CHARACTERIZATION AND MOLECULAR CONFIRMATION OF *PSEUDOMONAS AERUGINOSA* ASSOCIATED WITH SURGICAL SITE INFECTIONS IN SELECTED HOSPITALS IN BAUCHI STATE, NIGERIA

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AUGUST, 2021

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN MICROBIOLOGY, DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, AHMADU BELLO UNIVERSITY ZARIA.

AUGUST, 2021

DECLARATION

I declare that the work in this dissertation titled "**Characterization and molecular confirmation of *Pseudomonas aeruginosa* associated with surgical site infections in selected hospitals in Bauchi State**" has been carried out by me in the Department of Microbiology, Ahmadu Bello University under the supervision of Dr. E.E.Ella and Dr M.H.I.Doko. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institutions.

_____	_____	_____
Name of student	Signature	Date

CERTIFICATION

This dissertation titled "**Characterization and molecular confirmation of *Pseudomonas aeruginosa* associated with surgical site infections in selected hospitals in Bauchi State**" by Titus Onyi meets the regulations governing the award of the degree of Masters of Science in the Department of Microbiology, Ahmadu Bello University, Zaria and it has been approved for its contributions to knowledge.

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DEDICATION

This work is dedicated to God Almighty for His mercy, love and grace to me. It is also dedicated to my family for their love and support.

ABSTRACT

Pseudomonas aeruginosa associated with surgical sites infections (SSIs) is a serious public health concern and associated with patients' morbidities, longer wound management, high costs of treatment and deaths. This study aimed to isolate, characterize and confirm isolates, using PCR, *Pseudomonas aeruginosa* associated with surgical site infections in selected hospitals in Bauchi State of Nigeria. The study was a cross sectional and hospital-based study that cultured post-surgical wound swabs from 250 consented patients on admission who had surgeries within the past 30 days. Out of the 250 surgical wound swabs collected and cultured, an overall incidence of *Ps. aeruginosa* associated with surgical site infections was found to be 2.0%. The incidence of *Ps. aeruginosa* associated with SSIs was found to be statistically associated with the type of surgery (p=0.000), post-operative duration (p=0.041) and HIV status (p=0.031). The study also found that all the isolates of *Ps. aeruginosa* were confirmed with PCR analysis using the *oprL* gene with amplicon size of 504bp. All the isolates of *Ps. aeruginosa* were multi-drug resistant with antibiotic resistance ranging from 20 to 50%. The Multiple Antibiotic Resistance (MAR) indices of the isolates ranged from 0.2 to 0.5. All the isolates were susceptible to Ofloxacin and were all resistant to Cotrimoxazole. The study recommends enhanced post-surgical infection control and wound management especially for HIV positive patients and those who undergo below elbow amputation. The study recommends enhanced post-surgical infection control especially below elbow amputation as well as patient health education to reduce post-operative duration in hospital and increased cost.

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LIST OF ABBREVIATIONS

CDC: Centre for Disease Control

CHG: Chlorhexidine Gluconate

CLED: Cysteine Lactose Electrolyte Deficient

CLSI: Clinical and Laboratory Standard Institute

CS: Caesarean Section

DMACA: Di Methyl Amino Cinnam Aldehyde

HAI: Healthcare Associated Infection or Healthcare Associated Infection

HIV: Human Immunodeficiency Virus

KIA: Kligler Iron Agar

LPS: Lipopolysaccharide

MAR: Multiple Antibiotic Resistance

MBP: Mechanical Bowel Preparation

NICE: National Institute for Health and Care Excellence

NNIS: National Nosocomial Infections Surveillance System

PCR: Polymerase Chain Reaction

SAP: Administration of Surgical Antibiotic Prophylaxis

SSI: Surgical Site Infection

TAE: Tris Acetate Ethylene Diamine Tetra Acetate

WHO: World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Pseudomonas aeruginosa (*Ps. aeruginosa*) is a Gram-negative opportunistic bacterium commonly associated with nosocomial infections (Tang *et al.*, 2017). It is associated with increased mortality relative to *Staphylococcus aureus* and other Gram-negative bacteria in surgical site infections (SSIs) (Tran *et al.;* 2014; Turner *et al.*, 2014).The bacterium is particularly pathogenic and equipped with various virulence factors that confer on it a wide range of antibiotics resistance (Mohammad, 2013). *Pseudomonas aeruginosa* is prominent among other gram-negative bacilli in the etiology of surgical site infections (SSIs) (Andreassen *et al*., 2002; El Zowalaty & Gyetvai,2016).

Pseudomonas aeruginosa may infect surgical wound either from endogenous route or from normal flora of patients especially those with immune compromised status. Surgical Site Infections associated with *Ps. aeruginosa* may also occur from exogenous contamination of medical devices used for wound dressing and the environment (Kaye *et al.*, 2005). Surgical Site Infections usually occur within 30 days of surgery and are characterized by purulent discharge from the wound or insertion site of wound drain or spreading cellulitis from the wound (Mangram *et al.*, 1999; WHO, 2011).

Surgical site infections account for 25% of nosocomial infections (Shuaibu *et al.*,2017) Although prevalence and bacteriology of surgical site infections vary with geographical location and facilities, SSIs associated with *Ps. aeruginosa* are very common.The World Health Organization (WHO) estimated that in the low and middle income countries, the pooled incidence of SSI is 11% (WHO, 2019). The World Health Organization (WHO) estimated that the overall incidence of SSI in Nigeria is 16-31 % (WHO, 2011).Most of the

studies conducted in Nigeria, determined pooled incidences of SSIs while few studies determined the incidence of *Ps. aeruginosa* in the SSIs. This underscores the need for further studies on *Ps. aeruginosa* associated with SSI. This is also in line with the WHO recommendation for more research on hospital-specific SSIs for infection control and to build national data on Hospital Acquired Infections (HAIs) (WHO, 2011).

1.2 Statement of Research Problem

The disease burden of SSI associated with *Ps. aeruginosa* is enormous. *Ps. aeruginosa* is a leading pathogen that causes serious infections at various tissues including surgical wounds. It causes a spectrum of health problems including wound pains, fever, septicemia and death (El Zowalaty & Gyetvai, 2016). There is also huge financial burden of the cost of extra hospital admission and treatment of SSIs.

Globally, the rate of occurrence of surgical site infections range from 2.5% to 41.9% (Mawalla *et. al.,* 2011). In Europe, hospital acquired infections (including SSI) caused 16 million extra days of hospital stay, 7 billion Euros of medical costs and 37,000 deaths annually (WHO, 2011). In developing countries, health care associated infections (including SSIs) account for an average of extra 5 to 29.5 days per patient. They also account for up to 18.5% of mortality of adult patients in Africa and huge amount of extra medical bills (WHO, 2011).

Multi-drug resistant strains of *Ps. aeruginosa* in post- surgical wounds have been classified by several healthcare organizations as serious threat to public health. Considering its high prevalence with associated high mortality rates and limited treatment options, *Ps. aeruginosa* has been identified as a critical research priority for the development of novel therapies (Tacconelli *et al.*, 2017; WHO, 2017).

There is a huge gap in extensive research on SSIs. World Health Organisation reported that as many as 66% of developing countries have no imprinted data related to the burden of SSI e.g. the prevalence on a national level (Tariq *et. al.,* 2017).

Studies conducted in Nigeria have reported significant rates of occurrence of SSIs. Some studies have also determined significant rates of occurrence of *Ps. aeruginosa* associated with SSIs. Ohajuru *et al.* (2011) reported an incidence of 21% for SSIs at Obafemi Awolowo University Teaching Hospital Complex in Osun state. Oni *et. al.* (2006) reported an incidence of 9.4% for SSIs in University College Hospital, Ibadan Oyo state. In Aminu Kano Teaching Hospital, Kano, Jido and Garba (2012) reported an incidence of 9.1% for SSI among women that had caesarean sections (CSs) Yasidi *et al.* (2015) reported an incidence rate of 19.6% for *Ps. aeruginosa* associated with SSIs in Federal Medical Centre, Nguru in Yobe State of Nigeria

1.3 Justification for the study

Accurate information on the incidence and etiology of infections acquired within the hospital is essential for the articulation of effective preventive measures (Sanjay *et. al.,* 2010). The heavy disease burden of SSIs underscores effective treatment and management in hospital and community setting with detailed epidemiological knowledge of the infecting bacterial pathogens and the drug susceptibility pattern that is peculiar to the environment (Mulu, 2006; Yasidi *et. al.,* 2015). Similarly, the WHO recommends continuous studies on SSIs at various health-care settings for proper infection control and to build a national data on Hospital Acquired Infections, HAIs (WHO, 2011).

Although the association of *Ps. aeruginosa* with SSIs is well documented, only few studies associated with surgical site infections in health facilities have been conducted in Nigeria. Many of these facilities do not have empirical data on the rate of occurrence of *Ps. aeruginosa*, the drug susceptibility studies and some of the molecular characteristics of the

Ps. aeruginosa strain(s) at the facilities. It was pertinent, therefore, to conduct this study in Bauchi State where it had not been conducted before. The study provides pioneer empirical data and fills the gap in the knowledge of SSIs associated with *Ps. aeruginosa* in these facilities. The findings would provide crucial data for surgical site infection control at the study sites and also provide data for further studies.

1.4 Aim of the study

The aim of the study was to isolate, characterize and confirm isolates using PCR and determine antibiotic susceptibility of *Pseudomonas aeruginosa* associated with surgical site infections in selected hospitals in Bauchi state, Nigeria.

1.5 Specific objectives of the study

The specific objectives of the study were to:

1. isolate and characterize *Ps. aeruginosa* from surgical site infections in selected hospitals in Bauchi LGA of Bauchi State, Nigeria;
2. Confirm the isolates using Polymerase Chain Reaction, PCR;
3. determine the antibiotic susceptibility pattern of *Ps. aeruginosa* isolates by the Kirby Bauer Disk Diffusion method;
4. determine some socio-demographic patterns and risk factors associated with *Ps. aeruginosa* infections at surgical sites.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacillus that belongs to the *Pseudomonadaceae* family. This family is a member of the Gamma proteobacteria class of bacteria. The family *Pseudomonadaceae* has 211 correctly described species, though 56 species have been reclassified to other genera. They are constantly undergoing continuous taxonomic revision due to improvements in methodologies of species identification (Pitt *et al.,* 2006; Henry *et al.,* 2011). The genus Pseudomonas has other species including *Ps. putida, Ps. fluorescens, Ps. monteilii, Ps. otitidis, Ps. mosselii, Ps. stutzeri, Ps. mendocina, Ps. alcaligenes, Ps. pseudoalcaligenes, Ps. luteola, Ps. oryzihabitans* (Henry *et.al,* 2011).

Ps. aeruginosa is ubiquitous in nature and found in water, soil, plants and animals including man (Klockgether and Tümmler, 2017). It is monoflagellated in motility and adherence to the epithelial cells (Köhler *et al.*, 2000; Schwarzer *et al.*, 2016). The twitching movement of *Ps. aeruginosa* is assisted by type IV pilli (Kazmierczak *et al.,* 2015). *Pseudomonas aeruginosa* is readily adaptive to unfavourable and hostile environments. It has the adaptive feature such as biofilms formation, genetic modifications/mutations and antibiotic resistance due to chromosomal mutations and horizontal transfer of resistance genes. Consequently, there are various phenotypes (Kazmierczak *et al.,* 2015).

Ps. aeruginosa produces a spectrum of pathogenicity factors for infection. These may be structural or toxins secreted into host cells.The outer membrane of *Ps. aeruginosa* has the protective lipopolysaccharide (LPS) complex that confers structural pathogenecity. The virulence factors are elastases (LasA and LasB elastases) and pigments including pyocyanin (Hauser, 2009, Galle *et al.*, 2012).

Ps. aeruginosa infections can be classified into acute and chronic (Furukawa *et.al,* 2006; Valentini *et al.,* 2018). In acute *Ps. aeruginosa* infections, there is a quick dissemination of the pathogen to the bloodstream, causing bacteremia and possibly death within hours or days (McManus *et al.,* 1985). Acute *Ps. aeruginosa* infections usually involve G proteins, lipopolysaccharide, elastase or type IV pili that introduce toxins directly into host cells (Comolli *et al.,* 1999; Turner *et al.,* 2014).

2.2 *Pseudomonas aeruginosa* Infections

Pseudomonas aeruginosa is a significant pathogen in patients with compromised host defense mechanisms. It is the causative agent of many nosocomial infections.These nosocomial infections include:

2.2.1 Skin and soft tissue infections

These are wound infections that include pyoderma and dermatitis. Predisposing factors of these infections are burns, trauma, surgery, dermatitis and high moisture conditions (eg. in the toe webs of athletes, ears of swimmers, perineal region and under diapers of infants. Localized and diffused infections of skin caused by *Ps. aeruginosa* are common in immune compromised patients. These include acne vulgaris and ecthyma gangrenosum of the skin and surgical site infections (Weinstein and Mayhall, 2003; Mihai *et al.,* 2014).

2.2.2 Respiratory infections

Respiratory infections caused by *Ps. aeruginosa* occur almost exclusively in the lower respiratory tract of individuals with compromised immunity.These include primary pneumonia in patients with congestive heart failure and chronic lung disease; bacteremic pneumonia and complicated cystic fibrosis by mucoid strains of *Ps. aeruginosa* (Fujitani *et al.,* 2011*;* Heltshe *et al.,* 2018).

2.2.3 Urinary tract infections (UTIs)

UTI caused by *Ps. aeruginosa* are usually nosocomial and follow invasive surgery with instruments, urinary tract catheterization, instrumentation or surgery. *Ps. aeruginosa i*s the third leading cause of hospital-acquired UTIs. Nearly half (40%) of UTI caused by *Ps. aeruginosa* is disseminated to the blood causing bacteremia.

2.2.4 Bacteraemia and septicaemia

Bacteraemia and septicaemia predisposed by haematologic malignancies, immunodeficiency due to AIDS, diabetes mellitus, and severe burns**.**

2.2.5 Otitis externa and media (ear infections).

Although the bacterium is occasionally found in the normal ear, infection may occur following ear injury, inflammation and wet and humid conditions for example following swimming.

2.2.6 Meningitis and brain abscess of central nervous system.

These could be secondary to head trauma, surgery or invasive procedures, or secondary spread for example from urinary tract.

2.2.7 Bone and joint infections.

These usually occur after bone surgeries or puncture wounds of the foot.The joints commonly implicated are knee joints, elbow joints and hib joints.

2.2.8 Endocarditis

This may result from infection of heart valves including prosthetic heart valves or by direct invasion from the blood stream causing endocarditis.

2.2.9 Diarrhoea, gastroenteritis and necrotizing enterocolitis

These are usually caused by *Ps. aeruginosa* in immune compromised patients. The gastrointestinal tract is also an important route of entry for *Pseudomonas* bacteraemia and septicemia (Medscape, 2018).

2.3 Characteristics of *Pseudomonas aeruginosa*

2.3.1 Physical characteristics

Ps. aeruginosa is a Gram-negative bacillus measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm. Each of the strains has a single polar flagellum for motility.

Ps. aeruginosa is ubiquitous in habitat.It is found in the soil and water and other surfaces that are in contact with these environment. Naturally, *Ps. aeruginosa* occur as biofilms on substrate or on specific epithelial surfaces of host. It could also appear as unicellular organism, ie. Planktonic form, that actively swims with flagellum in ponds and other water surfaces.

The genome of *Ps. aeruginosa* is a relatively large one (5.5–7 Mb) with high G+C content (65–67%). The genome consists of a single circular chromosome that codes a large number of enzymes for various metabolic pathways, conferring high nutritional versatility. The genome of *Ps. aeruginosa*, which is especially large for a prokaryote, has provided an understanding of the metabolic and pathogenic mechanisms that underlie the success of this versatile pathogen, and it has become a model for understanding microbial genomic variation and evolution in chronic disease (Paul, 2018).

2.3.2 Colonial characteristics

Ps. aeruginosa is a strict aerobe. However, in some cases, where oxygen is absent, for example in Cystic Fibrosis, nitrate may be used as a respiratory electron acceptor. *Ps. aeruginosa* are glucose non-fermenters.

The bacterium has very simple nutritional requirements and may grow in distilled water. Optimal growth is in moist environments in soil and water.The growth temperature ranges from 5-42°C. The optimum temperature for growth is 37°C but it can grow at temperatures as high as 42°C.The growth of *Ps. aeruginosa* in the laboratory utilizes acetate as a source of carbon and ammonium sulfate as a source of nitrogen.

Ps. aeruginosa is tolerant to a wide variety of physical conditions, including high temperature, high concentrations of salts and dyes, commonly used antibiotics and weak antiseptics. This feature is wielded by the organism for ubiquitous nature and as a hospital acquired pathogen (Paul, 2018).

Ps. aeruginosa can produce many colonial types. Paul (2018) reported three types of colonies while the UKSMI (2018) reported six colonial types after aerobic incubation on nutrient agar for 24hr at 37°C.

The natural isolates from water or soil form a small and rough colony. The clinical isolates are likely to be smooth colony types, occasionally with a fried-egg appearance that is large, smooth, flat edges and an elevated appearance. Respiratory and urinary tract secretions may show a mucoid-type (alginate slime) (Paul, 2018). The colonial variation from one type to another does not necessarily indicate the presence of more than one strain (United Kingdom Standards for Microbiology Investigations, UKSMI, 2015)

The characteristic blue-green appearance of colonised/infected pus or culture is due to the mixture of pigments produced by *Ps. aeruginosa*. These are the pyocyanin (blue) and pyoverdin (fluorescein, yellow). Pyocyanin (from "pyocyaneus") refers to "blue pus", which is a characteristic of suppurative infections caused by *Ps. aeruginosa*. Pyocyanin is redox-active (oxidant) phenazines that play an important role in electron transport especially under microaerophilic conditions. It increases the bioavailability of iron by scavenging for it, and enhances virulence through oxidative stress.The mucoid strains of *Ps. aeruginosa*, may not

produce pyocyanin. Other pigments that could be produced by *Ps. aeruginosa* strains are pyorubin (red), pyomelanin (brown) and Pyochelin. Pyochelin is a siderophore and a derivative of pyocyanin that can acquire iron from the host or in low-iron habitat for growth of the pathogen (Lee & Zhang,2015).

2.3.3 Biochemical characteristics

The biochemical characteristics of *Ps. aeruginosa* include the following:

1. Oxidase test*: Pseudomonas aeruginosa* is oxidase positive. The organism contains cytochrome that produces intracellular oxidase which is part of respiratory chain.During oxidase test, the reagent (1% dimethyl-p-phenylenediamine) is reduced to deep-purple colour.

2. Citrate (utilization) test: *Pseudomonas aeruginosa* is citrate positive.In citrate test, the organism produces citrate-permeate which catalyses the formation of citrate to pyruvate.Citrate,which is the only source of energy (carbon source),converts ammonium salts to ammonia.The ammonia produced leads to an increase in alkalinity.So the bromthymol blue indicator turns from green to blue.

2. Catalase test**:** *Pseudomonas aeruginosa* is catalase positive. The test demonstrates the presence of catalase that releases oxygen from hydrogen peroxide and hence reduces the lethal effect of hydrogen peroxide, an end product of aerobic respiration. The test is positive if gas bubbles (of oxygen) are produced when a drop of 3% hydrogen peroxide is put in a glass slide having a small amount of colony growth.

3. Urease test*:* *Pseudomonas aeruginosa* is Urease negative. Urease test demonstrates the presence of urease which splits urea to ammonia and carbon dioxide. This reaction causes an increase in the alkalinity of the test medium which changes colour from yellow (negative) to red (positive).

4. Coagulase test: *Pseudomonas aeruginosa* is coagulase negative. Coagulase test demonstrates the presence of coagulase which converts fibrinogen to fibrin.

5. Indole test*:* *Pseudomonas aeruginosa* is Indole negative. The Indole test demonstrates the ability to convert amino acid tryptophan to indole using the enzyme tryptophanase. In the spot test, this reaction changes the blue colour of Kovac's Reagent to blue-green.

6. Sugar fermentation*:* Pseudomonads are oxidative in their respiratory strategy, unlike the Enterobacteriaceae. The organisms do not ferment sugars that include glucose, fructose, lactose or sucrose. Thus, in media that detect pH change by acid production like MaConkey, Kligler Iron Agar (KIA) and Cysteine Lactose Electrolyte Deficient Agar (CLED), *Ps. aeruginosa* forms pale coloured colonies with no indication of fermentation (acid or gas) (UK SMI,2015).

2.4 Epidemiology of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is ubiquitous but prefers aquatic habitat. The reservoirs of *Ps. aeruginosa* include water pools, fruits, flowers, unsterilized medical and hospital devices and instruments. It occasionally colonizes and lives as normal flora on the human skin, external ear, the large intestine and the respiratory tract. However, in compromised host immune defense, *Ps. aeruginosa* that lives as the normal flora may become infective (endogenous infection). *Ps. aeruginosa* may also be inoculated from the environment for example from hospital devices, instruments and materials infected with *Ps. aeruginosa*. In the latter case, it leads to exogenous infection.

Globally, the rates of SSI infections associated with *Ps. aeruginosa* vary among patients, health facilities and geographical regions. However, *Ps. aeruginosa* is a major pathogen associated with SSIs (Medscape,2019). It was ranked as the fourth most common cause of SSIs (Medscape,2019). In low- and middle-income countries, 11% of patients who undergo surgery get SSIs (WHO, 2019).

A Systematic Review and Meta-Analysis, Olowo-Okere *et al.* (2019) found that the pooled cumulative incidence of SSIs in the 6 geopolitical regions of Nigeria was 14.5% (95%

confidence interval [CI]: 0.113-0.184). These SSIs include those caused by *Ps. aeruginosa*. The highest incidence as reported at the north-eastern region (27.3%, 95% CI: 0.132-0.481).The majority of SSIs occurred in among elderly patients and in patients with co-morbid conditions. Most of the surgeries were colorectal and abdominal surgeries (Olowo-Okere, 2019). Murphy *et al.* (2016) found that the rate of occurrence of *Ps. aeruginosa* was 16% of pathogens of SSIs in an orthopedic hospital in Portharcourt Nigeria. Olowo-Okere et *al.* (2019) found that *Ps. aeruginosa* was 35.0% and the most prevalent of all the extended-spectrum β-lactamase (ESBL)-producing. Gram-negative bacterial isolates from patients with surgical site infections (SSIs) at a tertiary healthcare facility in Abuja, Nigeria.

Oni *et al.* (2006) found that 27.9% of nosocomial infections were Surgical Site Infection at University College Hospital Ibadan, They also found that 21.7% of these SSIs was caused by *Pseudomonas* species .In Federal Medical Centre, Nguru, Yobe state, Yasidi *et al.,(*2015) found that the incidence of *Ps. aeruginosa* among the surgical wound sites was 19%.

2.5 Pathogenesis of *Pseudomonas aeruginosa* associated with Surgical Site Infections

Pseudomonas aeruginosa associated with SSIs usually occurs after some alteration of normal host defenses, in this case altered skin architecture. The pathogenesis of *Ps. aeruginosa* associated with SSIs starts with the introduction of the organism from the reservoirs to the surgical site. The reservoirs include water pools, fruits, flowers, unsterilized medical and hospital instruments and devices. The pathogenesis of *Ps. aeruginosa* associated with SSIs is multifactorial and associated with virulence factors possessed by the bacterium. Pollack (2000) described three distinct stages of the pathogenesis: (1) bacterial attachment and colonization (2) local invasion and (3) disseminated systemic disease. These stages will be discussed in terms of SSIs associated with *Ps. aeruginosa*.

2.5.1 Colonization

Epithelial tissues that protect organs from the environment are the first-line of defense. *Ps. aeruginosa* is a normal skin flora in humans but may become pathogenic in patients with immune compromised conditions, damaged skin epithelium from surgery, people with vascular disease and elderly (over 65years of age) who are at risk of developing skin infections (Turner *et al.,*2014; Serra *et al.,* 2015). Under these conditions, colonisation of the surgical wound site with *Ps. aeruginosa* commences with the attachment of the organism to the surface of the cutaneous epithelium of surgical incisions and subsequent formation of biofilms (Brandenburg *et al.,* 2015; Jensen *et al.,* 2017). The attachment is likely mediated by pili, flagella and the extracellular polysaccharide slime.The biofilms are surface-attached communities of *Ps. aeruginosa* bacteria within an extracellular matrix made up of polysaccharides, nucleic acids and siderophores (such as pyoverdine) (Maunders and Welch, 2017).

2.5.2 Invasion

Pseudomonas aeruginosa can invade the whole epidermis tissue and even the subcutaneous tissue (Garcia *et al.,* 2018). This leads to loss of epidermis, de-keratinization of the skin and partial loss of basement membrane (Shepherd *et al.,* 2009). The inhibition of wound healing of SSIs by *Ps. aeruginosa* may involve any or many of these successive processes of wound healing. These are coagulation, inflammation, cell proliferation, matrix repair, granulation tissue formation, re-epithelialization and remodeling or scar formation. (Serra *et al.,* 2015, Prevaldi *et al.,* 2016) The flagellum helps in the attachment of *Ps. aeruginosa* to these structures. Invasion is also aided by the biofilm layer and extracellular enzymes and toxins that breakdown the physical barriers of skin and subcutaneous tissue. Invasion damages the epithelia of skin and subcutaneous tissue, impairs the epithelial repair mechanisms and resists phagocytosis by the host immune system.

Invasion is aided by the *Ps. aeruginosa* slime layer and virulence factors.These are discussed below.

1. The bacterial capsule or slime layer shields the pathogen from host immune cells.

2. Virulence factors: Virulence factor production is coordinated by bacterial cell-to-cell communication systems, called quorum sensing (Lee and Zhang, 2015). There are types II (T2SS) and type III (T3SS) systems that release the vast majority of known virulence factors. Type II (T2SS) system allows *Ps. aeruginosa* to secrete bacterial elastases (LasA and LasB elastases). Elastase has several activities that relate to virulence. The enzyme cleaves collagen, IgG, IgA, and complement. Las B degrades types I and IV collagen protein and impairs wound healing secreted into the extracellular environment. Pyocyanin is a blue green pigment that has a pro-inflammatory effect on the phagocyte. Exotoxin A (Exo A) is a highly toxic enzyme and alters epithelial integrity in wound healing leading to necrosis at wound site (Galle *et al.*, 2012).

Type III (T3SS) system is a needle-like complex of proteins utilised by *Ps. aeruginosa* to inject several bacterial cytotoxins directly into epithelial cells. The secretion system has been identified as a major virulence determinant in the pathogenesis of acute *Ps. aeruginosa* infections (Engel and Balachandran, 2009; Hauser, 2009; Galle *et al.*, 2012). The T3SS secrete four exotoxins with various virulence activities: Exotoxin S,T,U and Y.Exotoxin S (ExoS) disrupts the structural proteins in wound healing (Shafikhani *et al.*,2008).Exotoxin T (ExoT) , Exotoxin U (ExoU), a phospholipase) and Exotoxin Y (ExoY) are associated with cytoskeleton disruption (Cowell *et al.*, 2005).

Another virulence factor secreted by *Ps. aeruginosa* is the Alkaline protease (AprA) an enzyme that cleaves various components including complement C1q and C3 (Hong and Ghebrehiwet,1992) and destroys the flagellin of the pathogen (Bardoel *et al.*,2011).The Alkaline protease and the LasB are invasion-mediating virulence factors of *Ps. aeruginosa.*

2.5.3 Dissemination

The host response to SSI associated with *Ps. aeruginosa* is mainly through cell mediated immunity. This involves phagocytosis by polymorphonuclear leukocytes following antibody-specific opsonization directed primarily at the antigenic determinants of lipopolysaccharide (LPS), a complex of glycolipid present in the outer membrane of *Ps. aeruginosa*.

Dissemination of *Ps. aeruginosa* in SSIs to the blood results in bacteremia and other specific organ infections is associated with diminished host antibody and immune compromised conditions like neutropenia and chronic diseases. *Ps. aeruginosa* is resistant to phagocytosis and the host's serum bactericidal response due to its mucoid capsule and possibly LPS. The bacteria proteases inactivate complement and lyse IgG antibodies. The Lipid A moiety of *Pseudomonas* LPS (endotoxin) mediates the usual pathologic aspects of Gram-negative septicemia, e.g. fever, hypotension and intravascular coagulation. The dissemination of *Ps. aeruginosa* in SSIs causes diseases like urinary tract infections (UTIs), otitis externa, meningitis, brain abscess osteomyelitis and endocarditis, Ecthyma gangrenosum etc. Ecthyma gangrenosum is a skin lesion that was first associated with *Pseudomonas* septicemia was later given the name "ecthyma gangrenosum" It is usually but not always associated with *Ps. aeruginosa* bacteremia in the critically ill and immune compromised patients. The lesions of Ecthyma gangrenosum is characterized by hemorrhagic vesicles or pustules that evolve into necrotic ulcers with a tender erythematous border (Medscape, 2018).

2.6 Drug Resistance of *Pseudomonas aeruginosa* associated with Surgical Site Infections

Pseudomonas aeruginosa is a well-established surgical site pathogen with multi-drug resistance. *Ps. aeruginosa* has been found to be resistant organism to many known antimicrobial agents including topical antibiotics such as B-lactams, cephalosporins, quinolones and gentamicin (Wang & Zang,2017). Hence antibiotic selected for susceptibility assay for the organism is determined by the resistance pattern in a location. The antibiotics

are also selected from various classes of antibiotics, For example, ofloxacin (Tarivid) is a quinolone antibiotic that is bactericidal and inhibits nucleic acid synthesis by inhibiting the enzyme DNA Gyrase which is essential in DNA replication and transcription. Streptomycin is a glycoside that inhibits protein synthesis by inhibiting the 50S ribosomal subunit of microorganisms. The 50S is implicated in the formation of the single stranded RNA also called ribosomal RNA (rRNA). Chloramphenicol also inhibits the 50S subunit while Gentamicin, a glycoside also inhibits protein synthesis by inhibiting the 30S ribosomal subunit. The 30S is implicated in the transfer of RNA to messenger RNA (mRNA). Cotrimoxazole (Septrin) inhibits nucleic acid synthesis by inhibiting dihydrohydrofolate reductace, an enzyme that catalyzes the formation of dihydrofolic acid from Para-Amino Benzoic Acid (PABA).Sparfloxacin, Ciprofloxacin and Pefloxacin are quinolones that have bactericidal activity by inhibiting nucleic acid synthesis and function. Amoxicillin is a B-lactam antibiotic and specifically a penicillin.It inhibits cell wall synthesis by binding to the penicillin binding protein of microorganisms.Augmentin is a combination of Amoxicillin and Clavulanic,a B-lactaminase inhibitor.B-lactaminase is produced by some bacteria to destroy B-lactam units of B-lactam antibiotics.These are penicillins,cephalosporins and carbapenems.

Pseudomonas aeruginosa has a spectrum of adaptations for drug resistance.These are:

1. *Pseudomonas aeruginosa*'s outer membrane offers a wide range of barrier to antibiotic permeability to the organism. Additionally, many strains of *Pseudomonas aeruginosa* have a multi-drug efflux system, an energy dependent pump that utilizes three protein components an example is the MexAB-oprM system that is responsible for extrusion of B-lactams and other disinfectants.

2. The biofilm formation offers the *Ps. aeruginosa* cells an impervious barrier to therapeutic concentrations of a wide range of antibiotics. This excessive extracellular matrix secretion

and metabolic changes that occur within the biofilm are highly tolerant to many antimicrobial agents.

3. *Pseudomonas aeruginosa* has plasmids containing genes that regulate antimicrobial resistance. These plasmids are acquired from the environment and transferred through gene transfer. Antibiotic resistance plasmids contribute to the multi-drug resistance of *Ps. aeruginosa associated* with SSIs (Bodey *et al.*, 1983).

2.7 *OprL* Gene of *Pseudomonas aeruginosa*

The outer membrane proteins of *Ps. aeruginosa* play important roles in the interaction of the bacterium with the environment (Qin *et al.*,2003).The L-peptidoglycan-associated lipoprotein) and I-lipoproteins are two outer membrane proteins of *Ps. aeruginosa* that alter membrane permeability and utilize efflux mechanism to confer antibiotic resistance for *Pseudomonas* species and *Pseudomonas aeruginosa* respectively. These L and I outer membrane proteins are coded by the *oprL and oprI* genes respectively. While the *oprI* is present in all *Pseudomonas* species, the *oprL* is specifically present in *Ps. aeruginosa. Therefore*, the *oprL* gene is a fairly specific (Osayande, 2008; Douraghi *et al.*, 2014) and accurate (De Vos *et al.*,1997; Anuj *et al.*, 2009) gene for molecular identification of *Ps. aeruginosa* in clinical samples including surgical wound swab.

2.8 Surgical Site Infection (SSIs)

Surgical Site Infection (SSI) also called post-surgical wound infection is a type of healthcare-associated infection in which a wound infection occurs after an invasive (surgical) procedure on part of the body where the surgery took place. Surgical site infection is defined as an infection that occurs within 30 days after the operation and involves the skin and subcutaneous tissue of the incision (superficial incisional) and/or the deep soft tissue (for example, fascia, muscle) of the incision (deep incisional) and/or any part of the anatomy (for

example, organs and spaces) other than the incision that was opened or manipulated during an operation (organ/space) (CDC, 2019). A surgical wound refers to a wound created when an incision is made with a scalpel or other sharp cutting device and then closed in the operating room by suture, staple, adhesive tape, or glue and resulting in close approximation to the skin edges (WHO, 2019).

Infection in the surgical wound may prevent healing, causing the wound edges to separate, or it may cause an abscess to form in the deeper tissues. The infection may range from a spontaneously limited wound discharge within 7 to 10 days of an operation to a life-threatening postoperative complication, such as a sternal infection after open heart surgery. Surgical site (wound) infection is characterized with local signs and symptoms of infection, for example, heat, redness, pain and swelling, and (in more serious cases) with systemic signs of fever or a raised white blood cell count. SSI is both the most frequently studied and the leading health associated infection reported hospital-wide in Low- and Middle-income countries. The risk factors of SSIs include diabetes mellitus, obesity, anemia, immune-suppressant drugs, use of corticosteroids and malnutrition (Labib, 2012).

2.8.1 Classification of surgical site infections based on body anatomy

Based on body anatomy, Surgical Site infection is categorized into three different types according to the Centre for Disease Control and Prevention (CDC's) and National Nosocomial Infections Surveillance System (NNIS). These are superficial, deep, and organ/space infections. They are discussed below.

1. Superficial infections which invade only the skin and subcutaneous tissue;

2. Deep infections penetrate into deep tissue, such as facial and layer of muscle. It also includes infections which involve both superficial and deep incision sites and

3. Organ/space infections occur in an organ or space other than the incision site (Singhal and Kanchan, 2015).

2.8.2 Classification of surgical site infections based on degree of infection

In 1964, National Research Council group (United States) categorized post-surgical lesion (wound), now commonly called SSIs, into four major categories considering the degree of microbiological contamination. The classification is still in use especially among clinicians. These are clean, clean-contaminated, dirty, contaminated and dirty wounds. They are discussed below.

1. Clean wound: This refers to an uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital or uninfected urinary tracts are not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow non-penetrating (blunt) trauma are in this category if they meet the criteria.

2. Clean-contaminated wound: This refers to operative wounds in which the respiratory, alimentary, genital or urinary tracts are entered under controlled conditions but with no contamination. Specifically, operations involving the biliary tract, appendix, vagina and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered

3. Contaminated wound: This refers to open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (for example, open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute, non-purulent inflammation is encountered, including necrotic tissue without evidence of purulent drainage (for example, dry gangrene) are included in this category.

4. Dirty or infected wound: This includes old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation (CDC, 2019).

Table 2.1 Classification of Surgical Site Infections based on degree of infection

Wound Type	Class	Definition/Major Characteristics
Clean	I	No inflammation stumbles upon and the gastrointestinal (GI), respiratory, genital & urinary tract is not involved. Discretionary (elective), not emergency, principally closed and without rupture/ break techniques involved.
Clean-contaminated	II	Operative method involved a colonized viscera or cavity (opening) of the body, although with controlled and elective situations with nominal spillage. Furthermore, emergency and urgent cases are clean otherwise, inconsequential break in technique.
Contaminated	III	Operative procedures are carried out with major interruption/breaks in desolate/aseptic/sterile method (like open cardiac massage) or gross/foul spillage/ drain from the GI tract, access into genitourinary or biliary system in the existence of contaminated bile/urine contents and incisions with non-purulent, sensitive and acute inflammation are integrated into this group.Operative procedures are carried out with major interruption/breaks in desolate/aseptic/sterile method.
Dirty	IV	Dirty wounds are demonstrated with surgical processes mainly involved active infections prior to surgery

(Singh *et al.*, 2014)

2.8.3 Pathogens of surgical site infections

Generally, SSIs are associated with Gram-negative and gram positive bacteria including facultative anaerobic Gram-negative bacilli, *Streptococci* and *Staphylococc*i and *Ps. aeruginosa* (Bertrand, 2002). But the most frequently reported nosocomial pathogens have been *E. coli, S. aureus, enterococci* and *Ps. aeruginosa* (Teresach *et al.*, 2008). Wound infection after contaminated surgical operations is usually caused by the patient's own normal flora, or gained entry while the patient is in a hospital. Infection caused by microorganisms from an outside source following surgery is less common (Trilla, 1994). However, the rate of occurrence of bacteria implicated in SSIs vary with geographicplocation, skin flora and fomites e.g. dressing materials (Andenaes *et al.*,1996; Ranyan *et al.*, 2010).

2.8.4 Complications of surgical site infections associated with *Pseudomonas aeruginosa.*

Pathogens including *Ps. aeruginosa* can be disseminated (Fig, 2.1) from the SSI site to other part(s) of the body including distant organs. Some of the complications of *Ps. aeruginosa* are bacteremia and septicemia, urinary tract infections (UTIs), otitis externa, meningitis, brain abscess osteomyelitis, endocarditis and death etc.

2.9 Presumptive Identification of *Pseudomonas aeruginosa* from Surgical Site Infections

This is based on the following sequence of procedures: isolation on primary isolation media (Cetrimide agar) based on colonial morphology, Gram stain and oxidase activity (Pitt and Simpson, 2006; Henry and Speert, 2011).

2.9.1 Isolation on primary media

The procedure involves inoculation of surgical site wound samples on primary isolation plate, incubation at specified conditions on agar and identification of the colony morphology. Cetrimide agar is the selective agar for *Ps. aeruginosa*. The sample is incubated in the

Cetrimide agar aerobically at 37°C for 24hrs. Colonies are flat and spreading with serrated edges and a metallic sheen. The colonies are surrounded by blue-green pigment and fluoresce under short wavelength (254nm) ultraviolet light. Colonies may also appear blue or non-pigmented. They also have a grape-like aroma (Pitt and Simpson, 2006; Henry and Speert, 2011).

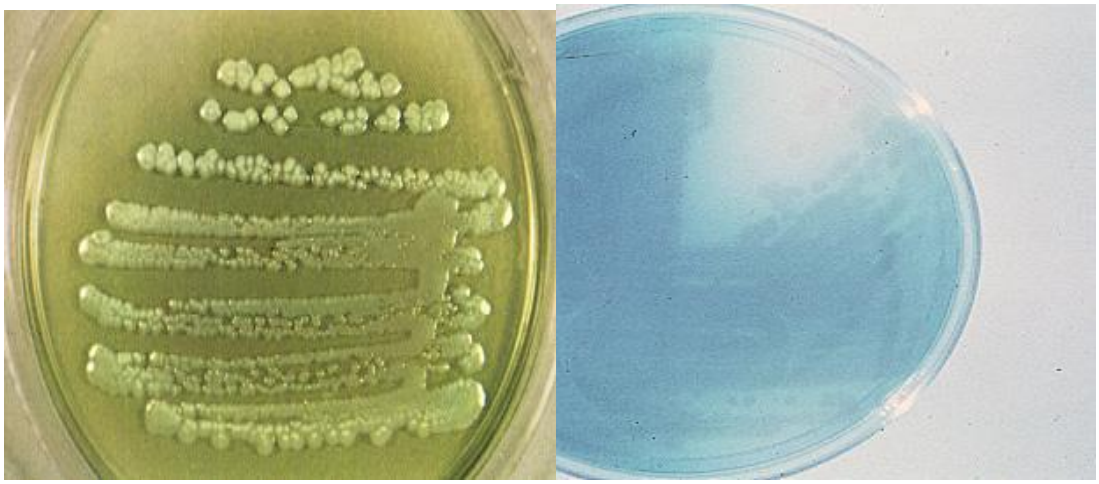

Figure 2.1 Colony appearances of *Pseudomonas aeruginosa* (Green (Left) or Blue (Right) (CDC,2019)

2.10 The Use of Polymerase Chain Reaction, PCR, to Confirm *Pseudomonas aeruginosa* in Surgical Site Infections

Some of the molecular methods of detecting *Pseudomonas aeruginosa* in Surgical Site Infections are: Conventional Polymerase Chain Reaction (PCR) method, Real-Time PCR, Multiplex Polymerase Chain Reaction and Fluorescence-Based quantitative PCR.

2.10.1 Conventional polymerase chain reaction method

Polymerase Chain Reaction (PCR) ensures accurate and rapid identification of *Ps. aeruginosa* species (Anuj *et al.*, 2009). This, to a large extent, minimizes the potential error of phenotype misidentification of *Ps. aeruginosa* and false negative culture of Ps. aeruginosa colonization due to sample overgrowth by other bacteria (Cornelis et al., 1989). Conventional PCR is based on three simple steps required for any DNA synthesis reaction: (1) denaturation of the template into single strands; (2) annealing of primers to each original strand for new strand synthesis; and (3) extension of the new DNA strands from the primers. These reactions may be carried out with any DNA polymerase and result in the synthesis of defined portions of the original DNA sequence. However, in order to achieve more than one round of synthesis, the templates must again be denatured, which requires temperatures well above those that inactivate most enzymes (Delidow *et al.*,1993).

The common target genes in conventional PCR for *Ps. aeruginosa* include *oprI, oprL, ecfX, gyrB, algD* and *fliC* (Deschaght *et al.*, 2011).

2.10.2 Real-time, fluorescence-based quantitative PCR

In 1996, Applied Biosystems, Inc. (USA) invented the Real-Time, Fluorescence-Based Quantitative PCR (real-time qPCR) method. This method enables quantitative detection via real-time monitoring of PCR reactions by introducing fluorescent molecules into the reaction mixture. Currently, it has become one of the most widely used nucleic acid-based molecular

detection techniques for pathogens including *Ps. aeruginosa* associated with SSIs (Johnson *et al.*, 2013).

2.10.3 Multiplex polymerase chain reaction

The main advantage of multiplex PCR is its ability to simultaneously amplify multiple PCR products in a single reaction, thereby enabling multiplex detection and significantly reducing the detection cost and time requirements. This could involve *Ps. aeruginosa,* with other bacteria (De Vos *et al.* (1997) simultaneously detected *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* in clinical samples using a duplex PCR assay. This method had 100% sensitivity and 74% specificity, with a detection limit of 102 cells per mL in skin biopsy specimens from patients with burns and sputum samples from cystic fibrosis patients.

2.11 Treatment of Surgical Site Infections associated with *Pseudomonas aeruginosa.*

Pseudomonas aeruginosa associated with *Ps. aeruginosa* are generally treated with antibiotics. Unfortunately, because of the prevalent multi-drug resistance of *Ps. aeruginosa* in SSIs, the antibiotic treatment is difficult. Ideally, the antibiotic treatment of SSI associated *Ps. aeruginosa* is influenced by the antibiotic susceptibility of the *Ps. aeruginosa* from wound swab analysis. Other factors are potential side effects of the antibiotics, drug-drug interactions of the antibiotic with other drugs used by the clinician in the systemic treatment of the patient and cost of the drugs. In poor resource setting, the selection of antibiotics may be influenced by knowledge of the previous laboratory results, if any, of the antibiotic susceptibility patterns of *Ps. aeruginosa* from surgical site wounds at the health facility (CDC, 2019).

2.12 Prevention of Surgical Site Infections associated with *Pseudomonas aeruginosa*

The general prevention of SSI associated with *Ps. aeruginosa* are related to the general protocol for infection control in the hospital. The prevention is also specifically related to the

2019 revised WHO Global Guidelines for the Prevention of Surgical Site Infection and the 2019 guidelines on prevention and treatment surgical site infections by the National Institute for Health and Care Excellence (NICE). The recommendations include:

1. Practice of infection control protocol at health facilities.

2. Hand hygiene by both health provider and patients using soap and under running water or use of alcohol-based hand sanitizer, particularly before and after caring for wounds or touching a medical device.

3. Environmental sanitation for example daily and periodic patients' rooms cleaning and disinfection and disinfection of shared equipment (CDC, 2019).

The specific recommendations by CDC and WHO in the prevention of SSIs associated with *Ps. aeruginosa* could be divided into three: pre-operative, intra-operative and post -operative measures (CDC, 2019)

2.12.1 Pre-operative measures

1. Preoperative health education to patients and patients' relatives on SSIs and the treatment.

2. Preoperative bathing of patients through shower or bed bath using soap or appropriate antimicrobial agent, either the day before or on the day of surgery.

3. Removal of hand jewellery, artificial nails and nail polish by any member of operating team before surgery to reduce SSI.

4.Surgical hand preparation either by scrubbing with a suitable antimicrobial soap and water or aqueous antiseptic surgical solution with a single-use brush or pick for the nails before donning sterile gloves.

5. Surgical site preparation of the skin at the surgical site immediately before incision using appropriate antiseptic preparation of either alcohol or Chlorhexidine Gluconate (CHG).

6. Administration of Surgical Antibiotic Prophylaxis (SAP) within 120 minutes before incision, while considering the half-life of the antibiotic to the surgical incision when indicated, depending on the type of surgical operation.

7. The simultaneous use of Mechanical Bowel Preparation (MBP) and the use of preoperative oral antibiotics are recommended to reduce the risk of SSI in adult patients undergoing elective colorectal surgery

8. Conditional removal of patient's hair around the surgical site.While hair removal for surgery is generally discouraged to reduce SSIs, when absolutely necessary, hair should be removed only with a clipper with a single-use head on the day of surgery. The use of razors for hair removal is discouraged because they increase the risk of surgical site infection (CDC, 2019; WHO, 2019).

2.12.2 Intra-operative measures

1. The use of both sterile, disposable, non-woven or sterile, reusable woven drapes and surgical gowns is indicated during surgical operations for the purpose of preventing SSI.

2. The use of triclosan-coated (antimicrobial-coated) sutures for the purpose of reducing the risk of SSI, independent of the type of surgery.

3. The application of antiseptic or antibiotic to surgical wounds before closure is not advised except as part of a clinical research trial. Currently, there is no empirical evidence supporting usefulness of antimicrobial sealants on surgical wound after surgery.

4. The maintenance of optimal oxygenation during major surgery and in the recovery period and maintain a blood hemoglobin saturation of more than 95%.

5. The practice of wound irrigation and intra-cavity lavage to reduce the risk of surgical site infection is discouraged because of lack of empirical evidence to reduce SSIs.

6. Incise drapes, when used, should be iodophor-impregnated drapes unless the patient has an iodine allergy. Non-iodophor-impregnated incise drapes used routinely for surgery may increase the risk of surgical site infection.

7. The use of antimicrobial triclosan-coated sutures, especially for pediatric surgeries, should be considered to reduce the risk of surgical site infection.

8. The use of sutures instead of staples to close the skin after caesarean section is useful to reduce the risk of superficial wound infection and breakdown (CDC, 2019; WHO, 2019).

2.12.3 Post-operative measures

1. The use of sterile dressing for 24 to 48 hours post operatively offers protection of the surgical wound.

2. The practice of adequate hand washing before and after surgical wound dressing is recommended.

3. The practice of aseptic non-touch technique in the changing or removing of surgical wound dressings is recommended.

4. It is beneficial to advise patients on the safety of having bath/shower 48 hours after surgery.

5. Wound cleansing with sterile saline up to 48 hours after surgery may be required.

6. The application of topical antimicrobial agents for surgical wounds that are healing by primary intention is not advised to reduce the risk of surgical site infection

7. The use of Eusol and gauze, or moist cotton gauze or mercuric antiseptic solutions to manage surgical wounds that are healing by secondary intention is discouraged.

8. Tap water may be used for surgical site wound cleansing after 48 hours if the wound has broken down and suppurating pus. Appropriate interactive dressing may be used to manage surgical wounds that are healing by secondary intention.

9. The use of Eusol and gauze, or dextranomer or enzymatic treatments for debridement in the management of surgical site infection is not advised. (CDC, 2019; WHO,2019)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

Bauchi State is located in the North-Eastern geo-political region of Nigeria, on the latitude coordinate of 10.314159 (10° 18' 50.9724" N) and longitude coordinate of 9.846282 (9° 50' 46.6152" E). It is the fifth largest state in Nigeria with a land mass of 45,837km^2. It shares boundaries with Kano and Jigawa States at the north, Taraba and Plateau States at the south, Gombe and Yobe States at the East and Kaduna State at the west. It has savannah vegetation and an estimated population of about 6,537,300 people who are mostly farmers (Latlong.net, 2018). The map of the study area is presented in Figure 3.1.

The study was conducted in two locations in Bauchi Local Government of Bauchi State. These were Bayara, a rural village and Shadawanka, an urban town. The inhabitants of Bayara are predominantly poor village farmers and traders. There is a secondary hospital facility, New General Hospital Bayara which provides medical services for them. Shadawanka is located in the Bauchi capital metropolis and is dominated by middle class. Specialist Hospital Bauchi, a tertiary hospital facility is located in the Shadawanka .

3.2 Study Design

The study was a cross-sectional and hospital based one in which the hospital files of consented patients with surgical site infections were assessed for relevant information for the study. The information obtained from the patients' files were recorded in a proforma.

3.3 Study Population

The study was carried out among consented admitted patients with surgical site infections and who were within 30days post-surgery at the selected hospital facilities.

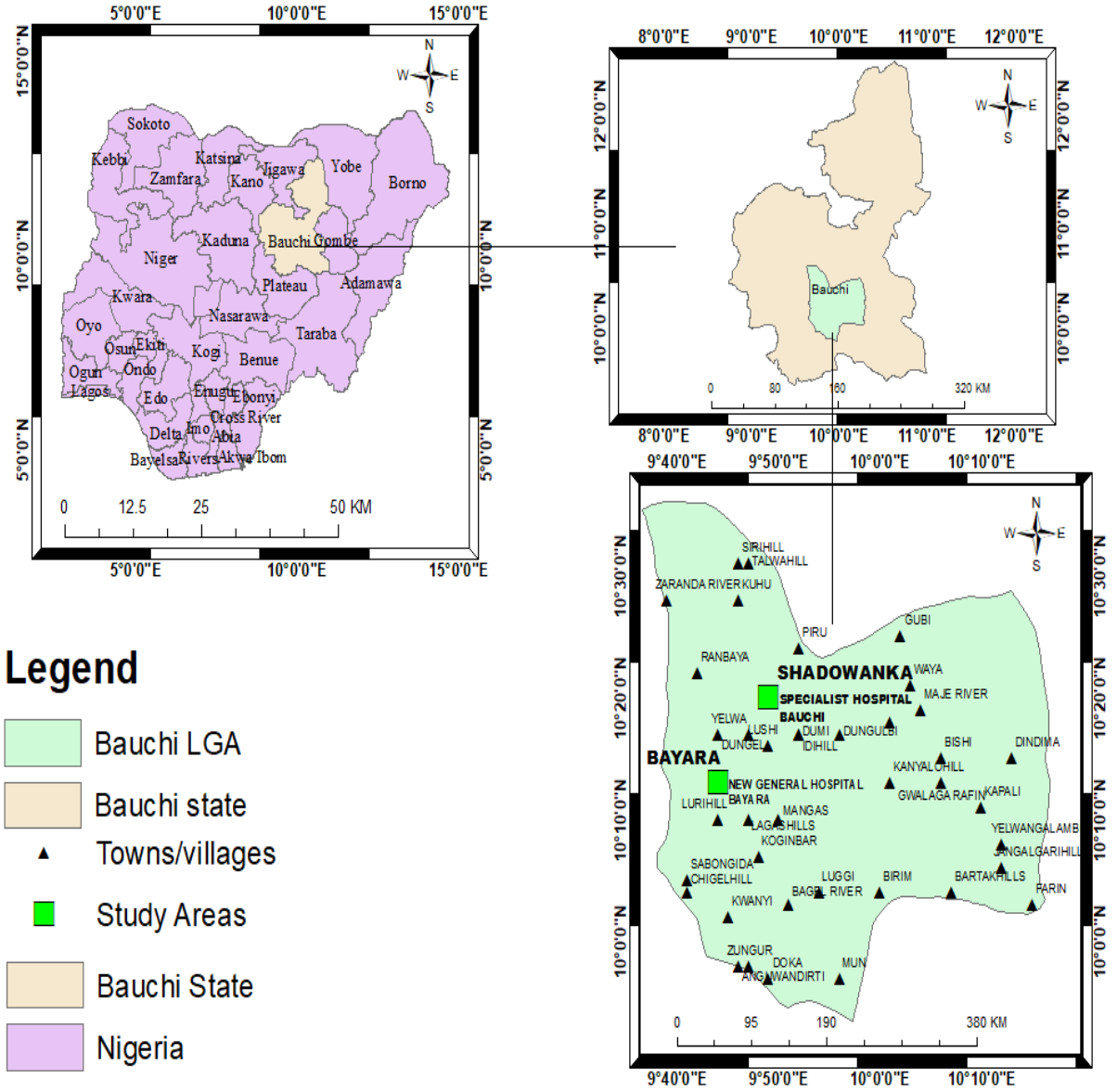

Fig 3.1: Map of Bauchi State showing the sampling area

3.4 Ethical Approval and Consent

Approval for the study was obtained from the Bauchi State Health Research and Ethics Committee (Appendix I). Permission was also obtained from the heads of the two facilities. Consent for the study was obtained and the consent form was duly signed by every patient that met the inclusion criteria for the study (Appendix II).

3.5 Sample Size Determination

The sample size for the study was determined using the formula by Thrushfied (2007). A prevalence of 19% reported for *Ps. aeruginosa* in surgical site infection by Yasidi *et al.* (2015) was used for estimating the minimum sample size.

$n=Z^2Pq/L^2$

Where

n = number of samples

Z = standard normal deviate at 95% CI = 1.96

P = 19% (Yasidi *et al.*, 2015) = 0.19

q = 1 – 0.19 = 0.81

L = allowable error of 5% (0.05)

$n = Z^2Pq/L^2$

$n = 1.96^2 * 0.19*0.81/0.05^2$

= 0.59122224/0.0025

= 236.48 ~ 237

However, 250 samples were used for the study to accommodate for bias and sample errors.

3.6 Data Collection

3.6.1 Inclusion and exclusion criteria

The inclusion criteria were based on the guideliness for identifying SSI by CDC (CDC, 2018). All consented patients who had surgery and were on admission within 30days of surgeries and who had purulent discharge or/and at least had one of the following: pain, tenderness, or high temperatures (greater than 40^{o}C) were included.

Patients who did not have surgical site infections or had SSI or had antibiotics two weeks before the surgery or did not consent to the study were excluded.

3.6.2 Data collection with proforma

Informed consent was obtained from each patient. Relevant demographic data including health facility, ward, age, sex, surgery type, duration in hospital and occupation were obtained from patients' hospital files and recorded in the proforma (Appendix III).

3.7 Collection of Samples and Processing

The surgical wound swabs of patients that met the inclusion criteria were collected when their wounds were opened for wound review or wound dressing at the wards. The swabs were labeled and transported in ice packs to the Microbiology Unit Abubakar Tafawa Balewa University Teaching Hospital. At this laboratory, swabs were inoculated into Cetrimide Agar and incubated at 37°C for 24hrs.

A total of two hundred and fifty (250) wound swab samples were collected and inoculated into Cetrimide agar.

3.8 Analysis of Samples

The samples were cultured on cetrimide agar, Gram stained and biochemically characterized.

3.8.1 Culture on cetrimide agar

The swabs samples were inoculated on the Cetrimide Agar by surface swabbing then incubated at 37°C for 24hours. After the incubation, the culture plates were examined and read after 24hours and extended to 48 hours for plates with no appreciable growth after 24 hours. The plates were examined for colonial morphology, shape colour and odour.

3.8.2 Biochemical characterization of isolates

Two biochemical tests were conducted for further identification of the isolates. These are discussed below:

1. Oxidase test: A colony of the test organism was picked using a wire loop and smeared on the oxidase strip paper (impregnated with the substrate tetramethyl-p-phenylenediamine dihydrochloride). The smeared area of the oxidase strip paper changed change to deep blue colour or purple (indophenols) within 10 seconds indicating that *Ps. aeruginosa* is Oxidase positive.

2. Citrate (utilization) test: A light inoculum was picked from the center of the *Ps. aeruginosa* colony and inserted on the test slant. Next, the slant is incubated aerobically at 37°C for 4 days. It was observed that there was no colour change-the slant changed from green to intense blue, indicating that *Ps. aeruginosa* is citrate positive.

3. Catalase test**:** In the test, a drop of 3% hydrogen peroxide is inserted in a glass slide having a small amount of *Ps. aeruginosa* colony. There were bubbles (of Oxygen gas) indicating a positive test. A negative control that had no colony but emulsified in one of the drops, served as a negative control. *Ps. aeruginosa* is catalase positive.

4. Urease test*:* In the Rapid Urease Test (RUT), a colony of *Ps. aeruginosa* is inserted into a slant containing Urease reagent. The cap is left loose and incubated aerobically for 48 hours at 37°C. There is no change of colour of the slant from the yellow, indicating that is *Ps. aeruginosa* is Urease negative.

5. Indole test: The Procedure for Indole Spot Reagent (Dimethylaminocinnamaldehyde, DMACA) test: Several drops of Indole Spot Reagent were placed on a piece of filter paper. Next, a colony of *Ps. aeruginosa* is picked with an inoculation loop and rubbed onto the reagent saturated area of the filter paper. There is no change of colour of the filter paper that still remained pink, indicating that *Ps. aeruginosa* is Indole negative.

6.Sugar Fermentation: In the test, the Purple Broth (with glucose) was warmed to room temperature and then inoculated with pure culture of *Ps. aeruginosa*. A control of only Purple Broth Base was also prepare. Both media were incubated for 37ºC for 3 days. It was observed that there was no change in the colour of the broth.

3.9 Confirmation of Isolates using Polymerase Chain Reaction (PCR)

PCR analysis was done using *oprL* as the target gene.The primer sequence for the PCR is shown in Table 3.1.The PCR steps involved the DNA extraction, amplification and Gel electrophoresis.

3.9.1 DNA extraction

In order to minimize contamination, the procedure was carried out on separate benches. DNA extraction was conducted using QIAGEN DNA extraction kit. The DNA extraction involved three processes: collection of cells, lysing bacteria and DNA purification.

1. Collection of cells: One mililiter of incubated bacterial culture was pipetted into a 2.0 ml sterile Eppendorf tube. This was centrifuged for 5 minutes at 7500 rpm. The supernatant was discarded into a 50 ml conical tube with 10% commercial bleach marked “biohazard waste".

2. Lysing bacteria: 180 µl of Buffer ATL was added to the mixture followed by addition of 20 µl of Proteinase K. The bacteria cell pellet was resuspended by vortexing and incubated for 30 minutes. This was followed by addition of 200 µl of Buffer AL and then mixed by vortexing

Table 3.1: Primer Sequence for detection of *oprL* gene

Target Gene	Primers sequences (5'-sequence-3')	Size of target gene
oprL-F	ATGGAAATGCTGAAATTCGGC	504 bp
oprL-R	CTTCTTCAGCTCGACGCGACG	

(Jami Al-Ahmadi & Zahmatkesh Roodsari,2016)

1. Collection of cells: One mililiter of incubated bacterial culture was pipetted into a 2.0 ml sterile Eppendorf tube. This was centrifuged for 5 minutes at 7500 rpm. The supernatant was discarded into a 50 ml conical tube with 10% commercial bleach marked “biohazard waste".

2. Lysing bacteria: 180 µl of Buffer ATL was added to the mixture followed by addition of 20 µl of Proteinase K. The bacteria cell pellet was resuspended by vortexing and incubated for 30 minutes. This was followed by addition of 200 µl of Buffer AL and then mixed by vortexing

3. DNA purification: The mechanism of DNA purification is the selective absorption of DNA to the silica of the spin column. The debris and proteins are the discarded materials.

The lid of a DNeasy spin column was uniquely labelled (recorded identifier) for each of the 5 samples. Using a P1000 pipette, the full volume of pre-treated bacterial cells was transferred to the corresponding spin column. This was centrifuged at 8000 rpm for 1 minute and then the collection tube and its contents were discarded. The DNeasy spin column was placed into a new 2.0 ml collection tube.Next, there was addition of 500 µl of Buffer AW and then the mixture was centrifuged at 8000 rpm for 1 minute. Next, the collection tube and its contents were discarded. The DNeasy spin column was again placed into a new 2.0 ml collection tube. There was addition of 500 µl of Buffer AW2 and then centrifuged at 13000 rpm for 3 minutes. The collection tube and its contents were discarded and the spin column examined to ensure that no liquid remains on the spin column. The spin column was placed in a new, sterile 1.5 ml elution tube labelled with a unique, recorded identifier. There was addition of 100 µl of Buffer EB to the spin column. The Buffer EB was pre-incubated at 37°C to improve elution. The new mixture was incubated at room temperature for 5minutes and centrifuged at 8000 rpm for 1 minute. The eluate is the purified DNA.

3.9.2 PCR amplification

Eight (8) microlitres of extracted DNA was dispensed in aliquoted 0.2mls of nuclease free microtubes.

Preparation of Reaction mix: The cocktail was prepared in1.5ml nuclease free microfuge tube by the steps below. First 5 x 12.5µl of Qiagen Toptaq PCR master mix was added. Then, there was addition of 5 x 0.5 µl of 20mM of Forward Primer of *oprL* Gene to all the tubes before the addition of 5 x 0.5 µl of 20mM of Reverse Primer of *oprL* Gene.The next step was addition of 2.5 µl of coral load and then addition of 1uL of Nuclease free water. The whole cocktail was carefully mixed. Then, 14.2µl (71 µl /5) of the cocktail was dispensed into each of the 5 PCR tube (containing the template) and carefully mixed.

The PCR tubes were capped and transferred to the applied Biosystem 9700 Thermocycler. Initial denaturation was at 95ºC for 5 minutes. This was followed by 30 cycles of denaturation at 95ºC.Annealing temperature was at 57ºC for 30 seconds and extension at 72ºC for 1minute.Final extension was at 72ºC for 10 minutes. Cooling was at 4ºC for four minutes.

3.9.3 Gel Electrophoresis

The amplified products were resolved by 1% agarose gel electrophoresis stained with ethidium bromide, and photographed using a Ultraviolet transilluminator.

Preparation of 1% Agarose gel.1g of Agarose powder was measured out and placed in a clean beaker and then 100ml of water was added and then mixed. There was a subsequent addition of 2ml of 1 X Tris Acetate Ethylne Diamine Tetra Acetate (TAE buffer) to it and the heating of the mixture for 3 minutes in a microwave. There was then addition of 5 µl of ethidium bromide before casting and cooling of the gel in the electrophoresis chamber. Two hundred and fifty milliliters of 1X TAE were added in the chamber before the loading of the samples into the microwells using the micropipette.

The electrophoresis was conducted at 80volts for 45 minutes visualized visualized under the ultraviolet illuminator.

3.10 Antibiotic Susceptibility (Kirby Bauer Disc Diffusion Method)

All detected *Ps. aeruginosa* isolates were assayed for antibiotic susceptibility by the Kirby Bauer Disk Diffusion Method. The multi disc method utilized was the Maxi Disc and had ten antibiotics that were selected based on the patients' antibiotic susceptibility pattern in the study area. The test organisms were picked up with a sterile loop then suspended in peptone water and incubated at 37°C for 2 hours. The turbidity of the suspension was adjusted to 0.5 McFarland's Standard (1.5×10^8 CFU/mL). A sterile swab stick was dipped into the bacterial suspension and excess fluid removed by pressing the swab stick against the wall of the tube, the swab was then used to carefully swab the entire surface of Mueller Hinton Agar (MHA) plates. The surface was then allowed to dry for 3 minutes. The commercially prepared antibiotic multidisc (Maxi Disc) of selected 10 antibiotic agents were then placed on the inoculated MHA 25mm away from each other. The antibiotics were :Streptomycin,S (30µg); Cotrimoxazole, SX (30µg); Sparfloxacin,SP (10µg); Ciprofloxacin,CPX (10µg); Amoxicillin, AM (30µg); Augmentin, AU (30µg); Chloramphenicol, CH (30µg); Gentamicin, CN(10µg); Peflacin, PEF (30µg) and Ofloxacin, OFX(10 µg).The plates were then incubated at 35°C for 24hrs after which the zones of inhibition for each of the antibiotic were recorded. The diameter of zone of inhibition was measured in millimetres and interpreted as sensitive, intermediate or resistant based on the chart by Clinical and Laboratory Standard Institute (CLSI) guidelines 2017.

3.11 Data Analysis

Data on various variables extracted from patients' hospital files and recorded on the proforma were transferred to the Statistical Package for Social Sciences (SPSS) version 23 for analysia. The results obtained were presented in tables and percentages.

CHAPTER FOUR

4.0 RESULTS

4.1 Result of isolation and characterization of *Ps. aeruginosa* from surgical site infections

Out of the 250 surgical site wound swabs from the two selected hospital facilities, 2.0% (5/250) were positive for *Ps. aeruginosa* (Figure 4.1). This gave the total incidence of *Ps. aeruginosa* associated with the SSIs at the hospital sites to be 2.0%. All the 5 positive samples were obtained from Specialist Hospital Bauchi. No positive sample was obtained from New General Hospital Bayara. There was significant statistical difference between incidence of *Ps. aeruginosa* infection and SSI and the hospital facilities (χ^2 = 21.12 , df= 1, p= 0.029).(Table 4.1)

Table 4.1: **Incidence of *Pseudomonas aeruginosa* associated with Surgical Site Infections in relation to the selected hospitals in Bauchi State, Nigeria. (n=250)**

Variables	No. Tested	No. Positive (%)	χ^2	Df	p-value
Specialist Hospital					
Bauchi	205	5(2.4)	1.12	1	0.029
New General Hospital					
Bayara	45	0(0.0)			

Key: χ^2=Chi Square; P-value < 0.05 is statistically significant; $p \geq 0.05$ is statistically not significant

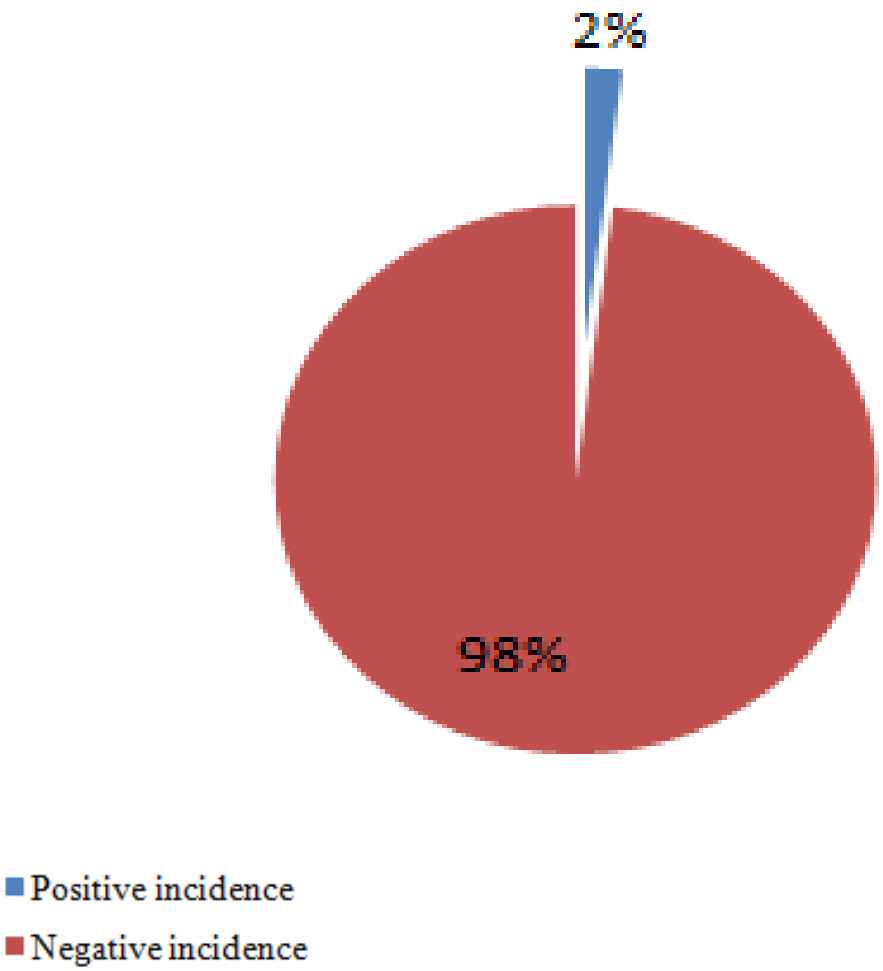

Fig 4.1:Overall incidence of *Pseudomonas aeruginosa* associated with surgical site infections in selected hospitals in Bauchi State,Nigeria.

4.2 PCR Result

All the isolates were successfully confirmed to be *Pseudomonas aeruginosa* with PCR at an amplicon size of 504 bp.

Plate 4.1 shows Gel electrophoresis graph of *oprL* gene with an amplicon size of 504 bp.

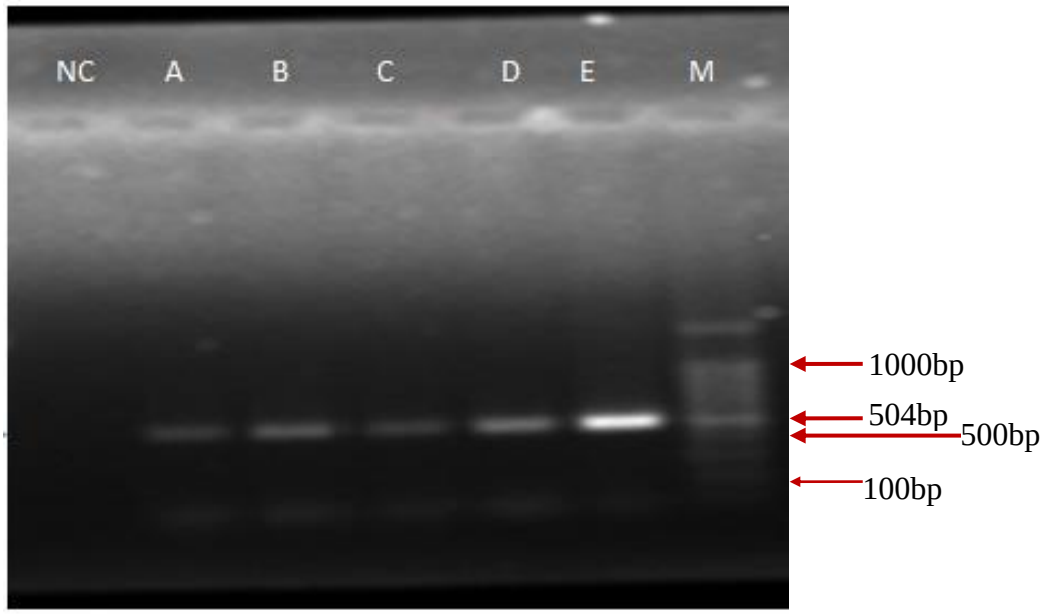

Plate 4.1: Gel electrophoresis graph of *oprL* gene with amplicon size of 504 bp

Key:

Lane NC = Negative control

Lanes A-E = Samples

Lane M = 100bp molecular marker

4.3 Antibiotic Susceptibility Pattern of the *Pseudomonas aeruginosa* isolates

Table 4.2 shows the antibiotic susceptibility pattern of *Ps. aeruginosa* isolated from SSI from selected hospitals in Bauchi State.

From the table, all the isolates were all susceptible to Ofloxacin. All the isolates were resistant to cotrimoxazole.

4.4 Antibiotic Resistance Pattern of the *Pseudomonas aserugin* :s

The result of the antibiotic resistance pattern is shown in Table 4.3. The number of antibiotics resisted ranged from 2 to 4. Four out of five (80%) of the isolates were multi-drug resistant (antibiotic resistance of at least 1 antibiotic from 3 or more antibiotic classes (Magiorakos *et al.*,2012). These classes include penicillins (Amoxicillin and Augumentin), aminoglycosides (Gentamicin), Flouroquinolones (Ciprofloxacin, Pefloxacin, Sparfloxacin and Ofloxacin), Sulfonamides (Cotrimoxazole) and anti-50S ribosome synthesis inhibitor (Chloramphenicol and Streptomycin).

The Multiple Antibiotic Resistance (MAR) index (ratio of number of antibiotics resisted to number of antibiotics tested) ranged from 0.2 to 0.5. Isolates A, B and D had a MAR index of 0.5 each while isolates C and E had MAR indices of 0.2 and 0.4 respectively.

Table 4.2: **Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* associated with Surgical Site Infections in selected hospitals in Bauchi State, Nigeria. (**Clinical and Laboratory Standard Institute (CLSI) guidelines 2017.)

Antibiotics (Disc potency)	**No.(%) Susceptible**	**Intermediate**	**No.Resistant (%)**
Gentamicin (10µg)	3(60)	0(0)	2(40)
Pefloxacin (30 µg)	4(80)	0(0)	1(20)
Ofloxacin (10µg),	5(100)	0(0)	0(0)
Streptomycin (30µg)	2(40)	0(0)	3(60)
Cotrimoxazol(30µg)	0(0)	0(0)	5(100)
Sparfloxacin (10 µg)	2(40)	0(0)	3(60)
Ciprofloxacin(10µg)	4(80)	0(0)	1(20)
Amoxicillin (30µg)	3(60)	0(0)	2(40)
Augmentin (30µg),	4(80)	0(0)	1(20)
Chloramphenicol (30µg)	1(20)	0(0)	4(80)

Table 4.3 Antibiotic Resistance pattern of *Pseudomonas aeruginosa* associated with Surgical Site Infections in selected hospitals in Bauchi State, Nigeria

Isolates ID	No of antibiotics tested	No of antibiotics resisted	Resistance Pattern	MAR Index	No of Antibiotic classes resisted
A	10	5	PEF,SXP,AM,AU,CH	0.5	4
B	10	5	CN,S,SXT,AM,CH	0.5	4
C	10	2	S,SXT	0.2	2
D	10	5	S,SXT,SP,CPX,CH	0.5	4
E	10	4	CN,SXT,SP,CH	0.4	4

KEY:

PEF=Pefloxacin

SXT=Cotrimoxazole

AM=Amoxicillin

AU=Augmentin

CH=Chloramphenicol

CN=Gentamicin

S=Streptomycin

SP=Sparfloxacin

CPX=Ciprofloxacin

MAR=Multiple Antibiotic Resistance

4.5 Incidence of *Pseudomonas aeruginosa* in Relation to Demographic Variables

Some demographic variables of patients were assessed in relation to *Ps. aeruginosa* associated with SSIs as shown in Table 4.4.

The incidence of positive samples was 2.80 among females and 1.40% among males. The difference in the prevalence based on sex was not statistically significant (χ^2=0.617, p-value=0.432).

The incidence of positive samples was highest at 11.11 and among the age bracket of 51-60years. It was lowest at 0.00 and at the age bracket of 21-30 years. The difference in frequencies based on ages was not statistically significant (χ^2=10.014, p-value=0.075).

The incidence of positive samples was highest at 8.33 and among participants who had tertiary education. It was lowest at 2.27 and among participants who had no formal education. The difference in incidence based on educational status was statistically not significant (χ^2=3.543, p-value=0.315).

The incidence of positive samples was highest at 9.09 and among drivers. It was lowest at 0.00 and among participants who were unemployed, manual labourers, hotel managers football players and civil servants. The difference in incidence based on occupation was not statistically significant (χ^2=9.120, p-value=0.521).

Table 4.4: Incidence of *Pseudomonas aeruginosa* associated with surgical site infections in relation to demographic variables among selected hospitals in Bauchi State, Nigeria.

Variables	No. Tested	No. Positive (%)	χ^2	Df	p-value
Sex					
Female	107	3 (2.80)	0.617	1	0.432
Male	143	2 (1.40)			
Total	250	5			
Age					
1-10	10	1 (10.00)	10.014	5	0.075
11-20	62	1 (1.61)			
21-30	89	0 (0.00)			
31-40	61	1 (1.63)			
41-50	19	1 (5.26)			
51-60	9	1 (11.11)			
Total	250	5			
Education Status					
No formal education	119	1 (0.84)	3.543	3	0.315
Primary	88	2 (2.27)			
Secondary	31	1 (3.22)			
Tertiary	12	1 (8.33)			
Total	250	5			
Occupation					
Unemployed	9	0 (0.00)	9.120	10	0.521
Domestic work	43	1 (2.32)			
Student	46	1 (2.17)			
Manual labour	9	0 (0.00)			
Trading	85	0 (0.00)			
Hotel management	1	0 (0.00)			
Professional Soccer	1	0 (0.00)			
Public relations	3	0 (0.00)			
Civil service	14	0 (0.00)			
Driving	11	1 (9.09)			
Farming	28	2 (7.14)			
Total	250	5			

Key: χ^2=Chi Square; P-value < 0.05 is statistically significant (*); p $\geq$ 0.05 is statistically not significant.

4.6 Incidence of *Pseudomonas aeruginosa* in Relation to Risk Factors

Table 4.5 show the incidence of *Pseudomonas aeruginosa* in relation to risk factors. The highest incidence was 100.00% in a single patient with below elbow amputation. The least incidence of 0.00% was found among patients who had sutured laceration at the upper limb/arm, amputation of the right toe, inguinal herniorrhaphy, incisional hernia, sutured lacerations of the scalp and lower limb/foot. The difference in prevalence based on surgery type was statistically significant (χ^2=57.708, p-value=0.000).

An incidence (positive samples) of 12.5 % was obtained among patients who were 11-15 day post-operative admission. The least prevalence of 0.97% was obtained among patients who were 1-5 days post-operative. The difference in prevalence based on post-operative duration was statistically significant (χ^2=6.366, p-value=0.041).

An incidence (positive samples) of 12.50% was obtained among patients who were HIV positive. The prevalence of 1.65% was obtained among patients HIV negative patients. The difference in prevalence based on HIV status was statistically significant (χ^2=4.649, p-value=0.031).

Table 4.5: **Incidence of *Pseudomonas aeruginosa* associated with surgical site infections in relation to risk factors among selected hospitals in Bauchi State, Nigeria**

Risk factors	No. Tested	No. Positive (%)	χ^2	Df	p-value
Surgery					
Caeserean section	105	2 (1.90)	57.708	11	**0.000***
Laparatomy	38	1 (2.63)			
Sutured Laceration Upper limb/arm)	20	0 (0.00)			
Amputation (Right toe)	1	0 (0.00)			
Inguinal Hernionrraphy	19	0 (0.00)			
Breast lumpectomy	6	1 (16.67)			
Incisional hernia	3	0 (0.00)			
Appendisectomy	5	0 (0.00)			
Amputation (Below Knee)	28	0 (0.00)			
Sutured laceration (scalp)	18	0 (0.00)			
Amputation (Below elbow)	1	1 (100.00)			
Sutured laceration (Lower limb/foot)	6	0 (0.00)			
Total	250	5			
Post- Operative Duration (Days)					
1-5	206	2 (0.97)	6.366	2	**0.041***
6-10	28	1 (3.57)			
11-15	16	2 (12.50)			
Total	250	5			
HIV Status					
Positive	8	1 (12.50)	4.649	1	**0.031***
Negative	242	4 (1.65)			
Total	250	5			

Key: χ^2=Chi Square; P-value < 0.05(*) is statistically significant $p \geq 0.05$ is statistically not significant

CHAPTER FIVE

5.0 DISCUSSION

In this study, an overall incidence of *Ps. aeruginosa* associated with surgical site infections in the selected hospitals in Bauchi State was found to be 2.0%. The low overall incidence of 2.0% reported from the study may be due to good surgical wound management in Specialist Hospital Bauchi and New General Hospital, Bayara.

The overall incidence of 2.0% reported from the study is much lower than the 19.0% reported in the study by Yasidi *et al.* (2015) in Nguru,Yobe State, Nigeria.Similarly, the overall incidence is lower than the 19.4% reported by Abdulmutallib *et al.* (2019) in three selected hospitals in Sokoto, North-Western Nigeria. It is also lower than the prevalence rate of 29.6% reported by Ranjan *et al.* (2010) in India. The differences in the incidences may be due to variation in the population studied as well as the sample size utilized. For example, in the study by Yasidi *et al.* (2015), there was a larger sample of 392 compared to the 250 in the present study. More so, the frequencies of *Ps. aeruginosa* associated with SSIs vary with patients, geographic regions and hospital facilities due to for example, variations in laboratory protocol for the detection of the pathogen (WHO,2019).

The incidence reported at Specialist Hospital Bauchi and New General Hospital Bayara were 2.4% and 0.0% respectively. New General Hospital Bayara had a lower incidence than Specialist Hospital Bauchi probably because of lower patient attendance and number of surgeries in the former compared to the latter. New General Hospital Bayara is located at a low populated area at the outskirts of Bauchi metropolis. The facility is attended by fewer number of patients compared to Specialist Hospital Bauchi. Specialist Hospital Bauchi is a tertiary hospital facility located in Bauchi capital metropolis and attends to higher number of patients.

All the isolates of *Ps. aeruginosa* were confirmed by PCR results. They all had the *oprL* gene, which is a resistance gene that is very common in the outer membranes of *Ps. aeruginosa* strains (Douraghi *et al.,* 2014). The finding is similar to the study by Jaffe *et al.*, 2001; Levenir *et al.* ,2007 and Anuj *et al.* (2009) who all reported a 100% confirmation of positive *Ps. aeruginosa* culture positive isolates with PCR using *oprL* target gene. The finding reaffirms the reliability and specificity of *oprL* gene in the molecular detection of *Ps. aeruginosa* in wound and other clinical samples (Anuj *et al.*, 2009; Deschaght *et al.*, 2011).

Five classes of antibiotics were utilized in the antibiotic susceptibility assay. This was to have an extensive evaluation of the susceptibility pattern and determine multi-drug resistance. Eighty percent of the isolates were multi-drug resistant similar to 81.0% reported by Hassuna (2016). It is however lower than the 8.5% reported by Abdulmutallib *et al.*(2019) in selected hospitals in Sokoto,North-Western Nigeria.The multi-drug resistance of the isolates reported in this study is however higher than the 15.9% reported by Weiner *et al.* (2016). The difference in the antibiotic resistance may result from differences in antibiotic resistance genes and plasmids of the various isolates of *Ps. aeruginosa*.The 100% resistance to septrin by all the isolates in this study is similar to the report by Chir (2017). The antibiotic resistance to ciprofloxacin, gentamicin, amoxicillin, streptomycin is comparable to the ones reported by Chir (2017). The high level of antibiotic resistance was probably as a result of excessive and indiscriminate use of broad-spectrum antibiotics. The antibiotic susceptibility pattern in this study confirms the *Ps. aeruginosa* isolates obtained in the study were multi-drug resistant (Oli *et al.,* 2017). The finding underscores the need for antibiotic susceptibility test for any suspected case of SSI. In poor resource setting the known antibiotic susceptibility pattern of previous assays in a health facility should be a guide for the antibiotic management of SSIs associated with *Ps. aeruginosa* (Bangera *et al.,* 2016).

There was no statistically significant relationship between the sociodemographic variables and the incidence of *Ps. aeruginosa* in the study. However, there was a statistically significant relationship (p=0.041) between the type of surgery and prevalence of *Ps. aeruginosa* in the study. This high incidence of below elbow amputation was probably because of involvement of deep body layers including bone tissues which are prone to SSIs.The result is similar to the finding of Wang & Zhang (2017. who reported caesarean section, CS, as the most frequent surgery associated with SSI. Caesarean section is a similar surgery as elbow amputation in terms of involvement of not only the skin, but also deeper body tissues including muscles.

The study established a statistically significant relationship (p=0.041) between post–operative duration and incidence of *Ps. aeruginosa* associated with SSI.The post-operative duration with the highest incidence of *Ps. aeruginosa* associated with SSI was 11-15 days (12.5%). This could be because of higher SSIs associated with complicated surgeries. This is related to the study by Wang & Zhang (2017) that found a higher risk of developing *Pseudomonas aeruginosa* in SSI after 7 days post-operation. This finding highlights the importance of proper post-operative management of surgeries to attain early surgical wound healing as recommended by the 2019 WHO guidelines on the prevention of SSIs.

The study found a statistically significant relationship (p=0.031) between HIV status and prevalence of *Ps. aeruginosa* in SSIs.The study reported a higher prevalence of *Ps. aeruginosa* (12.5%) among HIV positive patients than HIV negative patients (incidence of 1.7%). This could be because the HIV infection compromised the immunity of the patients and favoured the pathogenesis of SSIs. This prevalence is lower than that reported by Zhang *et al*. (2012) who reported an HIV prevalence of 47.5% among SSIs. Immune compromised status is an established risk factor in the pathogenesis of SSI through disruption of the cell mediated mechanism of phagocytosis of *Ps. aeruginosa* (Labib, 2012).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The study isolated *Ps. aeruginosa* from surgical wound sites in selected hospital facilities in Bauchi Local Government of Bauchi State, Nigeria. The study found the overall incidence of *Ps. aeruginosa* associated with surgical site infections in the selected hospital facilities to be 2.0%. There was statistical relationships between the incidence of *Ps. aeruginosa* associated with the surgical site infections and the surgery type (p=0.000), post-operative duration (p=0.041) and HIV status (p=0.031).

The study also demonstrated the presence of *oprL* gene with 504bp in all the isolates and confirmed the *Ps. aeruginosa* isolates. The finding reaffirms the reliability and specificity of the *oprL* gene in the PCR detection of *Ps. aeruginosa* in SSIs and other clinical samples.

The study also found that the MAR indices that ranged from 0.2 to 0.5. All the isolates were susceptible to Ofloxacin and all resistant to cotrimoxazole.

6.2 Recommendation

1. Although there is a low prevalence of *Ps. aeruginosa* associated with SSIs in the facilities in thi study, there is need to strengthen surgical site wound management to further reduce the infection in the hospital facilities.

2. Appropriate post-operative management of surgical wounds is recommended to enhance early wound healing, reduce hospital stay and in turn the prevalence of *Ps. aeruginosa* associated with SSIs the hospital facilities.

3. Special post-surgical wound care should be provided for patients who undergo below knee amputation and HIV positive patients to reduce SSIs.

4. In the antibiotic management of surgical site patients with surgical sites infected with *Ps. aeruginosa*, clinicians at the facilities should take cognizance of the drug resistance to cotrimoxazole. Oxofloxacin could be the drug of choice if the antibiotic susceptibility result from surgical wound swab is not available in the facility in the facility.

5. Further studies is recommended with larger sample size to monitor changes in the drug resistance pattern of *Ps. aeruginosa* associated with SSIs.

6.3 Contribution to Knowledge

1. This study found that 2.0% was the overall incidence of *Ps. aeruginosa* associated with SSIs in Specialist Hospital Bauchi and New General Hospital Bayara all in Bauchi Local Government of Bauchi State.
2. The incidence of *Ps. aeruginosa* associated with SSIs in Specialist Hospital Bauchi was 2.4% and 0.0% in New General Hospital Bayara.
3. The *oprL* gene with a base pair of 504 bp detected and confirmed all the isolates of *Ps. aeruginosa* in the study.
4. Eighty percent (80%) of the *Ps. aeruginosa* isolates associated with SSIs in study were multi-drug resistant.

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APPENDIX 1

GOVERNMENT OF BAUCHI STATE

MINISTRY OF HEALTH

Bello Kirfi Road, Off Murtala Mohammed Way,
P.M.B. 065, Bauchi.

E-mail: bauchismoh@gmail.com

Reference MOH/GEN/S/1409/I *Date* 13th November

PROTOCOL REG. N0: BSMOH/NREC/04/2018
PROTOCOL APPROVAL N0: NREC/12/05/2013/2018/05

Titus Onyi
Deparment of Microbiology
Ahmadu Bello University
Zaria, Kaduna State.

ETHICAL CLEARANCE FOR SUBMITTED PROTOCOL:
"Isolation, Characterization and Molecular Detection of Pseudomonas aeruginosa Associated with Post-surgical Site Infections in selected hospitals in Bauchi State"

The Bauchi State Health Research Ethics Committee (HREC) under the State Ministry of Health has received the above named protocol for ethical clearance and approval in line with the guidelines set by the Committee. The protocol was reviewed and the committee noted that that the research does not entails clinical trials or any invasive procedures.

2. Consequently, the Committee hereby granted expedited approval for the research to be conducted. However, you should share with us your workplan clearly indicating the start date, where and when to visit the research site(s) and also **the final results of your findings.**

3. The Committee requires you to comply with all Institutional Guidelines, Rules and Regulations and with the tenets of the National Health Research Ethics Committee Code including that all adverse events are reported promptly to the Committee. **No changes are permitted in the research without prior approval by the Committee** except in circumstances outlined in the Code. The Committee reserves the right to conduct compliance visit to your research site without prior notice.

4. Thank you

(Usman U. Muhammad)
For: Hon. Commissioner.

APPENDIX II

INFORMED CONSENT FORM

Address of Researcher: Department of Microbiology, Ahmadu Bello University Zaria
Research Title:

CHARACTERISATION AND MOLECULAR CONFIRMATION OF *PSEUDOMONAS AERUGINOSA* ASSOCIATED WITH SURGICAL SITE INFECTIONS IN SELECTED HOSPITALS IN BAUCHI STATE.

This research is being conducted by Titus Onyi, an MSc student of the department of Microbiology of Ahmadu Bello University Zaria, Kaduna State. The research is part of the requirement for the award of the MSc degree.

Pseudomonas aeruginosa is a multidrug resistant bacterium that is implicated in surgical site infections. When it infects post-surgical wounds, it causes non-healing of the wounds, longer hospital stays for treatment, higher medical cost and sometimes death from the wound infection. Accurate information of the occurrence and aetiology of infections acquired within a hospital is essential for articulation of effective preventive measures. This is why it is important to conduct this study. The study seeks to isolate, characterize and confirm *Pseudomonas aeruginosa* associated with surgical site infections in selected hospital facilities in Bauchi State. Specialist Hospital Bauchi and New General Hospital Bayara are the study sites for the study. Patients that have surgeries in the hospital within 30days and who have pains, purulent discharge from the wound, hotness of the surgical wound site and other inclusion criteria may wish to voluntarily participate in the study. Their wound swabs may be taken for analysis in the laboratory. All the wound samples and information collected in this study will be given code and no names will be recorded. This cannot be linked in any way to you and your name. No identifier will not be used in any publication or reports from the study. Your participation is entirely voluntary. If you choose not to participate, this will not affect your treatment in the hospital in any way. You may also voluntarily withdraw from participation at any time.

Statement of person obtaining informed consent:

I have fully explained this research to……. and have given sufficient information including risks and benefit to make informed decision.

Name……. Date……Signature……

Statement of person giving informed consent:

I have read the description of the research or have had it translated in the language I understand. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may stop being part of the study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

Name……. Date……Signature……

APPENDIX III

rgery	Post-op duration	Sex	Age	Occupation	Ward	HIV Status	Growth on Cetrimide Agar	Gram Staining	Oxidase test	Citrate test	Catalase test	PCR assay