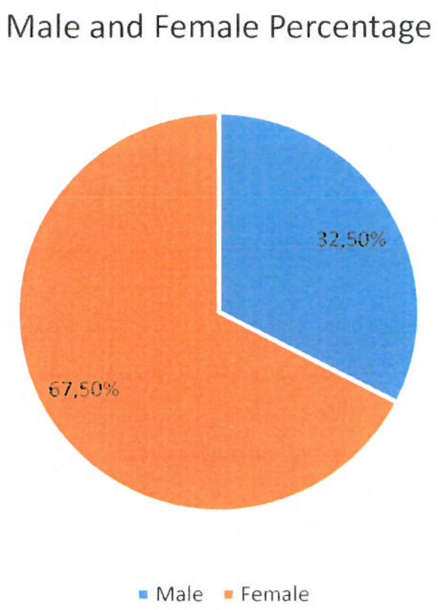

Figure I: Male and Female percentage in the orthopedic Department.

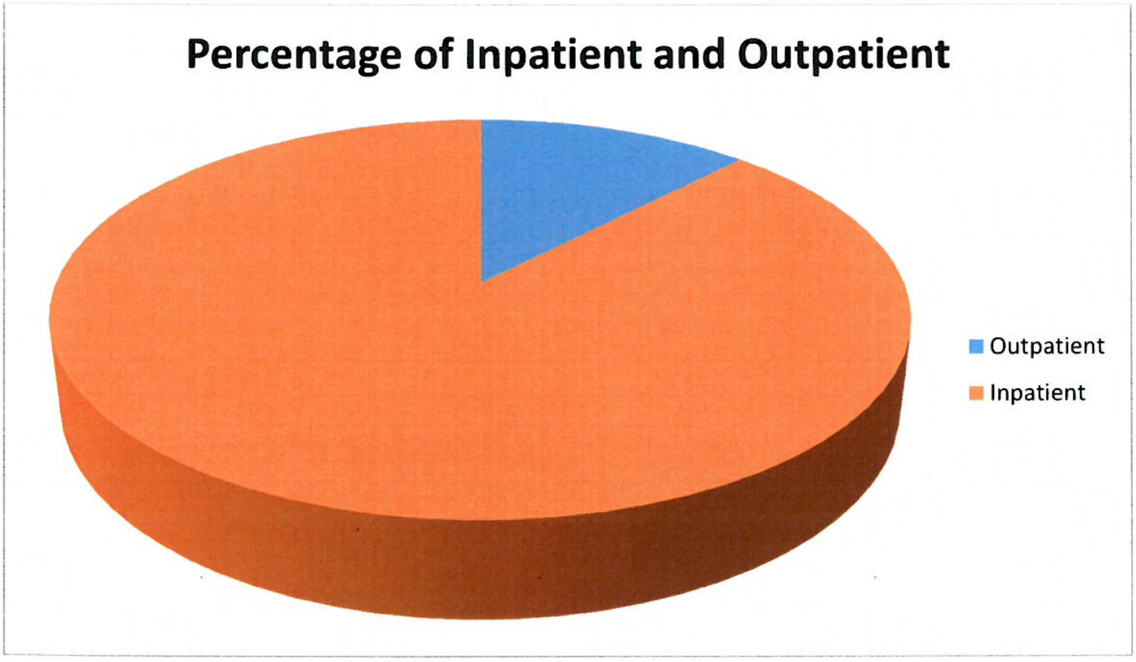

Figure II: Percentage of Inpatient and Outpatient in the orthopedic department.

In total, 13 different isolates were identified, but a total 124 microorganisms were isolated out of 123 patient samples with 1 patient sample having *E.coli* and *Candida* specie together. The predominant bacteria being *E. coli.* (11.3%) (Figure III). It was followed by *P.aeruginosa* (8.1%), *S. aureus* (8.1%), CoNS (4.8%), *Enterococcus faecium (E. faecium)* (2.4%), *Enterococcus faecalis (E. faecalis)* (2.4%), *Candida species* (1.6%), *Citrobacter species* (1.6%), *A. baumannii* (1.6%), *Proteus species* (1.6%), *Enterobacter species* (0.8%), *K. pneumonia* (1.1%) and *Burkholderia cepacia* (0.8%).

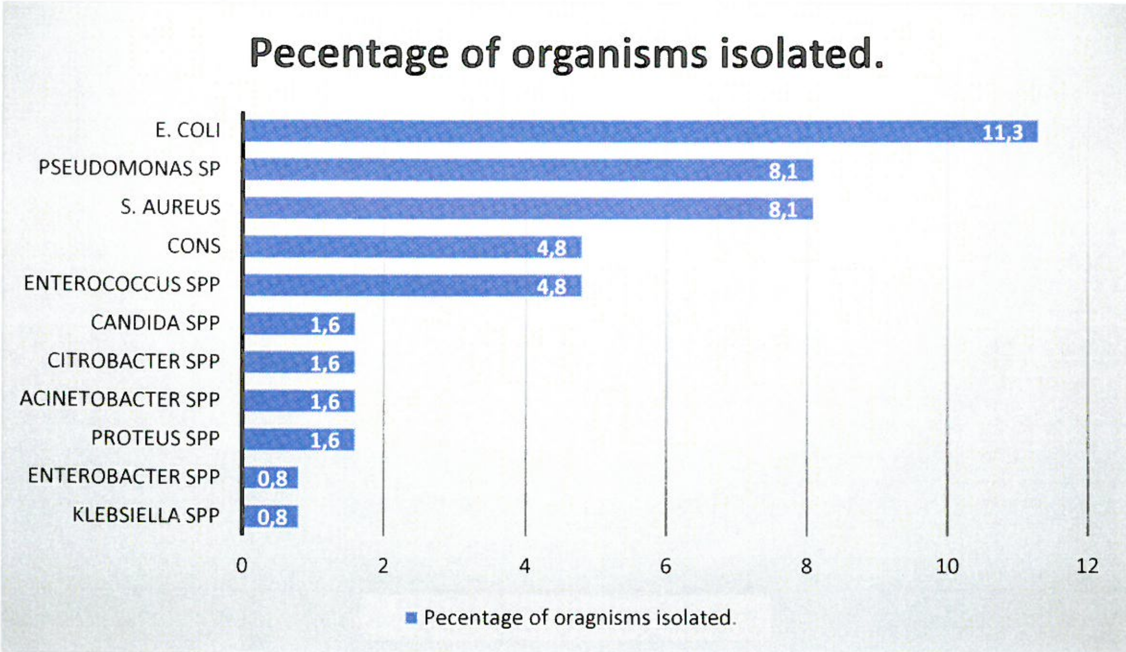

Figure III: Percentage of organism isolated from the orthopedic department.

After the data collection from the orthopedic unit, the susceptibility and resistance pattern of antimicrobials were analyzed for both Gram positive and Gram negative bacteria. For the Gram Negative bacteria A. *baumannii* (n=2) showed resistant to almost all the antibiotics used and showed only sensitivity to trimethoprim-sulphamethoxazole (SXT) 100%, Tigecycline 100%. *P. aeruginosa* (n=10) showed varying sensitivity and resistance values to the antibiotic used but has the highest sensitivity to Amikacin (AK) 100% and Piperacillin/ Tazobactam (TPZ) 100% but high resistance to Aztreonam (ATM) 80%. The *Enterobacteriacae* (n=20) also showed varying sensitivity and resistance to antibiotic but had the highest sensitivity to Amikacin (AK) 100%, Imipenem (IPM) 95% but had the highest resistance to trimethoprim- sulphamethoxazole (SXT) 63%. *Burkholderia cepacia* (n=1) showed sensitivity to only trimethoprim-sulphamethoxazole

(SXT) 100% but was resistant to Ceftazidime (CAZ) 100%.

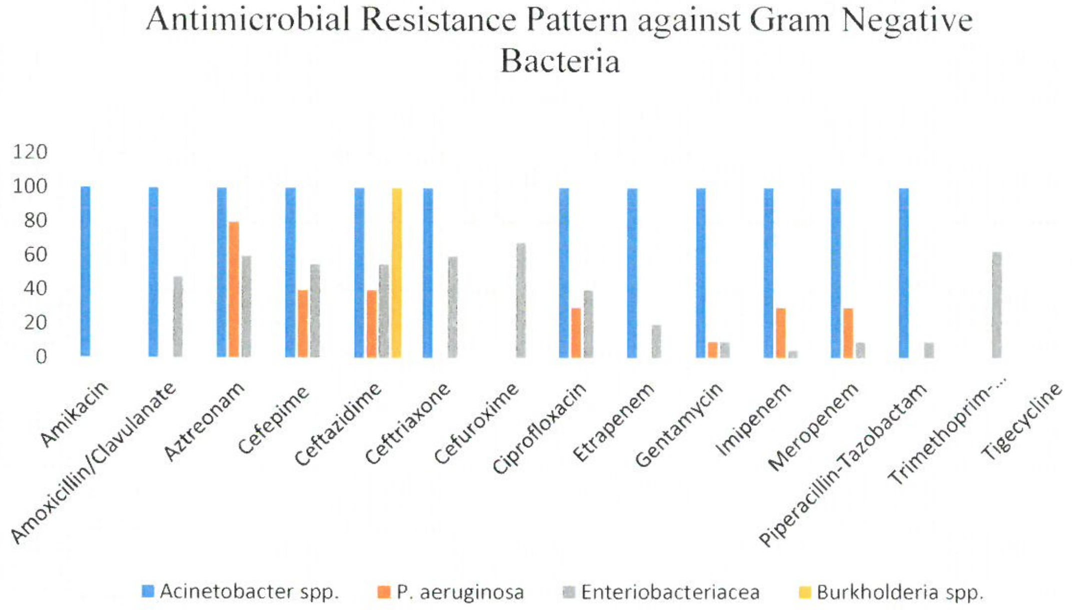

Figure IV: Antimicrobial Resistance Pattern against Gram Negative Bacteria

While for Gram Positive bacteria *CoNS* (n=6) showed varied sensitivity percentage but had the highest to Linezolid (LNZ) 100%, Teicoplanin (TEC) 100%, Vancomycin (VAN) 100%, but highest resistance to Ciprofloxacin (CIP) 83%, Trimethoprim-Sulphamethoxazole (SXT) 83%, and Erythromycin (E) 83%. *S. aureus* (n=10) showed highest sensitivity to Levofloxacin (LEV) 100%, Vancomycin (VAN) 90%, and Teicoplanin (TEC) 90% but high resistance to Erythromycin (E) 60%. *E. faecalis* (n=3) had high sensitivity to Ciprofloxacin (CIP) 100%, Linezolid (LNZ) 100%, Vancomycin (VAN) 100% but high resistance to Ciprofloxacin (CIP) 100%, and Erythromycin (E) 100%. *E. faecium* (n=3) had high sensitivity to only Vancomycin (VAN) 100% but highest resistance to Ciprofloxacin (CIP) 100%, Levofloxacin (LEV) 100%.

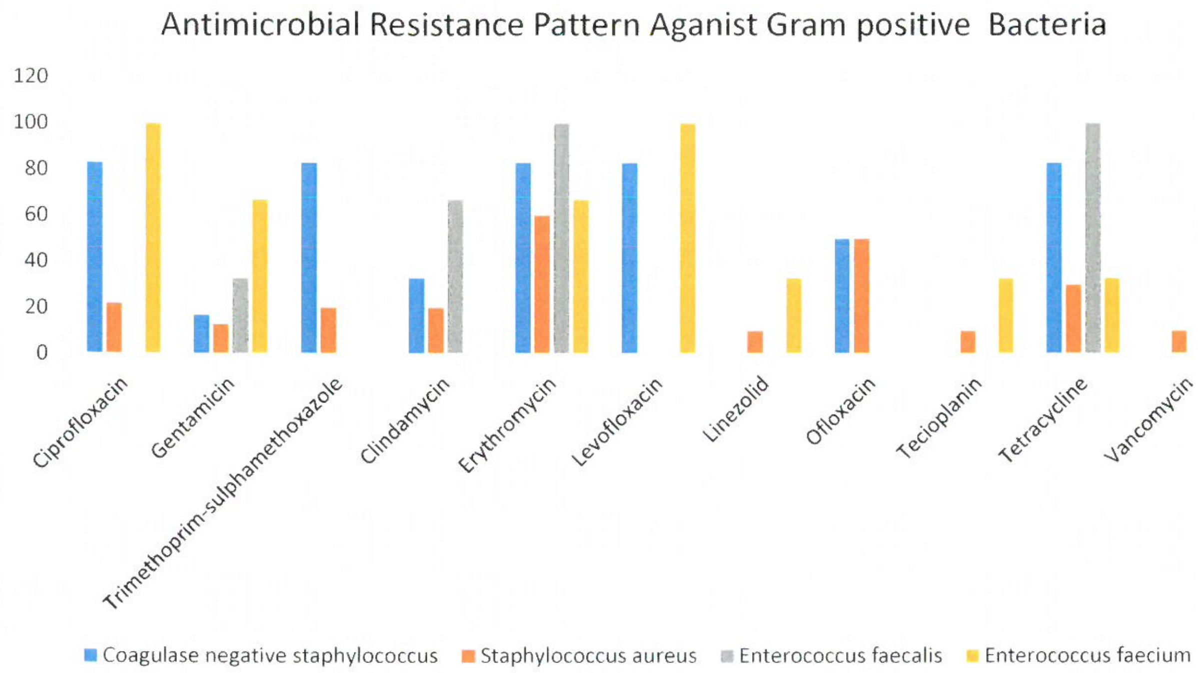

Figure V: Antimicrobial Resistance Pattern against Gram positive Bacteria.

The total number of microorganisms isolated which is 13 different microorganisms, with the addition of the samples that showed no growth makes a total of 124 microorganisms, but we worked with a total of 123 samples from the orthopedic department which means 1 sample in the department had a ploy microbial colonization. (1 patient samples have *E.coli* and *Candida spp.* together).

Enteriobacteriacea that were isolated had a total number of 20 and the extended spectrum beta-lactamase (ESBL) had a total number of 11 from the Enteriobacteriacea. Therefore ESBL is 11/20 to be (55%). Also in the orthopedic department the gram positive bacteria isolated shows that the *CoNS* had a total number of 6 and *MRCNS* had a total number of 3 from the *CoNS* isolated. Therefore, *MRCNS* is 3/6 (50%). *S. aureus* had a total number of 10 and *MRSA* had a total number of 5 from the *S. aureus* isolated. Therefore, *MRSA* is 5/10 (50%), S. aureus had a total number of 10 and *VRSA* had a total number of 1 from the *S. aureus* isolated. Therefore, *VRSA* is 1/10 (10%).

4.2 General Surgery Department Results

Table IV shows that in the general surgery department there are a total of 62 patient samples were considered for this study and a total of 41(66.1%) were culture positive and a total of 21(33.9%) were culture negative.

Table III; Percentage of growth of microorganisms in the General surgery Department.

	Frequency	Percent (%)
Growth	41	66.1
No Growth	21	33.9
Total	62	100.0

A total of 62 patients from the general surgery unit, the minimum age in the department is 19 while the maximum is 89 with the mean age of study being 52 shown in (Table IV). Both male and female were considered for the study. The males have a total number of 23 (37.1%) and females with 39 (62.9%) shown in the (Figure VI). However the sex had no significance to growth in this study as the P value is 0.907(<0.05) The total number of outpatient is 30 (48.4%) and inpatient is 32 (51.6%) in the orthopedic Department shown in (Figure VII) However patients being in or out patient had no significance to growth as the P vaule is 0.652.

Table IV- The mean Age of patients in the General surgery Department.

	Number	Minimum	Maximum	Mean	Standard deviation
	62	19	89	52	20,990
	62				

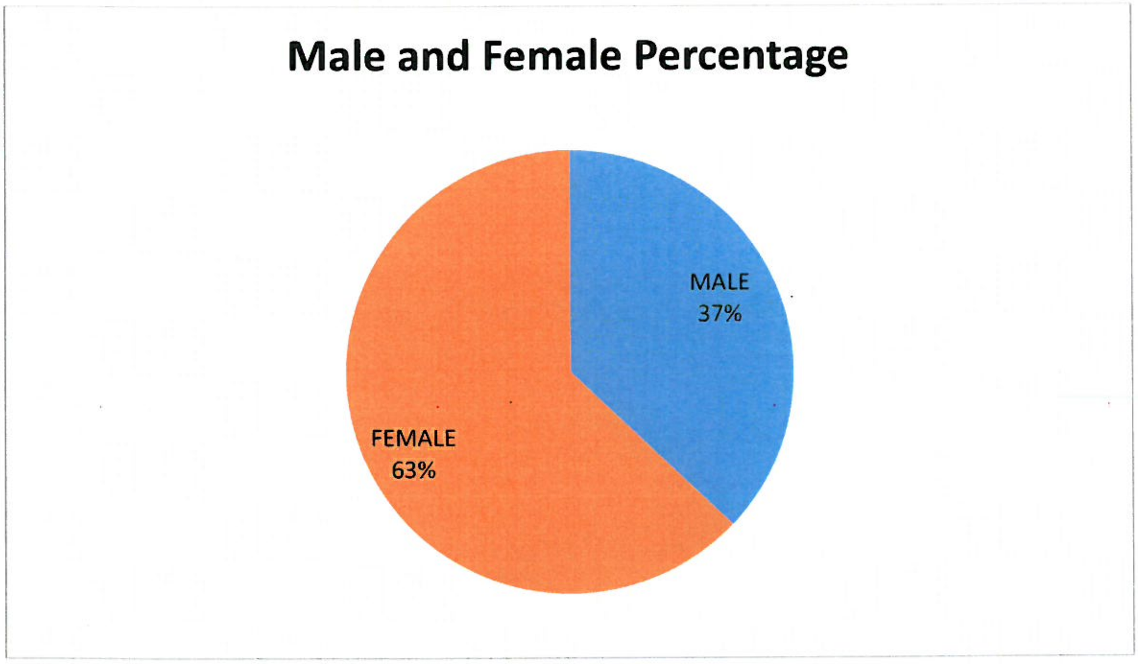

Figure VI: Male and Female Percentage from the General Surgery Department.

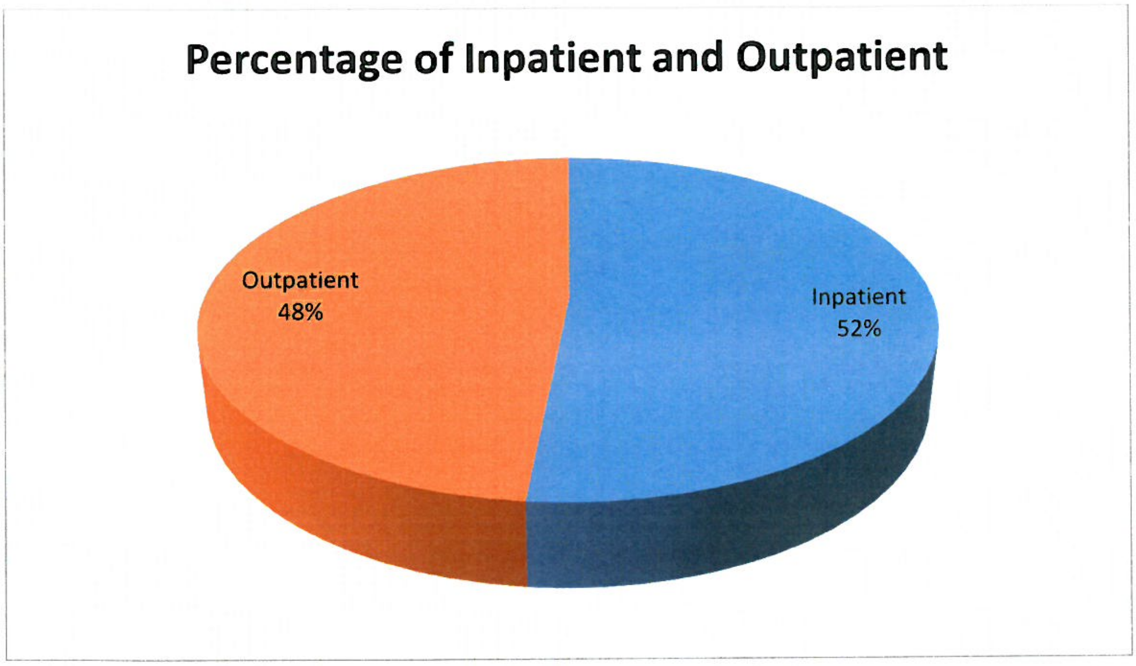

Figure VII: Percentage of Inpatient and Outpatient in the General surgery Department.

In total, 11 different isolates were identified also from the general surgery department, with the predominant bacteria being *E. coli*. (22.6%) (Figure VIII). It was followed by *P. areuginosa* (9.7%), *CoNS* (9.7%), *Proteus species* (4.8%), *S. aureus* (4.8%), *Citrobacter species* (4.8%), *K. pneumoniae* (3.2%), *E. faecium* (1.6%), *Candida species* (1.6%), *A. baumannii* (1.6%), and *Enterobacter cloacae* (1.6%).

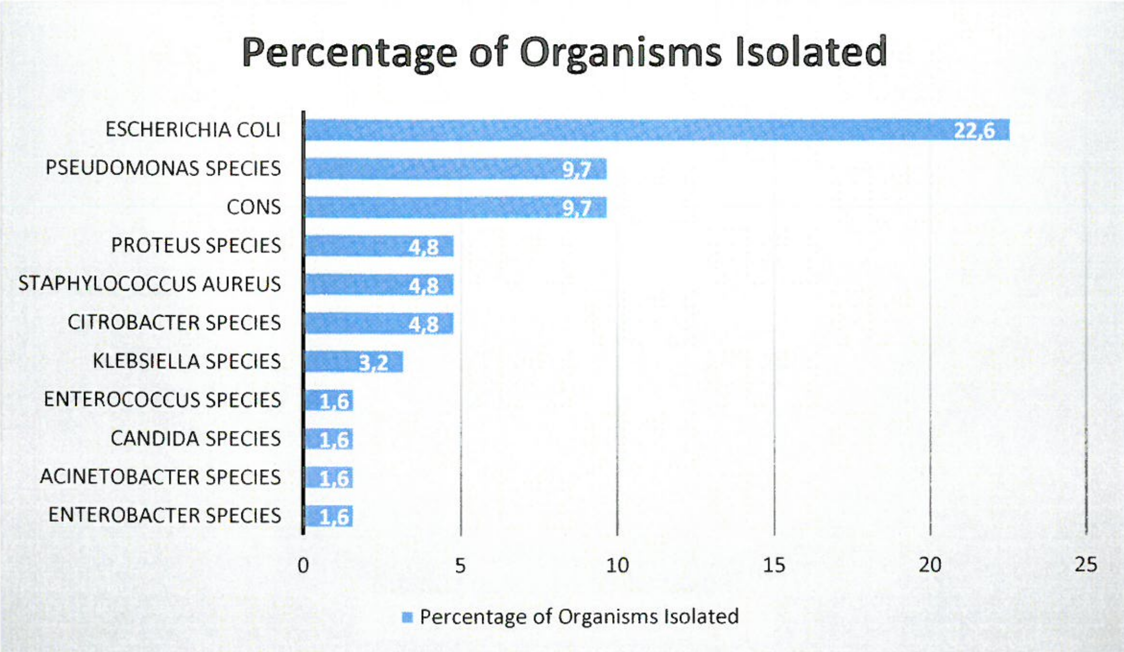

Figure VIII: Percentage of Organisms Isolated from the General Surgery Department.

After the data collection from the General surgery unit, the susceptibility and resistance pattern of antimicrobials were analyzed for both Gram positive and Gram negative bacteria. For the Gram Negative bacteria *A. baumannii* (n=1) showed resistant to almost all the antibiotics used and showed only sensitivity to Tigecycline 100%. *P. aeruginosa* (n=6) showed varying sensitivity and resistance values to the antibiotic used but has the highest sensitivity to Imipenem (IMP) 100%, but high resistance to Aztreonam (ATM) 50%. The *Enterobacteriacae* (n=23) also showed varying sensitivity and resistance to antibiotic but had the highest sensitivity to Amikacin (AMK) 100% and Meropenem (MEM) 100% but had the highest resistance to Amoxicillin/Clavulanate (AMC) 100%.

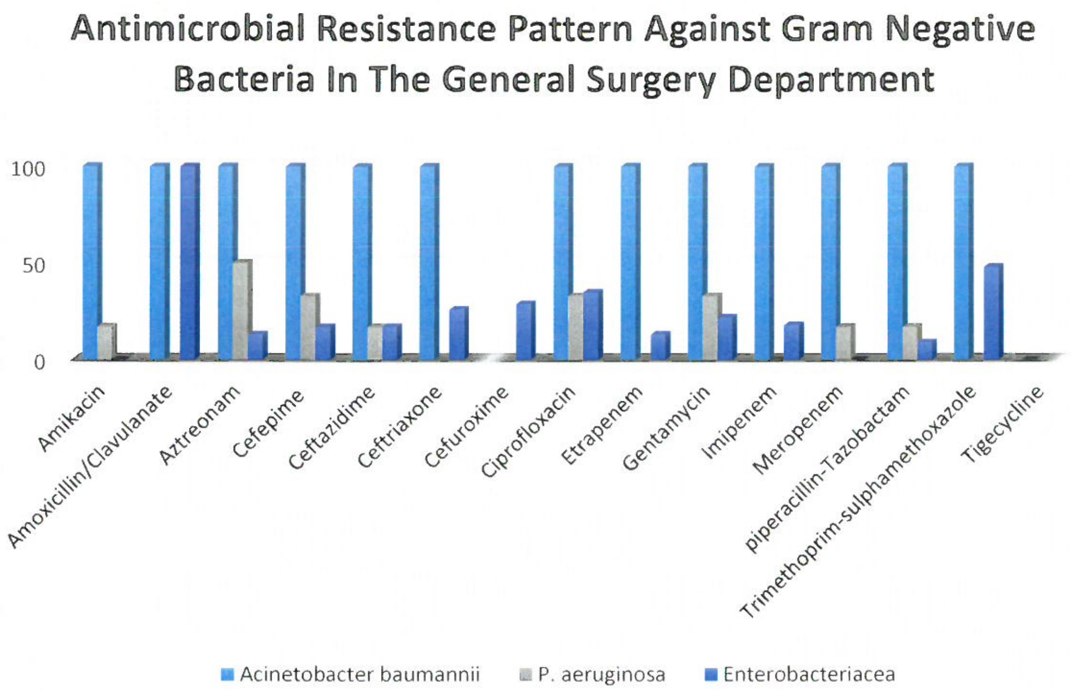

Figure IX: Antimicrobial Resistance Pattern against Gram Negative Bacteria in the General Surgery Department

The Gram Positive bacteria sensitivity and resistant pattern in *CoNS* (n=6) shows a varied sensitivity percentage but had the highest to Linezolid (LNZ) 100%, Teicoplanin (TEC) 100%, Vancomycin (VAN) 100%, but highest resistance to Ciprofloxacin (CIP) 33%, Trimethoprim-Sulphamethoxazole (SXT) 33%. *S. aureus* (n=3) showed high sensitivity to almost all the antibiotic used Levofloxacin (LEV) 100%, Vancomycin (VAN) 90%, and Teicoplanin (TEIC) 90% but only had high resistance to Ciprofloxacin (CIP) 33%. *E. faecium* (n=1) had high sensitivity to Linezolid (LNZ) 100%, Teicoplanin (TEC) 100%, Vancomycin (VAN) 100% but high resistance to Ciprofloxacin (CIP) 100%, and Erythromycin (E) 100%.

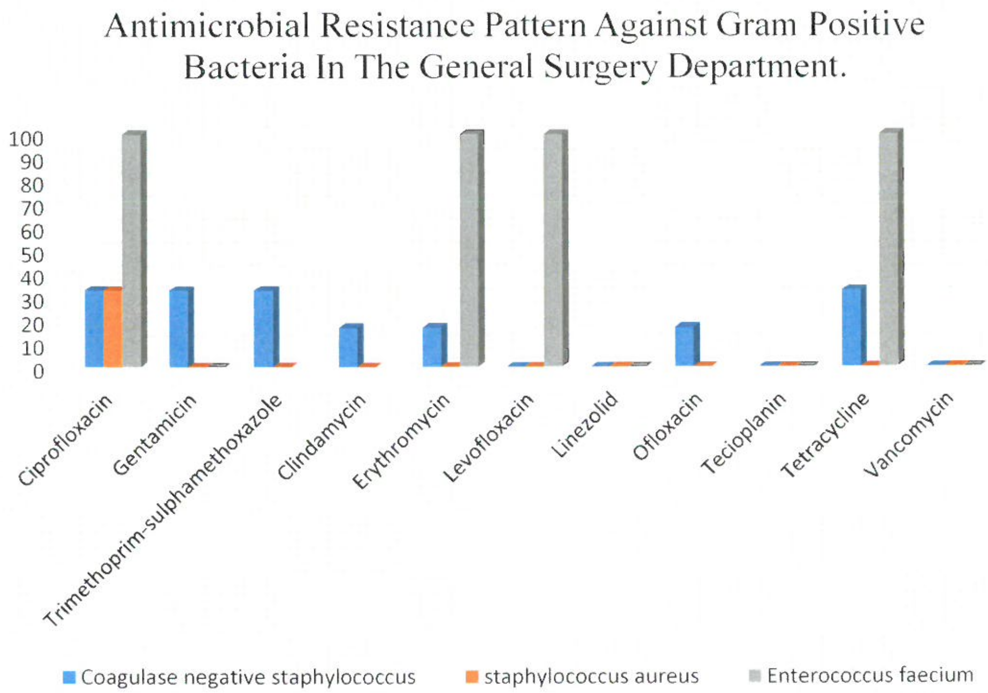

Figure X: Antimicrobial Resistance Pattern against Gram Positive Bacteria in the General Surgery Department.

The total number of microorganisms isolated which is 11 different microorganisms, with the addition of the samples that showed no growth makes a total of 62 microorganisms, but we worked with a total of 62 samples from the general surgery department which means no sample in the department had a ploy microbial colonization.

In the general surgery department, the *Enteriobacteriacea* has a total number 23 and 3 ESBL were isolated from the *Enteriobacteriacea*. Therefore ESBL (+): 3/23 (13%), while in the gram positive result shows *CoNS* to have a total number of 6 and 1 MRCNS was isolated from the *CoNS*. Therefore MRCNS: 1/6 (%17).

4.3 Discussion

Surgical wound infections are the commonest complications and one of the most frequently encountered nosocomial infections despite the gradual progress in surgery, surgical techniques and antibiotic prophylaxis thus they are supposed to be an important cause of morbidity and mortality worldwide. (Tesfahunegn et al, 2009; Ashby et al, 2010). Wound infection has been a major concern among health care practitioners not only in terms of increased trauma to the patient but also in view of its burden on financial resources and the increasing requirement for cost effective management within the health care system (Alexander, 1994). Whatever the cause, wounds have a substantial but often unrecognized impact on those who suffer from them and on the health care system. In fact, the phenomenon of wounds has been called the 'Silent Epidemic' (Smith & Nephew Foundation, 2007).

This study shows that the most frequently isolated microorganisms from both department considered for this study is *E. coli* which had the highest percentage. In the orthopedic department the gram negative bacteria *Enteriobacteriacea* that were isolated had extended spectrum beta-lactamase (ESBL) 11/20 to be 55% which should be a cause of concern as these microorganisms are known to be resistant to antibiotic which in turns causes a problem in effective wound healing. Also in the orthopedic department the gram positive bacteria isolated shows that the percentage of MRCN: 3/6 (50%), *MRSA*: 5/10 (50%), *VRSA*: 1/10 (10%). While in the general surgery department the isolated gram negative bacteria shows that the *ESBL* (+): 3/23 (13%) while in the gram positive result shows *MRCNS*: 1/6 (%17).

Although, it is an opinion among practitioners that primary cause of delayed healing and infection in wounds, are aerobic or facultative pathogens such as *S. aureus*, *P. aeruginosa*, and beta-hemolytic streptococci. The incidence of occurrence of *S. aureus* is high in wounds therefore it is considered as the most problematic (Periti et al, 1998; Mayhall, 1993). But according to a review of the literature colonized wounds contain one-third of anaerobic bacteria while infected wounds contain 50% of anaerobic bacteria. Therefore, antimicrobial treatment of wounds should have coverage of a variety of potentially synergistic aerobic or facultative and anaerobic microorganisms and should not only target specific pathogens that are often supposed to be the causative agents (such as *S. aureus* and *P. aeruginosa*). Hence both aerobic and anaerobic pathogens may contribute to infection in poly microbial wounds (frequently via combined interactions), broad-spectrum antibiotics assist the most successful treatment in the management of wound infections.

Our result shows that clindamycin or metronidazole along with an aminoglycoside (e.g., gentamicin) or a cephalosporin (e.g., cefuroxime or Cefotaxime) has proved to be very effective. In the United States cefoxitin or cephamycin are used as a single agent for treatment of already established infection not for prophylactics. But the origination of new classes of antibiotics such as the ureidopenicillins, the carbapenems, and the B-lactam/B-lactamase inhibitor combinations has enlarged the choice for both prophylactic and therapeutic treatment (Periti et al, 1998; Surucuogluet al, 2005). Since *S. aureus* is mostly the most isolated microorganisms involved in infected wounds, the most frequent treatments of choice are cephalosporin, macrolides, clindamycin, and semi synthetic penicillin such as oxacillin (Surucuoglu et al, 2005). If strains of MRSA involved, then the glycopeptide antibiotics vancomycin and teicoplanin are alternative choices (Periti et al, 1998). In a previous study poly microbial growth detected from 59.6% of cultures and 61.5% multidrug-resistant organisms isolated. Our results are not very different from previous studies (Otokunefor and Datubo-Brown, 1990; Uçkay et al, 2008). As some cultures revealed poly microbial and multidrug-resistant organisms, the overall antibiotic susceptibility was highly variable.

Selecting the suitable treatment for wound infections needs and requires an understanding of the normal infectious flora, susceptibility patterns of the pathogen, and antimicrobial agents as these would be the determining factors in the selection of empiric antimicrobial therapy and also a good infection control measures to be taken by the health institutions. Factors involved in the wound continuum from colonization to infection even up to healing can help practitioners to interpret clinical findings and microbiological investigations of wounds thus may aid in the development of more effective methods of treating infected and poorly healing wounds. As the controversy related to the use of topical antiseptics persists and the development of bacterial resistance to antibiotics continues, the desire for recognition and establishment of new antimicrobial agents that are safe and broadly effective and have a low tendency to induce resistance becomes increased abruptly.

Furthermore the microbiology of wounds has been actively researched in recent years, and there is still quiet much to be learned and discovered about the microbial mechanisms these pyogenic microorganisms use that induce infection and prevent wound healing (Nichols, 2004; Surucuoglu et al, 2005). As a result, debate and theories respecting microbial involvement in wound healing is likely to continue. With the desire to communicate a summarized analysis of wound microbiology, together with current opinion and controversies respecting wound evaluation and treatment, this retrospective study has attempted to approach microbiological aspects that are important to the management of microorganisms in wounds (Collier, 2001; Periti et al, 1998)

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS.

The study presents a clear understanding to the causative pathogens of wound infections in this hospital and their sensitivity and resistance profiles. It has been concluded that wound infections in this were poly microbic in nature and, in most cases, associated with *S. aureus, CoNS*, and *E.coli* and *P.aeruginosa*. Although *Pseudomonas* sp. showed varying resistance levels to gentamicin and ciprofloxacin, all the *Acinetobacter* sp. were resistant to most of the tested antibiotics used. Resistant gram negative bacteria are the most common isolates associated with wounds infection. Hence a careful selection of antibiotics to control the wound infection is required for proper management of wounds infections in order to help reduce morbidity and mortality. Results also displayed that there is a high rate of antibiotic resistance in all pathogens isolated. All the antibiotics tested, vancomycin showed to be the one most likely to be effective in treating infections as, in contrast to other antimicrobial agents tested in this study, not a single bacterial isolate was found to be resistant to its activity. A continuous inspection should be carried out to monitor the susceptibility of these pathogens and choose appropriate regimens both for prophylaxis and treatment of surgical wound infections. There is a need to create a viable antibiotic policy and draft guidelines to prevent or reduce undirected use of antibiotics, and conserve their effectiveness for better patient management.

Consistent dialogue between the microbiology department and the surgeons is strongly advised in keeping with preventing and controlling surgical wound infections at little cost. This will force rational use of antimicrobial agents and help in curbing the unsureness of resistance to these agents. The present study shows that the clinical isolates of *E. coli*, *P. aeruginosa*, *S. aureus*; *MRSA* & *MSSA*, *Enterococcus species*, *Candida species*, *CoNS*, *Citrobacter* species, *A. baumannii*, *Proteus* species, *Enterobacter* species ,and *Klebsiella* species are becoming resistant to commonly used antibiotics and also achieving more and more resistance to newer antibiotics. *MRSA* is a serious threat to hospitalized patients globally and it now represents a challenge for public health, as community associated infections appear to be on the increase in both adults and children in different regions and countries.

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APPENDIX

1. List of Chemicals and equipment

S.No	Chemicals	Manufactured
1.	Eosine methylene blue agar	Oxide
2.	Blood Agar	Oxide

S.No	Equipment	Manufactured
1.	BD Phonex	USA
2.	Laminar flow- hood	K&k scientific supplier Korea
3.	Incubator	Pansonic UK
4.	Electronic balance	Kern Germany
5.	Autoclave	Wisd Korea
6.	Hot plate stirrer	Jenway England
7.	Microscope	Motic BA210 USA- CANADA

2. Eosin Methylene Blue Agar (OXIDE)

Ingredients	Gm/Liter
Pancreatic Digest of Gelatin	10.0 g/L
Lactose	5.0 g/L
Sucrose	5.0 g/L
Dipotassium Phosphate	2.0 g/L
Eosin Y	0.4 g/L
Methylene Blue	65.0 Mg
Agar	13.5 g/L

3. Blood Agar

Ingredients	Percentage %
Peptone	0.5%
Beef extract/ Yeast extract	0.3%
Agar	1.5%
Sodium Chloride NaCl	0.5%
Sheep blood	5%