CLINICAL AND MOLECULAR EVALUATION OF *PSEUDOMONAS AERUGINOSA* NOSOCOMIAL INFECTIONS AMONG ADULT PATIENTS AT THE UNIVERSITY TEACHING HOSPITAL IN LUSAKA PROVINCE AND NDOLA TEACHING HOSPITAL IN COPPERBELT PROVINCE OF ZAMBIA

By

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A Thesis Submitted to the University of Zambia in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Infectious Diseases

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DEDICATION

This thesis is dedicated to my father, Alexandre Ngoy Kabungwe, who spared no effort from my early childhood to instill the spirit of excellence and hard work in my siblings and me. I am also grateful to my late mother, Esther Ilunga Mbidi, and my family, from whom I spent many hours away from home on clinical, teaching, and research duties.

DECLARATION

I, Patrice Ntanda Mukomena, do at this moment declare to the senate of the University of Zambia that this thesis is my original work and has not been previously submitted to any University for the award of a degree or any other qualification.

Signature---------- Date----------

CERTIFICATE OF APPROVAL

This thesis of Patrice Ntanda Mukomena has been approved as fulfilling the requirements for the award of the degree of Doctor of Philosophy in Infectious Diseases of the University of Zambia.

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ABSTRACT

Pseudomonas is a type of bacteria commonly found in the environment, like soil and water. Of the many types of *Pseudomonas*, the one that most often causes infections in humans is called *Pseudomonas aeruginosa,* accounting for about 10% of hospital-acquired infections. *Pseudomonas aeruginosa* is one of the most commonly isolated nosocomial pathogen, often found in numerous reservoirs in hospital settings, such as disinfectants and medical devices. It has limited susceptibility to antibiotics, leading to increased morbidity and mortality.

Despite the previous research reports on *Pseudomonas aeruginosa* in Zambia, there is a paucity of data on clinical *Pseudomonas aeruginosa* population diversity and molecular characterization of clinical isolates in correlation with clinical outcomes. Further, data on the genetic determinants of *Pseudomonas aeruginosa* and antimicrobial resistance (AMR) is scarce.

Therefore, this study aimed to evaluate hospital-acquired *Pseudomonas aeruginosa* infections in selected hospitals of Lusaka and Copperbelt provinces in Zambia. The study assessed the clinical and molecular characteristics of *Pseudomonas aeruginosa* and determined predictors of hospital-acquired infections with multidrug-resistant *Pseudomonas aeruginosa* in patients at various study sites. The study also evaluated antimicrobial resistance patterns in patients infected with *Pseudomonas aeruginosa* at different study sites. Frequencies were estimated, and the association between the outcome variable (positive culture) and categorical predictor variables was analyzed using the Chi-square test and logistic regression. Further, the study described phenotypic characteristics, assessed the genetic diversity of clinical isolates of *Pseudomonas aeruginosa,* and assessed the clinical relationship between *Pseudomonas aeruginosa* phenotypes and genotypes with antimicrobial resistance and treatment outcomes.

A year-long hospital-based cross-sectional study was conducted from April 2020 to April 2021 at two large tertiary-level hospitals in Zambia. Hospitalized and out-patients with previous

hospital contact were screened for nosocomial infections, followed by the collection of specimens (skin swabs, urine, or sputum) for bacteriological culture and Polymerase Chain Reaction (PCR) amplification of 16S rRNA gene fragments.

Nosocomial infections were defined according to the World Health Organization case definitions. Clinical assessment and follow-up of patients till discharge, 30 days, or death were conducted to establish the clinical outcomes. The Kirby-Bauer's disk diffusion method was used to evaluate antibiotic resistance patterns. The resistance genes, *βla IMP, βLaOXA 51,* and other resistance genes were detected using PCR.

Eight hundred and forty-one clinical specimens were collected and analyzed, 640 from the University Teaching Hospital in Lusaka district and 201 from the Ndola Teaching Hospital in Ndola district. Of the 841 participants, 116 (13.7%) were diagnosed with *Pseudomonas aeruginosa* nosocomial infections. Among these, 96 participants were males (71.2%), and 20 were females (28.8%). The study participants ranged from 15 to 98 years, with a mean age of 51 (SD ± 18). Catheter-associated urinary tract infections (57%) were the most common, followed by those from pressure sores (38.7%). Antibiotic sensitivity of *Pseudomonas aeruginosa* was best with amikacin and worse with cefepime. We observed a high prevalence of multidrug resistance (73.6 %). The Antimicrobial resistance (AMR) was associated with carbapenem-hydrolyzing *β*-lactamase gene *βlaOXA-51*, literacy level, and ward attendance.

Conclusions: This study showed that multidrug-resistant *Pseudomonas aeruginosa* is highly prevalent in the hospital settings at the University Teaching Hospital in Lusaka province and at the Ndola Teaching Hospital in Ndola in the Copperbelt province of Zambia. This calls for establishing and implementing antimicrobial stewardship programs, using antimicrobial sensitivity to treat infection, and strengthening the AMR surveillance system and awareness at the two hospitals.

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LIST OF ABBREVIATIONS AND ACRONYMS

%	Percentage
°C	Degree Celcius
Ab	Antibodies
AMA	American Medical Association
AMR	Antimicrobial resistance
AMST	Antimicrobial Susceptibility Test
Bp	Base pairs
BSI	Bloodstream infection
cELISA	Competitive-Enzyme Linked Immuno- Sorbent Assay
CFT	Compliment Fixation Test
CFU	Colony Forming Units
CLS	Clinical and Laboratory Standards Institute
CO_2	Carbon Dioxide
CSO	Central Statistical Office
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
ECDC	European Centre for Disease Prevention and Control
ESBL	Extended-spectrum β-lactamases
ESKAPE	The ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*)
EU	European Union
HAIs	Hospital Acquired Infections
HIV/AIDS	Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome
ELISA	Indirect-Enzyme Linked Immuno-Sorbent Assay
ICA	Immunochromatography Assay
Ig	Immunoglobulin
KCl	Potassium Chloride
Mab	Monoclonal Antibodies
MDR	Multidrug- resistant
MIC	Minimum Inhibitory Concentration
MLST	Multi Locus Sequence Typing
mM	Milimoles
Ng	Nanograms
NH	Ndola Teaching Hospital
NI	Nosocomial infection
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PI	Percentage Inhibition
rRNA	Ribosomal Ribonucleic Acid
RT-PCR	Real-Time Polymerase Chain Reaction
S-LPS	Smooth Lipopolysaccharide
SNP	Single Nucleotide Polymorphism
SSI	Skin and Soft Tissue infection
U	Units
UTH	University Teaching Hospital in Lusaka, Zambia
VAP	Ventilator Associated Pneumonia

WGS	Whole Genome Sequencing
WHO	World Health Organization
µl	Microlitres
µM	Micromolar

CHAPTER ONE

INTRODUCTION

0.1 Background

Pseudomonas aeruginosa is a Gram-negative rod-shaped bacterium, a non-glucose fermenter, widespread in natural environments. It is a water and soil-borne organism with a propensity to moist environments. *Pseudomonas aeruginosa* is a strictly aerobic, ubiquitous free-living of relatively low virulence bacteria, with most pseudomonads known to cause disease in humans associated with opportunistic infections (Diggle *et al.,* 2020). *Pseudomonas aeruginosa* has a large genome of 5.5-7 million base pairs with remarkable plasticity (Sisk-Hackworth et al., 2020). Its ability to adapt to various environmental niches and its high nutritional versatility stems from this genome plasticity. In addition, many intrinsic and acquired resistance mechanisms exist within the *Pseudomonas aeruginosa* population (Langendonk *et al.,* 2021).

The genus *Pseudomonas* comprises more than 140 species, though the majority are saprophytic, with only about 25 species associated with human infections. These include *Pseudomonas aeruginosa, Pseudomonas cepacia, Pseudomonas maltophilia, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas stutzeri* and *Pseudomonas putrefaciens* (Botta *et al.,* 2014; Tuon *et al.,* 2022). *Pseudomonas aeruginosa* and *Pseudomonas maltophilia* account for approximately 80 percent of pseudomonads recovered from clinical specimens, with *Pseudomonas aeruginosa* receiving the most attention due to the frequency with which it is involved in human disease (Iglewski *et al.,* 1996; Esposito *et al.,* 2021).

Pseudomonas aeruginosa is a significant cause of nosocomial infection (NI), representing a major threat to hospitalized patients, particularly those with serious underlying diseases

such as patients suffering from neutropenia, cancer, human immunodeficiency virus infection (HIV/AIDS), cystic fibrosis, burns and those taking immunosuppressive drugs (Babu *et al.,* 2019). It exhibits innate resistance to a wide range of antibiotics due to its natural ability to find new ways to resist treatment. It can horizontally pass along genetic material that allows other bacteria to become drug-resistant (Pachori *et al*., 2019; Qin *et al*., 2022).

To curb the threat of drug resistance, the World Health Organization (WHO) published its list of antibiotic-resistant "priority pathogens"(WHO, 2017 and 2021). They included *Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacteriaceae as crucial threats to global public health. These are the most critical group as they have multidrug-resistant bacteria that pose a particular threat in hospitals and nursing homes and among patients requiring devices such as ventilators and urinary and blood catheters. *Pseudomonas aeruginosa* causes severe and often deadly nosocomial infections. These bacteria have become resistant to many antibiotics, including carbapenems and third-generation cephalosporins, the best antibiotics for treating multidrug-resistant bacteria (WHO, 2017).

Nosocomial infections (NI), also referred to as healthcare-associated infections (HAI), are infection(s) acquired during the process of receiving health care that was not present during the time of admission. They may occur in different healthcare delivery areas, such as hospitals, long-term care facilities, and ambulatory settings, and may also appear after discharge. NIs also include occupational infections that may affect staff (Sikora *et al.,* 2022). Among NIs caused by Gram-negative rods, *Pseudomonas aeruginosa* has a leading role, especially in critically ill and immunocompromised patients (Zakhour *et al.,* 2022).

According to the Centers for Disease Control and Prevention (CDC, 2023), *Pseudomonas aeruginosa* is the fourth most commonly isolated nosocomial pathogen, accounting for

10% of all hospital-acquired infections. *Pseudomonas aeruginosa* pathogen finds numerous reservoirs in a hospital setting, such as disinfectants and medical devices, leading to increased morbidity, mortality, prolonged hospital stay, and increased cost of care among hospitalized infected patients (Ng *et al.,* 2023). The high morbidity and mortality associated with nosocomial infections are due to weakened host defences and bacterial resistance to antibiotics. The production of extracellular bacterial enzymes and toxins also contributes to the disease burden of hospital-acquired infections (Montero *et al.,* 2020).

In Zambia, specific clinical and molecular epidemiologic data on *Pseudomonas aeruginosa* nosocomial infection is scarce and poorly understood (Loevinsohn *et al.,* 2021; Masich *et al*., 2020). Therefore, this study aimed to fill this knowledge gap using clinical, molecular, and genetic tools on *Pseudomonas aeruginosa* nosocomial infection. The study investigated nosocomial infections caused by *Pseudomonas aeruginosa* at the University Teaching Hospital (UTH) in the Lusaka district of Lusaka province and at the Ndola Teaching Hospital (NTH) in the Copper Belt province in Zambia.

1.1 Statement of the Problem

According to an estimate reported by WHO, approximately 15% of all hospitalized patients suffer from nosocomial infections (WHO, 2022); the frequency in low-income countries is three times higher than in high-income countries, with mortality as high as 75% in Southeast Asia and Sub-Saharan Africa (Khan *et al*., 2017). *Pseudomonas aeruginosa* accounts for about 10% of all nosocomial infections and is considered one of the most critical agents of Gram-negative bacterial infections, with reports of increasing antibiotic resistance worldwide (Pachori *et al*., 2019). The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* is a serious healthcare challenge with significant morbidity and mortality worldwide (Horcajada *et al.,* 2019). To highlight the extent of the problem, the World Health Organization (WHO) listed *Pseudomonas aeruginosa* as one of the

antimicrobial-resistant "priority pathogens" requiring new therapy (WHO, 2017; Reig *et al,* 2022).

Despite this alarming report, Tadesse *et al.* (2017) observed that data on AMR was not even available for 42.6% of countries on the African continent, even though the Centers for Disease Control and Prevention (CDC, 2023) estimated that one in every thirty-one hospital patients has NI.

Specific clinical and molecular epidemiologic data on *Pseudomonas aeruginosa* is scarce in Zambia. According to the Zambia National Antimicrobial Resistance Coordinating Committee (AMRCC) and the Zambia National Public Health Institute (ZNPHI), *Pseudomonas aeruginosa* is a routinely isolated bacterium in cases of bacteremia, urinary tract infections and wounds, and soft tissue infections in Zambia (WHO, 2023; ZNPHI, 2018). Further, according to clinical and laboratory audits, morbidity leading to high mortality lies in the appearance of drug-resistant strains, as observed at the University Teaching Hospital (UTH), Lusaka (UTH audits, 2018; ZNPHI, 2018). A systematic review found no available data on AMR for six of the ten provinces of Zambia, with the majority of studies showing a degree of resistance to more than one class of antimicrobials (Nowbuth *et al.,* 2022). The resistance level to commonly prescribed antibiotics was significant across the human, animal, and environmental sectors (Lister *et al.,* 2009).

Mwamungule *et al.* (2015) reported that drug-resistant *Pseudomonas aeruginosa* was the second most commonly isolated organism in the hospital environment and on doctor's white coats in a major teaching hospital in Zambia. Resistance to third and fourth-generation cephalosporins and carbapenem was documented on a limited sample, but no molecular data was recorded (Kaluba *et al.,* 2021). To fill the gap, we investigated the clinical and molecular characteristics of drug-resistant *Pseudomonas aeruginosa* in adult patients from Zambia. This was to evaluate the clinical outcomes, molecular epidemiology, and risk

factors of AMR in Zambia. This study aimed at investigating the clinical significance and molecular characteristics of *Pseudomonas aeruginosa* commonly isolated from patients at the University Teaching Hospital and the Ndola Teaching Hospital in Zambia." This was to provide crucial information to help develop novel strategies to control pathogens responsible for AMR to combat this public health challenge.

1.2 Study Justification

Worldwide, there is a rising concern over antimicrobial resistance (AMR), including that of nosocomial infections (NIs). Nosocomial infections affect globally more than 100 million patients yearly, with *Pseudomonas aeruginosa* as one of the important NI pathogens (Sikora *et al.,* 2020).

Worldwide, there are more than 700,000 deaths per year due to AMR (Dadgostar *et al.,* 2019). According to this author, if no adequate measures are taken to stop its progress, AMR will globally cost approximately 10 million lives and about US$100 trillion annually by 2050. Other than the financial cost, "we may soon be facing the end of the antibiotic era" if drug-resistant pathogens such as *Pseudomonas aeruginosa* are not researched enough to find new ways of preventing and treating resistant pathogens (Nathwani *et al.,* 2014).

Despite this alarming situation, only a few African countries have established national surveillance systems for NIs, as emphasized by WHO in the patient safety module (WHO, 2021). In Zambia, ZNPHI (2018) put up baseline information required for integrated surveillance of antimicrobial resistance in Zambia, but the actual surveillance still needs to be fully scaled up.

The early and unstoppable success of antibiotics, the fruit of advanced scientific research, has been challenged by an intensification of antibiotic resistance in bacteria. This critical

situation has raised concern over the possibility of an "unstoppable war" if no action is taken immediately, such as scaling up research on fastidious and resistant pathogens like *Pseudomonas aeruginosa*, especially in developing countries where data is scarce (WHO, 2021).

There is a need for more data on NIs in Sub-Saharan Africa (SSA), including Zambia, due to few available studies despite reports of the associated financial burden for health systems and increased AMR (WHO, 2020). Therefore, investigating multidrug-resistant organisms such as *Pseudomonas aeruginosa* has become a global emergency as new resistance mechanisms are emerging and spreading. This threatens the clinician's ability to treat diseases like *Pseudomonas aeruginosa* infection successfully.

The spread of antimicrobial-resistant pathogens such as *Pseudomonas aeruginosa* makes hospital stays, medical procedures, and significant surgery such as cesarean sections hazardous. Antimicrobial resistance also increases the cost of health care delivery with lengthier hospital stays and more intensive care requirements.

Against this background, this study was conducted to evaluate the clinical and molecular characteristics, including genetic diversity and molecular resistance profile of *Pseudomonas aeruginosa* infections in Zambia. This was done to generate the knowledge needed for intervention strategies and to guide policy concerning the management of *Pseudomonas aeruginosa* nosocomial infections and prevent the looming risk of associated AMR crisis.

1.3 Research Questions

1. What are the phenotypic and molecular characteristics of antimicrobial-resistant *Pseudomonas aeruginosa* nosocomial infection among adult patients in Lusaka and Copperbelt Provinces of Zambia?
2. What are the risk factors and predictors of drug resistance and clinical outcomes among adults with antimicrobial-resistant *Pseudomonas aeruginosa* nosocomial infection in Lusaka and Copper Belt Provinces of Zambia?

1.4 Research Objectives

1.4.1 General Objectives

This study aimed to evaluate *Pseudomonas aeruginosa* nosocomial infections at the University Teaching Hospital in Lusaka province and at the Ndola Teaching Hospital in the Copperbelt province in Zambia to generate the knowledge needed for policy formulation and intervention strategies.

1.4.2 Specific Objectives

1. To assess the clinical characteristics of *Pseudomonas aeruginosa* nosocomial infections;
2. To evaluate AMR patterns and predictors of nosocomial infections with multidrug-resistant *Pseudomonas aeruginosa* in patients;
3. To analyze the molecular characteristics and relatedness between *Pseudomonas aeruginosa* clinical isolates from UTH (Lusaka) and NTH (Ndola);
4. To examine clinical and molecular determinants of mortality among *Pseudomonas aeruginosa* NIs patients at UTH and NTH

CHAPTER TWO

LITERATURE REVIEW

2.1 General Overview

In 1882, Gessard first discovered *Pseudomonas* (Chen et al., 2022), a strictly aerobic, Gram-negative bacterium of relatively low virulence. The genus *Pseudomonas* contains more than 140 species, most of which are saprophytic. The pseudomonads are ubiquitous, with a preference for moist environments, primarily as water and soil-borne organisms. Pseudomonal species have been found in soil, water, plants, and animals. Among these, more than 25 species are associated with opportunistic human infections (Mena & Gerba, 2009; Chen *et al.,* 2022). The pseudomonads are bacteria that cause infections in humans include *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas cepacia*, *Pseudomonas stutzeri*, *Pseudomonas maltophilia*, and *Pseudomonas putrefaciens*. *Pseudomonas aeruginosa* and *Pseudomonas maltophilia* account for approximately 80 percent of pseudomonads recovered from clinical specimens (Sharma *et al.,* 2023). *Pseudomonas aeruginosa* has received the most attention because of the frequency with which it is involved in human disease (Tenover *et al.,* 2022). Although it seldom causes disease in healthy individuals, it is a significant threat to hospitalized patients, particularly those with serious underlying diseases such as cancer and burns (Fazeli *et al.,* 2012). *Pseudomonas aeruginosa* causes various infections with several clinical manifestations. They range from localized, benign skin infections to life-threatening systemic infections. The high mortality associated with these infections is due to weakened host defences, bacterial antibiotic resistance, and the production of extracellular bacterial enzymes and toxins (Tuon *et al.,* 2022). In this chapter, we aim to provide a comprehensive synthesis of the current knowledge about the manifestations of *Pseudomonas aeruginosa* infection.

2.1.1. Microbiology of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a species of Gram-negative bacilli measuring 0.5 to 0.8 μm by 1.5 to 3.0 μm belonging to the family Pseudomonadaceae. Obligate aerobes, these organisms grow best in ambient air at 37^0C, though they can grow at temperatures as high as 42^0C (Diggle *et al.*, 2020).

Colonial morphology typically features a metallic sheen, blue-green pigment, and a unique grape-like, fruity odor in the laboratory setting. The pigment pyoverdin, greenish-yellow and fluoresces under Wood's light, is expected to be the fluorescent group of Pseudomonas species (notably *Pseudomonas aeruginosa*, *Pseudomonas fluorescens, and Pseudomonas putida). Still, pyocyanin, which is non-fluorescent and greenish-blue, is unique to Pseudomonas aeruginosa and imparts the characteristic color* (Scales *et al.,* 2014). Biochemical features supportive of the identification include a positive oxidase test, an inability to ferment carbohydrates (alkaline over no change on a triple sugar iron (TSI) slant), and the ability to grow on Cetrimide agar. Growth at 42^0C remains a defining characteristic of *Pseudomonas aeruginosa* from the other fluorescent pseudomonads (Diggle *et al.,* 2020).

FIG 1. Electron microscopy picture of *Pseudomonas aeruginosa* (CDC, 2019)

2.1.2. Motility and Attachment

Pseudomonas aeruginosa has flagella and pili that are necessary for motility and respiratory infection, as they enable attachment to the epithelium via respiratory mucins and the glycolipid asialoGM1 (Reynolds & Kollef, 2021). Bacterial adhesion to the respiratory epithelium is an essential step for infection and is accomplished by interactions between bacterial adhesins and host receptors. For *Pseudomonas aeruginosa* infection, the main adhesins are the single flagellum necessary for motility, cell adhesion, biofilm formation, and the type IV pili appendages composed of pilin polymers. These allow the bacteria to move over surfaces in addition to serving relevant roles in biofilm formation and respiratory epithelial cell attachment (Tuon *et al.,* 2022).

Infection with *Pseudomonas aeruginosa* can occur in an acute phase in patients with abnormal respiratory epithelium from patients with chronic lung disease or with a critical illness that has led to respiratory failure and mechanical ventilation (Reynolds & Kollef, 2021). A more chronic infection can occur in patients with underlying lung disease, such as cystic fibrosis (CF). In the acute phase, *Pseudomonas aeruginosa* can attach to respiratory epithelium using its type IV pili and flagellum, and toxins secreted by the bacterium damage the host cell lung (Curran *et al.,* 2018). *Pseudomonas aeruginosa* then secrete an extracellular matrix, forming a biofilm, a structural matrix of bacterial cells encased within an extracellular matrix that adheres to the respiratory epithelium (Lau *et al.,* 2005). The matrix is primarily made up of polysaccharides, proteins, extracellular DNA, and lipids. As a result of biofilm formation, bacteria can act synergistically while protecting from phagocytosis by neutrophils and antibiotics (Curran *et al.,* 2018; Maurice *et al.,* 2018). Biofilm formation can also occur in the lungs of patients with chronic lung disease, such as in cystic fibrosis or chronic bronchiectasis,

where *Pseudomonas aeruginosa* organisms form a biofilm in thickened airway mucous and rarely need to travel to cell surfaces (Thi *et al.,* 2021).

2.1.3. Type III Secretions System

Amongst the most powerful mechanisms for increasing the virulence and morbidity from a *Pseudomonas aeruginosa* infection is the type III secretion system, which enables the bacterium to inject effector proteins into host cells, such as the respiratory epithelium (Qin *et al.,* 2022). The effector cells can alter host cell functions, disrupting innate immune response and altering the host actin cytoskeleton (Horna *et al.,* 2021). Four effector proteins, ExoS, ExoT, ExoU, and ExoY, are typically described as the effector proteins used by the *Pseudomonas aeruginosa* type III secretion system (Hardy *et al.,* 2022); the most clinically relevant effectors may be ExoS and ExoU, thought to be mutually exclusive. ExoS impairs cell-to-cell adhesion by disrupting the actin cytoskeleton of host respiratory epithelial cells while also inducing apoptosis of host cells (Bachir *et al.,* 2017). ExoU is thought to be responsible for the most significant virulence due to its cytotoxic activity that induces host cell death. It is more often found in patients in ICUs or burn units (Chaudhary *et al.,* 2019). Previous studies have demonstrated that a type III secretion system in *Pseudomonas aeruginosa* is associated with poor outcomes, including a more considerable burden of bacteria, the persistence of infection, and increased mortality (Khodayary *et al.,* 2019).

2.1.4. Pathogenesis

Pseudomonas aeruginosa, as a Gram-negative aerobic rod, is still one of the most resistant pathogens. Nosocomial infections caused by these bacteria are becoming more common, presenting as pneumonia, urinary tract infections, surgical site infections, and bacteremia, with a prevalence ranging between 7% to 11% among all nosocomial infections (Wood *et al.,* 2023; Ng *et al.,* 2023). It has enzymes encoded on both chromosomes and plasmids, often in

combination with other resistance mechanisms, such as reducing the permeability of the outer or cytoplasmic membrane (Wang *et al.,* 2021).

Due to carbapenemases, *Pseudomonas aeruginosa* loses sensitivity to carbapenem and becomes resistant to this antibiotic (Humphry *et al.*, 2014). It also becomes resistant to aminoglycosides, cephalosporins, and ureidopenicillins. *Pseudomonas aeruginosa* is also resistant to quaternary disinfectants (Halata & Moubareck, 2022) and can thrive under nutritionally stringent conditions, as evidenced by its ability to grow even in distilled water, using only dissolved carbon dioxide and residual ions as substrates for growth (Lebreton *et al.,* 2021). This hardiness makes it an incredibly effective opportunistic pathogen where host defences have already been compromised. In addition, it is hydrophilic and has a predilection for moist environments (Iglewski *et al.,* 1996). *Pseudomonas aeruginosa* infections have been associated with water-related reservoirs such as swimming pools, hot tubs, and contact lens solutions (Martinez *et al.,* 2014).

Despite its presence in the environment, *Pseudomonas aeruginosa* seldom colonizes healthy human hosts (Malhotra *et al.,* 2019). Still, colonization has been observed in individuals undergoing multiple courses of antimicrobial agents and in the respiratory tracts of mechanically ventilated patients (Moradali *et al.,* 2017).

Pseudomonas aeruginosa is highly effective in contaminating hospital-based water reservoir systems, and carriage on the hands of healthcare workers can further facilitate transmission (Mwamungule *et al.,* 2015). However, a Tanzanian study found no evidence of such contamination (Moremi *et al.,* 2017).

Pseudomonas aeruginosa is one of the major causes of nosocomial sepsis (WHO, 2017; WHO, 2022). It is one of the several nosocomial pathogens called ESKAPE organisms that tend to

develop antibiotic resistance (Ma *et al.,* 2020). ESKAPE is an acronym comprising the scientific names of six highly virulent and antibiotic-resistant bacterial pathogens, including *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa, and Enterobacter* spp. (Mulani *et al.,* 2020). This tendency to develop resistance to new antibiotics leads to multidrug-resistant (MDR) strains of *Pseudomonas aeruginosa*. MDR *Pseudomonas aeruginosa* strains are usually isolated in patients with nosocomial sepsis, particularly those in the intensive care unit (ICU) (Coyne *et al.,* 2020).

Pseudomonas aeruginosa has several features that contribute to its ability to cause disease. A study by Sato *et al.* (2003) showed that *Pseudomonas aeruginosa* strains deficient in pili and flagella could not establish infection and spread throughout the host (Wolfmeier *et al.,* 2022). Other factors that contribute to virulence include phospholipase C activity, surface expression of the ferripyochelin-binding protein, production of lipopolysaccharide, and the elaboration of exo-products secreted by the type III secretion system (Newman *et al.,* 2017). The biofilm, known as alginate, facilitates bacterial adhesion and immune evasion and is a significant virulence factor in airway colonization and chronic lung infection of patients with cystic fibrosis (Guillaume *et al.,* 2022).

2.2.Nosocomial infection

Nosocomial infection (NI), a health-care-associated infection (HAI), develops in a patient under surgical or medical management in the out-patient or in-patient medical facilities absent during admission or out-patient visit (Sikora *et al.,* 2023). These infections may develop during medical or surgical management of other health conditions. These could even occur after the patient's discharge. They also include occupational infections among the medical and support staff (Sikora *et al.,* 2022; Revelas *et al.,* 2012). Invasive devices such as urinary catheters,

cannulas, endotracheal tubes, and ventilators employed in modern healthcare facilities are associated with these infections (WHO, 2021).

In patients with nosocomial infection, symptoms occur within 48 hours of hospital admission, up to 3 days after discharge, or up to 30 days of a surgical procedure or medical visit in a healthcare facility when the patient was admitted for reasons other than the infection (Monegro *et al.,* 2024). Nosocomial infection may occur in different areas of healthcare settings, such as in hospitals, long-term care facilities, and ambulatory settings, and may appear after discharge within 30 days (Sikora *et al.,* 2021; Revelas *et al.,* 2012). According to the American Thoracic Society and the Infectious Diseases Society of America, any infection is considered to be nosocomial if a patient is admitted to an acute care medical facility for two or more days within 90 days of the infection, or resides in a nursing home, or received recent intravenous therapy, or wound care within the past 30 days of the current infection (Baggs *et al.,* 2018). Patients who have undergone invasive procedures like endotracheal intubation, insertion of intravascular lines, and urinary catheters are also at risk of acquiring NIs. Nosocomial infections can also present as fever in patients who became ill after being admitted for a non-febrile illness, those with septic pressure sores or burns, and those who developed clinical deterioration unexplained by the initial diagnosis (Sikora *et al.,* 2022).

According to the Centers for Disease Control and Prevention (CDC, 2020), NIs include complications or infections secondary to either device implantation, hospital exposure, or surgery. Several authors (Sikora *et al.,* 2022) consider nosocomial infections, acquired during the process of receiving health care, as the most common adverse event in healthcare delivery that affects patient safety. Nosocomial infections happen globally, during hospitalization or hospital contact as out-patient, in developing and developed countries in patients under medical and surgical care (Sikora et al., 2022; Szabó *et al.,* 2022). Often caused by multidrug-resistant pathogens, NIs have limited treatment options, resulting in high morbidity and mortality, more

extended hospital stays, and high cost of care (Agyeman *et al.,* 2022). According to the Centers for Disease Control and Prevention (CDC), Gram-negative bacteria, such as *Pseudomonas aeruginosa*, play an important role, especially in critically ill and immunocompromised patients (CDC, 2020). In most studies, Catheter-associated UTI (CAUTI) was the most common nosocomial infection (Mukomena *et al.,* 2023; Werneburg *et al.,* 2022). A disparity was, however, observed with other authors who observed that the most common sites of NI were the respiratory tract (Pezhman *et al.,* 2021). Other sites of NIs include decubitus and surgical site infections, central-line-associated bloodstream infections, and ventilator-associated types of pneumonia. NIs account for 7% in developed and 10% to 15% of patients in developing countries (Szabó *et al.,* 2022; Chandra *et al.,* 2017). NIs cause economic burden due to prolonged stay and increased risk of antimicrobial resistance and disability (WHO, 2021). Causes of NIs include bacteria, viruses, fungi and parasites, with bacteria causing about 90% (Ursula et al., 2021; Crum-Cianflone *et al.,* 2008). Of these, *Pseudomonas aeruginosa* accounts for 11 percent and has a high mortality and morbidity rate (Sikora *et al.,* 2022).

Among the most exposed patients are those admitted to Intensive Care Units (ICUs), dialysis units, burn units, neonatal units (NICU), and patients undergoing organ transplants (Murni *et al.,* 2022; Teerawattanapong *et al.,* 2018). The Extended Prevalence of Infection in Intensive Care study (Vincent *et al.,* 2009) estimated a higher proportion of nosocomial-infected patients within the ICU, often as high as 51%, with NI incidence ranging from 13.0 to 20.3 episodes per thousand patient days in developed countries. This leads to increased cost of care due to prolonged hospital stay, long-term disability, increased risk of antimicrobial resistance, socio-economic disturbance, and increased morbidity and mortality (Lakoh *et al.,* 2020; Peters *et al*., 2019; Cohen *et al.*,2010).

2.2.1. Types of nosocomial infections

The most frequent types of infections include catheter-associated urinary tract infections, central line-associated bloodstream infections, surgical site infections, and ventilator-associated pneumonia (Werneburg *et al.,* 2022).

Table 1. Classification and characterization of NIs according to type (Modified from Szabó *et al.,* 2022).

Characteristics	**Causative Organisms**
Catheter-associated urinary tract infections (CAUTI)	
Associated with indwelling bladder catheters	*Pseudomonas aeruginosa* *Escherichia coli* (*E. coli*) *Klebsiella pneumonia* *Enterococcus* spp. *Candida* spp.
Central line-associated bloodstream Infections (CLABSI)	
Associated with a catheter in the bloodstream	*Pseudomonas aeruginosa* *Acinetobacter baumannii* *Staphylococcus aureus (S. aureus)* *Candida spp.* *Coagulase-negative Staphylococcus* *Enterococcus spp.* *Streptococcus spp.* *Escherichia coli* *Bacteroides spp.*
The skin, gastrointestinal tract, and female genital tract serve as reservoirs of the healthy flora that may contaminate the surgical site.	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Klebsiella* spp. *Enterobacter* spp. *Enterococcus* spp. *Streptococcus* spp. Coagulase-negative *Staphylococcus*
Characteristics	**Causative Organisms**
Ventilator-associated pneumonia (VAP) and Hospital-acquired pneumonia (HAP)	
Ventilator-associated (VAP) and hospital-associated pneumonia (HAP) develop after 48 hours of intubation or admission.	*Pseudomonas aeruginosa* *Staphylococcus aureus* *P. aeruginosa* *Klebsiella oxytoca* and *K. pneumoniae Streptococcus* spp. *Enterobacter* spp.
Gastrointestinal infections with *Clostridioides difficile*	
The most common cause of NIs presenting with diarrhoea	*Clostridioides difficile*

2.2.2. Epidemiology of nosocomial infections

Nosocomial infection affects many patients globally, significantly elevating mortality rates and financial losses. WHO estimates that approximately 15% of all hospitalized patients suffer from these infections (Giacobbe *et al.,* 2020). These infections are responsible for increased morbidity and mortality. The frequency of overall infections in low-income countries is three times higher than in high-income countries (Maki *et al.,* 2021).

Nosocomial infections spread among hospitalized patients via environmental routes, and the hospital's design plays a critical part in preventing the incidence and spread of these infections. The built environment influences the incidence of infection in hospitals, so careful consideration of environmental transmission routes (air, surface, and water) should be considered in the design and operation of healthcare facilities to reduce nosocomial infections (Xiong *et al.,* 2021).

Vulnerable patient populations are exposed to a variety of infectious pathogens. Airborne pathogens are transmitted when an environmental reservoir of a pathogen (i.e., soil, water, dust, decaying organic matter) is disturbed. Microorganisms can spread directly from person to person. When droplets are produced during a cough or sneeze, a cloud of infectious particles is released into the air, potentially exposing susceptible persons within one meter of the source person (Argyropoulos *et al.,* 2022).

The microorganisms in the droplet residuals persist in dry, excellent conditions with little or no exposure to light or direct radiation. Susceptible individuals who come in contact with high concentrations of the microorganism may get infected (Gerba *et al.,* 2015). Many incidents and outbreaks of nosocomial infection have been linked to malfunctions and contamination of hospital ventilation systems (Langendonk *et al.,* 2021).

Although infections caused by airborne transmission pose a significant safety problem, most infections are now acquired in the hospital via the contact pathway (Nabarro *et al.,* 2019).

Microbiologically contaminated surfaces can be reservoirs of pathogens. However, these surfaces are generally not directly associated with the transmission of infection to patients or staff, but the transmission occurs through the hands of healthcare workers (Mwamungule *et al.,* 2015; Pegu *et al.,* 2021).

Therefore, handwashing is essential for healthcare workers to reduce nosocomial infections. Exposure of hospitalized patients to infection through healthcare workers' hands remains high due to the low rates of handwashing by healthcare staff, therefore representing a severe patient safety challenge (Krishnamoorthy *et al.,* 2023). Waterborne microorganisms such as *Pseudomonas aeruginosa* proliferate in moist environments and aqueous solutions, especially under warm temperature conditions and the presence of a source of nutrition. These infections spread through direct contact (e.g., hydrotherapy), ingestion of contaminated water, indirect contact, and inhalation of aerosols dispersed from water sources (Suleyman *et al.,* 2018).

An essential aspect of reducing infections spread through surface contact involves providing environmental support for visible handwashing, conveniently placed sinks, handwashing liquid dispensers, and alcohol rubs (WHO, 2020).

2.2.3. Reservoirs and Transmission

Microflora of patient

Bacteria in the patient's endogenous flora can cause infections if transferred to a tissue wound or surgical site. For example, Gram-negative bacteria, such as those in the digestive tract, cause skin and soft tissue infection (SSI) after abdominal surgery (Shakir *et al.,* 2021).

Patient and staff

Transmission of pathogens during the treatment is done through direct contact with the patients (hands, saliva, other body fluids) and by the staff through direct contact or other environmental sources such as water, food, and other body fluids (Khan *et al.,* 2017).

Transmission

The healthcare environment (i.e., water, food, and equipment) harboring pathogens such as *Pseudomonas aeruginosa* can transmit nosocomial infections. Transmission to other patients makes one more reservoir for the uninfected patient (Beganovic *et al.,* 2019).

An unhygienic environment is the best source for the pathogenic organism to prevail. Air, water, and food can get contaminated and serve as a source of transmission to the patients under healthcare delivery, especially in the absence of policies to ensure the cleaning and use of cleaning agents on walls, floors, windows, beds, baths, toilets, and other medical devices. Lack of adequately ventilated and fresh filtered air can increase airborne bacterial contamination. Poorly maintained filters and ventilation systems of general wards, operating theatres, and ICUs are risk factors for such infections (Beganovic *et al.,* 2019).

Infections attributed to water are due to the failure of healthcare institutions to meet the standard criteria in the absence of microbiological monitoring methods for water analysis (Yiek *et al.,* 2021).

Infected patients must be given separate baths; this could sometimes be difficult to meet when the ratio of patient to nurse is inadequate. The optimum nurse-to-patient ratio is of significant concern globally. The nurse-to-patient ratio is one of the determining factors of the patient outcome. Studies have shown that appropriate nurse staff helps to prevent falls in the incidences of pressure sores, nosocomial infections, patient mortality, hospital readmission and duration

of stay, patient care cost clinical and economic improvements in patient care (Butler *et al.,* 2019).

Improper food handling on the ward may cause food-borne infections (Beganovic *et al.,* 2019). Infections can be transferred from healthcare staff when healthcare professionals do not take a role in infection control to minimize transmission. When personal hygiene is not maintained for everyone, staff become a source of contamination and transmission. Experts agree that careful hand washing and disinfection are the most critical procedures to avoid transmission of pathogens during health care provision. Studies demonstrate that increasing compliance with hand disinfection reduces healthcare-associated infections (Haque *et al.,* 2020). This infection occurs when proper hand disinfectants are not used adequately after contact with infected patients. When hand and equipment sterilization become inadequate, the patient are exposed to nosocomial pathogens. Therefore, gloves, head covers, or a proper uniform is essential for healthcare delivery (Beganovic *et al.,* 2019).

Hospital waste can also be a potential reservoir for pathogens if improperly handled. Between 10 and 25% of the waste generated by the healthcare facility is termed as bio-hazardous, especially when it is not stored in an area with a restricted approach. Waste containing a high content of heavy metals and waste from surgeries, infected individuals, and contaminated with blood and sputum and diagnostic laboratories could be highly infectious. It must be disposed of separately (Beganovic *et al.,* 2019). Despite significant efforts to prevent nosocomial infections, it is estimated that in a day, one out of 25 patients can acquire at least a single type of nosocomial infection (Haque *et al.,* 2020).

2.2.4. Risk factors of nosocomial infections

Risk factors determining nosocomial infections depend upon the environment in which health care is delivered, the susceptibility and condition of the patient, and the lack of awareness of such prevailing infections among patients and health care providers (WHO, 2020). Factors that

affect the complex process of origin and spread of nosocomial infections may be divided into intrinsic and extrinsic (Sikora *et al.,* 2021). Intrinsic factors are associated with the biological balance of the patient, such as old age (over 65 years), immunodeficiency, hormonal disorders (diabetes), alcoholism, drug addiction, malignant tumors, obesity, malnutrition, circulatory disorders, poly-trauma, burns, pressure ulcers and other serious diseases (liver disease, AV shunt, cardiomyopathy) (Qin *et al.,* 2022). Extrinsic factors are related to prophylactic, diagnostic interventions, and therapeutic of patients in hospital facilities, such as length of hospital stay, surgery, transplantation, tracheostomy, endotracheal cannula, gastric tube, urinary catheterization, intravenous catheterization, infusion, transfusion, foreign bodies, drainage, instrumental procedure, repeated anesthesia, endoscopy, hemodialysis, radiation therapy, cytostatic therapy, immunosuppressive therapy, broad-spectrum antibiotic therapy, and hormonal therapy (Labovská *et al.,* 2021). Hospital admission is essential in transmitting nosocomial infections, with the highest incidence typical of the Intensive Care Unit (ICU) (Aiesh *et al.,* 2023).

The patient's age may affect the risk of nosocomial infection, with the incidence of nosocomial infections gradually increasing in patients over 65 years of age (Özdemir *et al.,* 2015). A significant relationship exists between increased age and a predisposition to nosocomial infections (Cristina *et al.,* 2021). Immunosuppressed patients after chemotherapy, human immunodeficiency virus infection, or steroid use are equally at risk for developing nosocomial infection (Safdar *et al.,* 2011). According to Revelas *et al.* (2012), nosocomial infections do not have an apparent sex predilection. They further observed that disinfection and sterilization in hospitals are of increasing concern. Nosocomial infections occur within 48 hours of hospital admission, three days of discharge, or 30 days of an operation (Khan *et al.,* 2017; Reveals *et al.,* 2012). They affect 1 in 10 patients admitted to the hospital and are associated with morbidity, mortality, and increased financial burden. Sikora *et al.* (2021) stated that risk factors

for developing a nosocomial infection are the length of hospital stay and initial antibiotic therapy. Staff shortages are a particular risk factor for the increased incidence of nosocomial infections due to increased staff workload and poor hand hygiene (Peters *et al.,* 2020).

In a prospective study, the incidence of nosocomial infection was estimated at 14.3% in patients with blood transfusions and 5.8% in patients without (Robert *et al.,* 2006; Mirouse *et al.,* 2017). In the group of patients with blood transfusions, there was a higher incidence of nosocomial infections, which was significant in seriously ill patients with a probability of survival of less than 25% (Sikora *et al.,* 2022).

2.3. Epidemiology *of Pseudomonas* aeruginosa nosocomial infections

Pseudomonas species usually inhabit soil, water, and vegetation and can be isolated from healthy persons' skin, throat, and stool. They often colonize hospital food, sinks, taps, mops, and respiratory equipment (Diggle *et al.,* 2020). Spread is from patient to patient via contact with fomites or ingesting contaminated food and water (Diggle *et al.,* 2020).

Pseudomonas aeruginosa has been isolated from the skin of some healthy persons and the throat (5 percent) and stool (3 percent) of non-hospitalised patients (Fazeli *et al.,* 2012). The gastrointestinal carriage rates increase to 20 percent in hospitalized patients within 72 hours of admission. Within the hospital, *Pseudomonas aeruginosa* finds numerous reservoirs: disinfectants, respiratory equipment, food, sinks, taps, and mops. Furthermore, it is constantly reintroduced into the hospital environment on fruits, plants, vegetables, and patients transferred from other facilities. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by ingesting contaminated foods and water (Fazeli *et al.,* 2012).

2.3.1. *Pseudomonas aeruginosa* and Nosocomial Infections

Pseudomonas aeruginosa is a common cause of nosocomial infections, including pneumonia, surgical site infections, urinary tract infections, and bacteremia (Ng *et al.,* 2023). *Pseudomonas aeruginosa is* thought to have a prevalence of 7.1%-7.3% among all healthcare-associated infections (Litwin *et al.,* 2021). The respiratory tract was reported in some studies as the most common site of *Pseudomonas aeruginosa* infection, and it is the most common Gram-negative organism identified in nosocomial pneumonia. Over the years, the prevalence has been increasing, especially over the past decade. In intensive care unit (ICU) patients, *Pseudomonas aeruginosa* is responsible for an even higher percentage of healthcare-associated infections (Reynolds *et al.,* 2021). A large international observational point-prevalence study of ICU patients found that *Pseudomonas aeruginosa* represented 16.2% of patient infections and was the cause of 23% of all ICU-acquired infections, with a respiratory source being the most common site (Vincent *et al.,* 2020).

Pseudomonas aeruginosa accounts for 10%-20 % of isolates in cases of ventilator-acquired pneumonia (VAP), second only to *Staphylococcus aureus* (Hurley *et al.,* 2018). Mortality from VAP secondary to *Pseudomonas aeruginosa* is estimated to be as high as 32%–42.8% (Lu *et al.,* 2014).

Globally, *Pseudomonas aeruginosa* accounts for approximately 26% of cases of VAP, with the most common risk factor being prolonged intubation and biofilm formation (Ramírez-Estrada *et al.,* 2016).

Pseudomonas aeruginosa is a common cause of nosocomial urinary tract infections (UTIs), particularly catheter-associated (CAUTIs). *Pseudomonas aeruginosa* accounts for approximately 10% of all CAUTIs and up to 16% of UTIs in ICU patients. Nosocomial UTI secondary to *Pseudomonas aeruginosa* is associated with high morbidity and mortality, and bacteremia is a potential complication (Kitagawa *et al.,* 2019). Additionally, *Pseudomonas*

aeruginosa CAUTI is associated with high rates of antimicrobial resistance, depending on local antimicrobial resistance patterns. Data from the International Nosocomial Infection Control Consortium report found resistance rates of > 40% for antibiotics such as fluoroquinolones, piperacillin-tazobactam, and meropenem in ICU patients. However, the report noted that these resistance rates were higher than have been reported in other study (Muller *et al.,* 2018). Amongst healthcare-associated infections reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention (CDC) from 2011–2014, 5.7% of surgical site infections were found to be secondary to *Pseudomonas aeruginosa,* with breast and cardiac surgeries being the type of surgery most associated with *Pseudomonas aeruginosa* (Reynolds *et al.,* 2021). Data from England from 2000 to 2013 showed that Pseudomonas species accounted for 4.3%–6.5% of all surgical site infections (SSI) annually (Elgohari *et al.,* 2017). Additionally, *Pseudomonas aeruginosa* infection after surgery is associated with worse outcomes, with a retrospective study of cardiac surgeries performed at a university hospital over seven years showing that *Pseudomonas aeruginosa* infection was associated with increased mortality (Massart *et al.,* 2020). *Pseudomonas aeruginosa* is the most common Gram-negative organism, leading to infection in burn wound patients, and it is associated with sepsis and death (Norbury *et al.,* 2016). Multi-drug resistant (MDR) *Pseudomonas aeruginosa* is an increasingly common cause of death in burn patients, with 86% of sepsis deaths in pediatric burn ICUs due to MDR organisms, with *Pseudomonas aeruginosa* as the responsible organism 64% of the time from 1999–2009 (Zakhour *et al.,* 2022). New therapeutic options must be developed as *Pseudomonas aeruginosa* infection is associated with poor outcomes and is estimated to represent 4.0% of all central line-associated bloodstream infection (BSI) (Horspool1 *et al.,* 2023).

2.3.2. Cystic Fibrosis and *Pseudomonas aeruginosa*

In patients with cystic fibrosis (CF), *Pseudomonas aeruginosa* is a critically important pathogen and is a predominant cause of morbidity and mortality (Beswick *et al.,* 2020). Complications of CF include chronic respiratory infections, structural lung disease and bronchiectasis, airflow obstruction, and death. Chronic infection with *Pseudomonas aeruginosa* is associated with worsening lung function, and guidelines recommend aggressive early therapy to treat *Pseudomonas aeruginosa* in CF patients to preserve lung function (Reynolds *et al.,* 2021).

Pseudomonas aeruginosa may thrive in the cystic fibrosis (CF) lung due to its ability to undergo mutations and genetic changes that allow it to survive in the typically anaerobic environment of the CF lung. Chronic infection with *Pseudomonas aeruginosa* typically produces a biofilm, and this conversion to a mucoid phenotype is associated with the production of the polysaccharide alginate (Moradali *et al.,* 2017).

Aggressive treatment regimens for *Pseudomonas aeruginosa* have been associated with a decrease in the prevalence of *Pseudomonas aeruginosa* in CF patients over recent years; however, data from the Cystic Fibrosis Foundation Patient Registry from 2006 to 2012 showed that *Pseudomonas aeruginosa* still had a prevalence of 49.6%, with increasing prevalence in older patients, as 74.1% of cultures were positive in patients aged > 26 years (Reynolds et al., 2021). Data from the European Cystic Fibrosis Society registry showed *Pseudomonas aeruginosa* had a prevalence of 29.8%, with over half of the patients aged > 40 years having a positive culture for *Pseudomonas aeruginosa* (Vongthilath *et al.,* 2019).

2.3.3. Non cystic Fibrosis Bronchiectasis and *Pseudomonas aeruginosa*

Bronchiectasis is characterized by the dilation and thickening of airways, which leads to chronic infection and airway inflammation (Polverino *et al.,* 2018). Non-CF bronchiectasis is more common than CF and has a variety of causes, such as post-infectious, primary ciliary

dyskinesia, immune deficiency, and idiopathic bronchiectasis (Beckeringh *et al.,* 2019). *Pseudomonas aeruginosa* is one of the most frequently isolated organisms in patients with non-CF bronchiectasis and is associated with worsening lung function and increased mortality (Reynolds *et al.,* 2021).

2.3.4. Immunocompromised Hosts

Pseudomonas aeruginosa is a significant pathogen in immunocompromised patients, particularly patients with neutropenia. It is a critically important pathogen in patients with hematological malignancies (Paprocka *et al.,* 2022). In one multicenter study, *Pseudomonas aeruginosa* was found to cause 17% of all Gram-negative bloodstream infection (BSI) in patients with hematological malignancies, with risk factors for *Pseudomonas aeruginosa* being prior surgery, neutropenia, use of steroids, and severity of underlying disease (Tofas *et al.,* 2017). Patients who have undergone organ transplantation are also at high risk of *Pseudomonas aeruginosa* infection and are at increased risk of adverse outcomes. Previous mortality estimates of bloodstream infection (BSI) with *Pseudomonas aeruginosa* in patients with a history of stem cell, liver, or lung transplant have been as high as 50% (Dettori *et al.,* 2022). The risk for *Pseudomonas aeruginosa* is highest immediately after transplantation, with over half (52%) of *Pseudomonas aeruginosa* occurring within three months of transplant (Liu *et al.,* 2022).

2.3.5. Emerging *Pseudomonas aeruginosa* Resistance

Antimicrobial resistance to *Pseudomonas aeruginosa* remains a severe health threat and is a significant source of morbidity and mortality, especially in ICUs and long-term care hospitals. MDR *Pseudomonas aeruginosa* is found in isolates from catheter associated urinary tract infection (CAUTI), Blood stream infection (BSI), and ventilator associated pneumonia (VAP). Data from the CDC show that 9% of *Pseudomonas aeruginosa* isolates were MDR in 2018, down from 15.7% in 2011 (CDC, 2021). Data from the World Health Organization (WHO,

2017) showed that antimicrobial resistance to *Pseudomonas aeruginosa* remains a serious concern. Carbapenem resistance in *Pseudomonas aeruginosa* can complicate treatment regimens, given how often *Pseudomonas aeruginosa* resists other antimicrobials (Gill *et al.,* 2022). In a study that investigated the prevalence of carbapenem-resistant *Pseudomonas aeruginosa* isolated over four months at multiple centers in the USA, 9% of *Pseudomonas aeruginosa* isolates were found to be carbapenem-resistant. Over 90% of the patients who had carbapenem-resistant isolates had healthcare exposures prior to their positive culture, emphasizing the relevance of nosocomial infections in *Pseudomonas aeruginosa* (Olalekan *et al.,* 2023; Walters *et al.,* 2019). One study found that resistance to one antimicrobial for a *Pseudomonas aeruginosa* BSI was highly correlated with other antimicrobial resistance. For example, 83% of *Pseudomonas aeruginosa* BSI isolates that were resistant to piperacillin-tazobactam were also resistant to ceftazidime, and 67% were resistant to ciprofloxacin (Reynolds *et al.,* 2021; Khan *et al.,* 2016). Another study investigating all *Pseudomonas aeruginosa* BSI at a single center over 13 years found that 28% of isolates were MDR, and 15% were extensively drug-resistant (Thaden *et al.,* 2017). Nosocomial pneumonia due to *Pseudomonas aeruginosa* has a high incidence of MDR strains, with an international multicenter retrospective study showing that 30.5% of nosocomial pneumonia secondary to *Pseudomonas aeruginosa* were MDR-strains, and this was associated with increased in-hospital mortality (Micek *et al.,* 2015).

2.3.6. Global distribution

Pseudomonas aeruginosa is a frequent causative pathogen in healthcare-associated infections (Raoofi *et al.,* 2023). Globally, it is estimated that *Pseudomonas aeruginosa* has a prevalence of 7.1–7.3%, as high as 11%, among all healthcare-associated infections in developing countries. One of the most common site of *Pseudomonas aeruginosa* nosocomial infection is the respiratory tract. The prevalence has increased over the past decade (Reynolds *et al.,* 2021).

Pseudomonas aeruginosa is a common cause of nosocomial infections, manifesting as pneumonia, surgical site infections, urinary tract infections, and bacteremia. *Pseudomonas aeruginosa* is the most common Gram-negative pathogen causing nosocomial pneumonia in the United States, and it is frequently implicated in hospital-acquired urinary tract and bloodstream infections. The Infectious Disease Society of America includes *Pseudomonas aeruginosa* in its list of 'ESKAPE' pathogens that pose the most significant public health threat due to a combination of increasing prevalence and ineffectiveness of existing antibacterial agents (Mulani *et al.,* 2019). A prospective observational study of 28 ICUs in the USA estimated that *Pseudomonas aeruginosa* was the cause of 11% of all HAP/VAP in ICU patients deemed to be at risk for developing nosocomial pneumonia, second only to *Staphylococcus aureus* (Zaragoza *et al.,* 2020). *Pseudomonas aeruginosa* is a common cause of nosocomial urinary tract infections (UTIs), particularly catheter-associated (CAUTIs). *Pseudomonas aeruginosa* accounts for approximately 10% of all CAUTIs and up to 16% of UTIs in ICU patients (Saleem *et al.,* 2022).

In mainland China, Ding *et al.* (2016) found *Pseudomonas aeruginosa* to be highly prevalent among patients with ventilator-associated pneumonia (VAP) and hospital-acquired pneumonia (HAP). It accounted for 19.4% (95% confidence interval (CI) 17.6–21.2%) of all isolates in VAP, which was similar to the proportion in HAP of 17.8% (95% CI 14.6–21.6%) but significantly more than the proportion in CAP of 7.7% (15/195, $p < 0.001$). Regarding VAP, the prevalence of *Pseudomonas aeruginosa* has decreased since 2007 (Ding *et al.,* 2016). *Pseudomonas aeruginosa* exhibited varying resistance to agents recommended for the initial management of VAP, with a high level of resistance to gentamicin (51.1%, 95% CI 37.7–64.4%) and a low level of resistance to amikacin (22.5%, 95% CI 14.3–33.6%). The prevalence of *Pseudomonas aeruginosa* isolates resistant to agents recommended for treating HAP ranged

from 22.2% (95% CI 13.8–33.6%) for amikacin to 50.0% (95% CI 30.2–69.8%) for cefoperazone.

In a point prevalence study conducted in Western European ICUs, *Pseudomonas aeruginosa* was one of the most common organisms, constituting nearly a third (29%) of all Gram-negative isolates, and was present in 17% of all positive cultures (Vincent *et al.,* 2009). According to Litwin *et al.* (2021), *Pseudomonas aeruginosa* has recently been shown to be one of the most important strains of bacteria and alert pathogens in Europe among Intensive Care Unit patients that provide severe therapeutic problems because of its multi-drug resistance. In Poland (Litwin *et al.,* 2021), a nearly 3-fold increase in *Pseudomonas aeruginosa* infections among Gram-negative strain infections and a 2-fold increase in the *Pseudomonas aeruginosa* HAIs frequency was observed between 2016 and 2019, as well as increased resistance. The European Centre for Disease Prevention and Control (ECDC report of 2017) reported *Pseudomonas aeruginosa* as the most common Gram-negative pathogen responsible for healthcare-associated infections acquired in European intensive care units. It constituted 19.9% of infections (33.3% in Slovakia, 32.4% in Hungary, 29.2% in Portugal, 24% in Spain, 23.1% in France, 19.4% in Italy, 17.1% in Belgium, 16.1% in Germany and 7.2% in the United Kingdom).

A multicenter observational study (Reynolds *et al.,* 2021) estimated the prevalence of VAP due to *Pseudomonas aeruginosa* as 4.1%. *Pseudomonas aeruginosa* was the most common cause of VAP globally, accounting for 26% of cases, with the most common risk factors for *Pseudomonas aeruginosa* VAP being prior colonization with *Pseudomonas aeruginosa* and prolonged hospitalization.

Nosocomial UTI secondary to *Pseudomonas aeruginosa* is associated with high morbidity and mortality, and bacteremia is a potential complication (Sikora *et al.,* 2021). Surgical site infections (SSIs) are one of the most significant complications in surgical patients and are

strongly associated with poorer prognosis. SSIs affect up to 10–20% of patients undergoing major surgery (Zukowska *et al.,* 2022; Sun *et al.,* 2021; Araújo *et al.,* 2018; Bn *et al.,* 2016). Data from England from 2000–2013 showed that Pseudomonas species accounted for 4.3%–6.5% of all surgical site infections annually (Reynolds *et al.,* 2021). Additionally, *Pseudomonas aeruginosa* infection after surgery was associated with worse outcomes.

Pseudomonas aeruginosa is a known complication in burn patients, with the moist environment of burn-wound patients believed to contribute to its predilection for burn patients (Gonzalez *et al.,* 2016). *Pseudomonas aeruginosa* is the most common Gram-negative organism leading to infection in burn wound patients, and it is associated with sepsis and death (Norbury *et al.,* 2016). Multidrug-resistant (MDR) *Pseudomonas aeruginosa* is an increasingly common cause of death in burn patients, with 86% of sepsis deaths in pediatric burn ICUs due to MDR organisms, with *Pseudomonas aeruginosa* as the responsible organism 64% of the time from 1999–2009 (Norbury *et a.,* 2016). Bloodstream infections (BSIs) due to *Pseudomonas aeruginosa* are associated with high morbidity and mortality rates, with estimated mortality rates of 43.2%–58.8% (Thaden *et al.,* 2017).

2.3.7. Distribution in Africa

The prevalence of nosocomial infections is higher in developing countries, varying between 2.5% and 14.8% of all hospital in-patients in Africa, compared with a 7.1% average in Europe (Raoofi *et al.,* 2023; Allegranzi *et al.,* 2011). Gram-negative bacteria in urinary tract infections, surgical site infections, and ventilator-associated pneumonia are common NIs (Nejad *et al.,* 2011). Kindu *et al.* (2020) conducted a systematic review with meta-analysis on carbapenemase-producing, non-glucose-fermenting, Gram-negative bacilli in Africa. This study reported the existence of studies on non-glucose fermenting Gram-negative bacteria, including *Pseudomonas aeruginosa***,** in Africa, but, in general, comprehensive data about the

molecular epidemiology were limited. In this systematic review and meta-analysis of the clinical isolates of *Pseudomonas aeruginosa* in Africa, the authors found that most of the studies were conducted in the North African region, but there was no report from Central Africa (Kindu *et al.,* 2010; Wassef *et al.,* 2016). This study reported a pooled prevalence of *Pseudomonas aeruginosa* among the clinical specimens in Africa of 21.36% for carbapenemase producers (Kindu *et al.,* 2020).

Hospital-wide *Pseudomonas aeruginosa* nosocomial prevalence varied between 2.5% and 14.8% in Algeria, Burkina Faso, Senegal, and the United Republic of Tanzania (Nejad *et al.,* 2011). The cumulative incidence of nosocomial infection in surgical wards ranged from 5.7% to 45.8% in studies conducted in Ethiopia and Nigeria (Samuel *et al.,* 2010). The latter reported an incidence as high as 45.8% and an incidence density equal to 26.8 infections per 1000 patient days in pediatric surgical wards. In a study conducted in the surgical wards of two Ethiopian hospitals, the overall incidence of patients affected by nosocomial infections was 6.2% and 5.7% (Allegranzi *et al.,* 2011; Nejad *et al.,* 2011).

Surgical site infection was the most common infection encountered in two studies investigating overall nosocomial infection incidence rates among surgical patients (Messele *et al.,* 2009; Kesah *et al.,* 2004). Similarly, a study from Burkina Faso on nosocomial infection prevalence among surgical patients reported surgical site infection as the most common type, followed by urinary tract infection and hospital-acquired pneumonia (Kakupa *et al.,* 2016; Nejad *et al.,* 2011).

In a systematic review on nosocomial infection in Africa, Nejad *et al.* (2011) reported an incidence of surgical site infection ranging from 2.5% to 30.9% following various surgical procedures. Superficial, deep, and organ/space surgical site infections accounted for 38.2% to 73%, 6.8% to 46.5%, and 10.4% to 20.5% of all surgical site infections, respectively. A survey conducted in the United Republic of Tanzania identified surgical site infection after discharge

in 21% of patients; one-third were re-hospitalised because of such infection (Nejad *et al.,* 2011). In the Democratic Republic of Congo (DRC), the overall prevalence of nosocomial infections was 34.5%. Surgical site infections were the most common (27,1%). The commonly isolated pathogens were Escherichia coli (11.9%), *Staphylococcus aureus* (6.8%), *Pseudomonas aeruginosa* (5.1%), *Shigella* spp. (5.1%) and *Salmonella typhimurium* (1.7%). (Kakupa *et al.,* 2016). In a study from Kenya, the cumulative incidence of surgical site infection after the cesarean section was 19% overall and 33% among women who had been in labor for more than 12 hours (versus 15% among women whose labor had lasted fewer hours) (Koigi-Kamau *et al.,* 2005). In a study from the Central African Republic, three of 51 patients who developed surgical site infections were identified after discharge. Of note, only 25% of all patients who were asked to return for a follow-up visit on the 30th day after surgery showed up (Sway *et al.,* 2019).

2.3.8. Distribution in Zambia

Pseudomonas aeruginosa nosocomial infections (NIs) are a significant public health problem in developed and developing countries, including Zambia. They pose a severe impact in resource-poor settings, where the infection rate is estimated to be relatively high. Data on the burden of *Pseudomonas aeruginosa* infections is scarce in Zambia (ZNPHI, 2018).

The burden of *Pseudomonas aeruginosa* nosocomial infections in Southern Africa, especially in rural areas, is poorly understood. According to Loevinsohn *et al.* (2021), nosocomial infections are of great concern in Zambia and the region due to frequently overcrowded healthcare facilities, open wards, scarce personal protective equipment, and limited infection control infrastructure. Another contributing factor is the low compliance with effective hand hygiene, which continues to fuel the high prevalence of nosocomial infection in Africa (Mutanekelwa *et al.,* 2019; Engdaw *et al.,* 2019).

In Zambia, though data on the burden of *Pseudomonas aeruginosa* infection is scarce, nosocomial infections remain a significant health burden, with cases of post-operative wound infections reported to be increasing steadily with report of post-operative wound infections among patients with a caesarian section as high as 30% (Ministry of Health, Zambia, 2003; Chu *et al.,* 2015).

Mwamungule *et al.* (2015) alerted the risk of nosocomial infection in a study on contamination of health-care workers' coats with *Pseudomonas aeruginosa* at a large tertiary hospital in Lusaka, Zambia. This study found that out of the 107 white coats worn by healthcare workers at a large tertiary hospital screened, 94 (72.8 %) were contaminated with different types of bacteria including *Pseudomonas aeruginosa*.

Mukwato *et al.* (2008) also conducted a study to determine the level of health-care workers' compliance with infection prevention guidelines and identify factors that influenced compliance at Ronald Ross General Hospital in Mufulira District of Zambia. The study findings suggested a need for the inclusion of infection prevention guidelines in the health workers' curricula; provision of in-service training in infection prevention protocols and improvements in the supply of materials for infection prevention (Mukwato *et al.* 2008). Further, Kaluba *et al.* (2021) conducted a study at a large tertiary hospital in Zambia. They found very low Carbapenem resistance in non-fermentative Gram-negative bacteria, demonstrating that imipenem is still a practical treatment choice for invasive infections caused by these organisms in our setting. However, this study was done on a very small sample size in our hospital, making it challenging to generalize the findings.

2.4. Clinical presentations of *Pseudomonas aeruginosa* nosocomial infections

Pseudomonas aeruginosa infections, both nosocomial and community-acquired, are primarily associated with debilitating medical conditions such as HIV infection, leukopenia, and

significantly advanced immunosuppression (Qin *et al.,* 2022). Both bacteremia (central lines, pneumonia, skin, UTI) and non-bacteremia (sinusitis, pneumonia, skin) forms of *Pseudomonas aeruginosa* infections may occur (Raoofi *et al.,* 2023).

2.4.1. Urinary tract infections (UTIs) caused by *Pseudomonas aeruginosa*

Urinary tract infections (UTIs) are among the most prevalent diseases in hospitalized patients, accounting for between 20% and 49% of nosocomial infections. UTIs are the second most common infection of any organ system in the body. These infections are more common in females than in men (CDC, 2022). Women are more likely to get a UTI than men. Nearly 1 in 3 women will have a UTI needing treatment before the age of 24. In women, the urethra is short, straight, and closer to the rectum, making it easier for germs to travel into the bladder. For some women, UTIs relate to changes in their hormonal levels (Kaur *et al.,* 2020). Incidence in women between the age of 20–40 years ranges from 25 to 30%, whereas in older women above 60 years of age, it ranges from 4 to 43% (Zilberberg *et al.,* 2022; Trześniewska-Ofiara *et al.,* 2022).

UTIs can present clinically uncomplicated or complicated infections. catheterization, which predisposes the host to these infections (Sabih *et al.,* 2022). Catheter-associated UTI is responsible for 40% of nosocomial infections (CAUTI), making it the most common cause (Rezai *et al.,* 2017). It accounts for more than one million cases in hospitals and nursing homes annually. UTIs often involve uropathogens other than *Escherichia coli,* such as *Pseudomonas aeruginosa,* a non-fermenter Gram-negative bacillus with a large intrinsic resistance to multiple antibiotics (Gajdács *et al.,* 2021). *Pseudomonas aeruginosa* is responsible for 12% of all nosocomial urinary tract infections (CAUTIs), making it the third most common organism after *Escherichia coli* and Enterococci isolated from UTI patients in the hospital setting (Rezai *et al.,* 2017; Bouza *et al.,* 2001; Djordjevic *et al.,* 2013). This characteristic and its quick ability

to acquire new antimicrobial resistance make this pathogen a growing problem in infectious disease pathology, especially when originating nosocomial (Raoofi *et al.,* 2023).

Urinary tract infections (UTIs) caused by *Pseudomonas aeruginosa* are frequently associated with a history of catheterization and/or instrumentation or surgery. According to Cole *et al.* (2014), catheterization of the urinary tract is the primary cause of hospital-acquired urinary tract infection by *Pseudomonas aeruginosa*. Pathogens use catheters as a source of host entry, attaching to the medical device surfaces in biofilms. Catheter and other medical device insertion may interrupt mucosal and urothelial layers, promoting pathogen colonization. *Pseudomonas aeruginosa* causes biofilm-mediated infections, including catheter-associated urinary tract infections, ventilator-associated pneumonia, infections related to mechanical heart valves, stents, grafts, and sutures, and contact lens-associated corneal infections (Garg *et al.,* 2016).

Nosocomial UTIs are catheter-associated, and the development of bacteriuria is directly related to the duration of catheterization (Flores-Mireles *et al.,* 2019). Between 15 and 25% of hospital patients are catheterised for 2 to 4 days during their stays, while many nursing home patients remain catheterised for months or years (Ndomba *et al.,* 2021). Biofilms are a serious problem, often refractory to antibiotic therapy. Antibiotic therapy can eliminate bacteria in a patient, but bacteria within biofilms survive antibiotic treatment (Lebeaux *et al.,* 2014). When antibiotic treatment ends, the biofilm can again shed planktonic cells, resulting in recurrent acute infection. This infection cycle is difficult to stop and often requires removing the contaminated device to eliminate the bacterial biofilm. However, removing the contaminated device is only a temporary solution, as a replacement with a new device again provides a surface for biofilm formation (Sharma *et al.,* 2019; Hughes *et al.,* 2017).

Ndomba *et al.* (2021) investigated the prevalence and indications of long-term indwelling urinary catheterization (IUC) at home in North-western Tanzania to determine the reasons for

staying long with an indwelling urinary catheter after the diagnosis of benign prostatic hypertrophy was confirmed. Almost 10% of the 2112 patients attending the urology clinic as out-patients in north-western Tanzania lived with a long-term indwelling urinary catheter, some for many years while awaiting surgery (Ndomba *et al.* 2021). This exposes patients in Africa and other parts of the world to CAUTI. They attributed this phenomenon of prolonged catheterization to a shortage of qualified urologists (Ndomba *et al.,* 2021). *Pseudomonas aeruginosa* is one of the important pathogens responsible for catheter-associated UTIs (CAUTI); it is associated with high mortality and morbidity (Pachori *et al.,* 2019).

2.4.2. Skin and soft tissue infections caused by *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a bacterium that causes a wide variety of infections that have characteristic skin manifestations. They range from localised, benign skin infections to life-threatening systemic infections that feature skin lesions with characteristic morphology (Morin *et al.,* 2021).

The cutaneous manifestations of *Pseudomonas aeruginosa* infection range from superficial to deep and can occur in both immune-compromised and healthy individuals. In the case of the immunocompromised host, however, more significant morbidity and mortality can result from untreated *Pseudomonas aeruginosa* infection (Sainz-Mejías *et al.,* 2020). Cutaneous manifestations of *Pseudomonas aeruginosa* infection can be classified as either primary infection due to cutaneous inoculation or secondary to *Pseudomonas aeruginosa* bacteremia (Wilson *et al.,* 2022). *Pseudomonas aeruginosa* is the most frequently isolated bacterium colonising severe burns and wound infections in hospitals (Wood *et al.,* 2023); multidrug-resistant (MDR) pathogens are associated with high morbidity and mortality worldwide.

In addition, the spread of *Pseudomonas aeruginosa* can disseminate from the initial infection site and enter the bloodstream, causing septicemia (Moremi *et al.,* 2017).

Infections of the skin by *Pseudomonas aeruginosa* can give rise to various mild skin infections with unique clinical presentations. These syndromes generally present in otherwise healthy individuals; some resolve spontaneously without specific antibacterial therapy. Green Nail Syndrome is one of the oldest cutaneous manifestations associated with *Pseudomonas aeruginosa* infection (Chiriac *et al.,* 2015). The classic clinical presentation consists of the triad of green discoloration of the nail plate, proximal chronic paronychia, and disto-lateral onycholysis (Wu *et al.,* 2011).

Interdigital infections of toe web spaces are most commonly associated with yeast; however, persistent colonisation by dermatophytes can increase susceptibility to further bacterial superinfection by pathogen such as *Pseudomonas aeruginosa* as the predominant causative bacterium (Gyamfi *et al.,* 2021). Patient reports of burning and pain often accompany cutaneous signs (Park *et al.,* 2020; Miró et al., 2011; Aste *et al.,* 2001).

Folliculitis is one of the best-known cutaneous entities ascribed to *Pseudomonas aeruginosa* infection; it is 'hot tub folliculitis' due to the recreational use of hot tubs, whirlpools, and swimming pools (Zichichi *et al.,* 2000). Hot tub folliculitis typically presents in previously healthy individuals exposed to contaminated water (Jacob *et al.,* 2020). It is characterized by the sudden onset of numerous, large, monomorphic, painful papules and pustules approximately 24 hours after prolonged immersion in contaminated water. The lesions are clustered on body areas in contact with the water surface, typically the upper trunk, axillary folds, hips, and buttocks. Hot tub folliculitis is more common after immersion in water with a temperature over 38C (Wu *et al.,* 2011).

Pseudomonas aeruginosa infections of the ear can vary from benign to life-threatening. On the one hand, studies of uncomplicated auricular perichondritis have suggested that *Pseudomonas aeruginosa* is the most common microorganism responsible (Reynolds *et al.,* 2021).

On the other hand, pseudomonal ear infections can progress to the severe condition known as malignant otitis externa, an invasive and potentially life-threatening condition that affects the external ear and skull base. In this setting, the patient is classically elderly, immunocompromised, and often has diabetes mellitus (Aaraj *et al.,* 2023).

Risk factors associated with the acquisition of *Pseudomonas aeruginosa* infections in hospitalised burn patients include the length of hospitalisation, previous use of broad-spectrum antibiotics such as Carbapenems, known presence of *Pseudomonas aeruginosa* on the unit, and total body surface area burned (Wood *et al.,* 2023; Hasannejad-Bibalan *et al.,* 2021).

2.4.3. *Pseudomonas aeruginosa* causes respiratory tract infections.

Nosocomial infections (NI) are a global problem in hospital care. This is a significant complication that worsens the prognosis of the underlying disease, increases mortality, prolongs hospitalisation, worsens the quality of life of patients, and increases the cost of treatment (Labovská *et al.,* 2021). Infections acquired in connection with hospitalisation can lead to significant morbidity and mortality, but preventive anti-infective measures can significantly affect these results. *Pseudomonas aeruginosa* develops respiratory infections in patients hospitalised in the intensive care Unit (ICU), who often have respiratory insufficiency and hemodynamic instability and require artificial lung ventilation (Reynolds *et al.,* 2021).

Mechanical ventilation is a significant risk factor for developing *pseudomonas aeruginosa* pneumonia (Ramírez-Estrada *et al.,* 2016). Contamination of the patient's respiratory tract may come from a device with which the patient has been in direct contact, namely an endotracheal tube, nasogastric tube, aspiration catheters, or bronchoscopes, but also from a device with which he has not been in direct contacts, such as a mechanical ventilator, ventilator hose, nebulisers and devices that supply oxygen (Labovská *et al.,* 2021). Much attention has been paid to ventilator-associated pneumonia (VAP) as the most common and potentially

preventable nosocomial infection (Divatia *et al.,* 2019). Other nosocomial respiratory infections include sinusitis, otitis media, and tracheitis. The most common risk factors are poor hand hygiene, insufficient isolation of patients, and contaminated objects such as stethoscopes. Family members and other patients may also transmit the infection to patients hospitalised in ICU or other wards (Sikora *et al.,* 2021).

Pseudomonas aeruginosa is notorious for its capacity to establish permanent residency in the airways of patients with a chronic respiratory condition such as cystic fibrosis (Prado-Martín *et al.,* 2021). This usually results in the recurrence of chronic lung infections, leading to progressive respiratory failure with increased morbidity and mortality. Ventilator-associated pneumonia (VAP) is defined as the development of new pneumonia for at least 48 hours after the start of mechanical ventilation. Other additional criteria are the development of new or progressive and persistent infiltrate or consolidation or cavitation that develops later than 48 hours on at least two serial CXR with post-initiation of mechanical ventilation (Shakeel *et al.,* 2022; Papazian *et al.,* 2020).

Independent risk factors for developing VAP are immunodeficiency, immunosuppression, and neuromuscular blockade. Other risk factors are genetic syndromes with neuromuscular weakness, burns, steroid administration, and total parenteral nutrition (Wu *et al.,* 2019). A nasogastric tube increases the risk as it provides a direct pathway from the upper gastrointestinal tract to the oropharynx. In-line nebulisers and manipulation of the ventilator circuit can affect the risk of nosocomial pneumonia. Although the duration of endotracheal intubation increases the risk of nosocomial pneumonia, the highest risk is during the first two weeks of intubation. Almost all intubated patients have a colonised endotracheal tube with nosocomial microorganisms within five days (Reynolds *et al.,* 2021). Minimising the patient's mechanical ventilation length is important in preventing nosocomial pneumonia. The presence of an endotracheal tube poses a risk of VAP and not the positive pressure ventilation associated

with it. Daily consideration is recommended regarding whether the patient can be extubated (Papazian *et al.,* 2020).

2.5. Laboratory diagnosis of *Pseudomonas aeruginosa* infections

Bacterial infections are among the leading causes of nosocomial infections, and identifying the causative organism can be challenging, especially for resistant strains, requiring fast and accurate detection methods. Deciding which test to implement for the correct and rapid identification of pathogens such as *Pseudomonas aeruginosa* can be challenging; laboratories consider the sensitivity and complexity of the method, the turnaround time, the expertise required for each test, and the cost of the analysis.

The diagnosis of *Pseudomonas aeruginosa* infection is made by clinical assessment and laboratory analysis. The gold standard for laboratory identification is bacterial culture, but several molecule targets could also be used to identify the pathogen indirectly. Figure 2 represents the Amp C β-lactamase—structure from the *Pseudomonas aeruginosa* PAO1 strain.

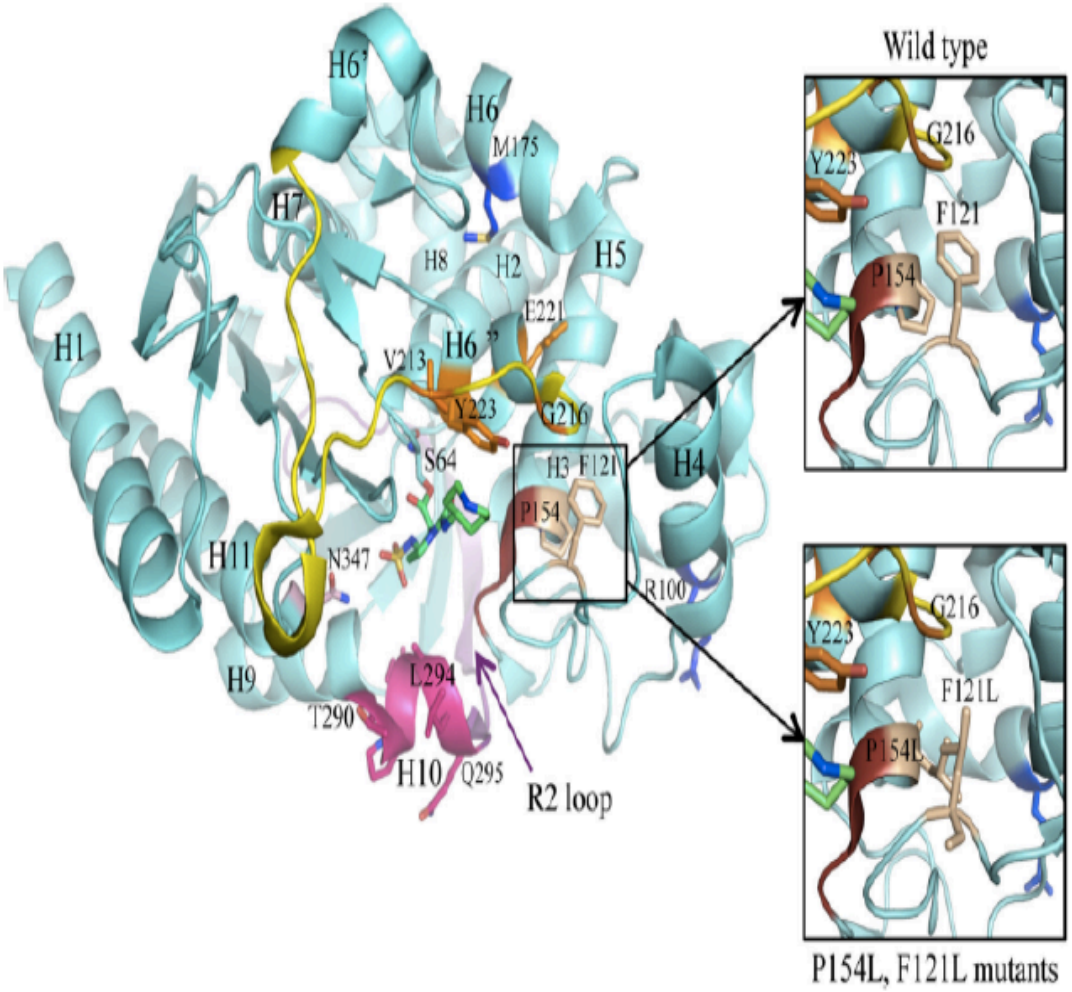


FIG 2 Representation of the AmpC β-lactamase structure from the *P. aeruginosa* PAO1 strain (light blue) in complex with an inhibitor (green) (PDB code 2WZZ) (41). The different structural regions lining the binding site are colored as follows: omega loop, yellow; helix H-10, pink; R2 loop, purple; and YSN, brick. The amino acids highlighted in this study as playing an important role in ESAC emergence are colored in terms of the function of their pertaining group (see the text): group I, orange; group II, pink; group III, wheat; and group IV, light pink. The two other mutants (M175L and R100H) found in combination with some from the other groups are colored in blue.

Figure 3 summarises common targets used by several laboratories to diagnose this infection.

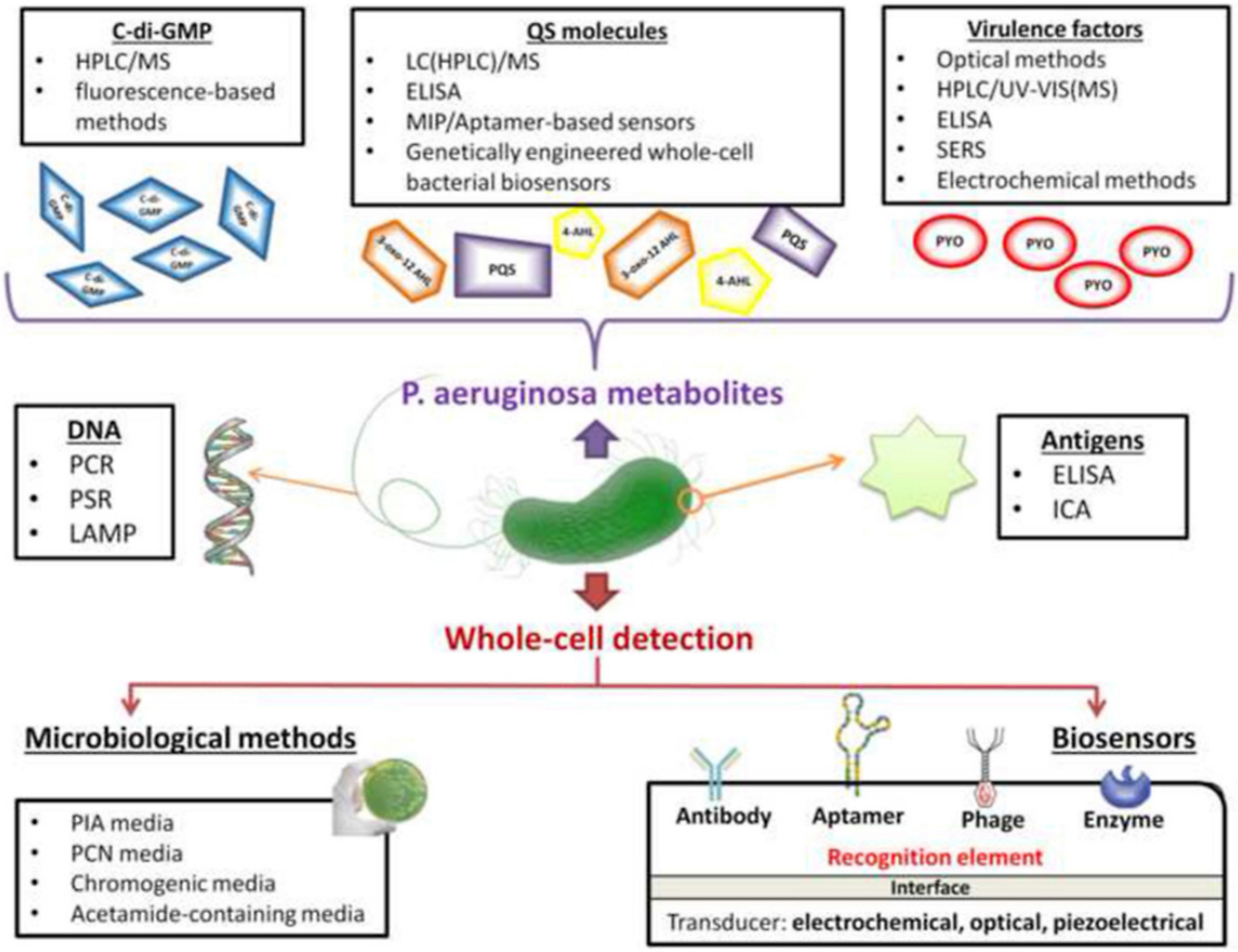


FIG 3: Several molecules targeted in the serological diagnosis of *Pseudomonas aeruginosa* infections. (Adapted from Capatina *et al.,* 2022).

2.5.1. Phenotypic bacteriological method: Cultural Isolation and Identification

The conventional approach used to detect and identify bacteria is based on traditional culture methods, which are still the gold standard due to their reliability, efficiency, sensitivity, and range of applications Past to Present (Franco-Duarte *et al.,* 2019). However, culture methods are laborious and require a long time for bacteria to grow, the results being reported after several days. Because of these limitations, other detection methods are required to supplement culture-based approaches to improve patient management (Lagier *et al.,* 2015). Microbiological techniques for rapid diagnosis allow quick identification of bacteria, which is necessary for the early management of patients.

Conventional methods for detecting pathogenic bacteria are based on bacterial culture and involve several steps: sample preparation, enrichment, dilution, plating, enumeration, and isolation of single species colonies on selective media for further characterization (Law *et al.,* 2015). Besides the lengthy analysis time and laborious steps, the conventional methods have other limitations that make these methods inappropriate for field applications or situations that require immediate results, such as intensive care management of patients, the need for special analysis conditions (temperature, light), low specificity compared to other methods, and the need for considerable quantities of consumables and qualified personnel (Ahmar *et al.,* 2020). Despite these limitations, phenotypic culture methods are still the most widely used tests in routine laboratories to identify bacteria, including those that produce nosocomial infections. Chromogenic agars, which contain antibiotics that allow only the development of resistant bacteria (selective media), represent an adaptation of the traditional culture methods and are increasingly used to detect several bacteria in clinical laboratories. This new media generation represents a sensitive, convenient, and relatively low-cost method for identifying pathogens based on a colour reaction produced in the bacterial culture, with a shorter turnaround time (Szabó *et al.,* 2022).

Molecular methods

In recent years, more powerful molecular, immunological, and biochemical analytical methods have emerged to overcome the limitations of conventional traditional methods. These diagnostic tools for detecting bacteria involved in nosocomial infections, such as *Pseudomonas aeruginosa*, are categorised into nucleic acid-based, biosensor-based, immunological-based and mass spectrometry-based methods (Szabó *et al.,* 2022). Various rapid detection methods have been developed and are generally more sensitive, specific, time-efficient, labor-saving, and reliable than conventional methods.

Nucleic acid-based methods such as PCR, mPCR, RT-qPCR, LAMP, NASBA and DNA microarray have high sensitivity and specificity. They can overcome the limitations of the traditional culture-based methods, but these methods require trained personnel and specialised instruments (Zhang *et al.,* 2022). Molecular methods, especially PCR techniques, help diagnose bacterial infections in routine laboratories and detect clinically relevant antibiotic-resistance genes and bacterial isolates growing in biofilms (Gerace *et al.,* 2022).

Polymerase Chain Reaction (PCR) is a simple molecular biology technique to amplify and detect DNA sequences. In recent decades, DNA amplification opportunities for detecting bacteria such as *Pseudomonas aeruginosa* in patients have increased substantially. Consequently, several studies have compared the specificity and the sensitivity of culture and PCR techniques (Liu *et al.,* 2019). Also, in the transmission of clonal *Pseudomonas aeruginosa* strains among patients, PCR may be helpful since the identification of these clones using a specific PCR can be achieved, which is not possible using culture (Wang *et al.,* 2022). PCR-based techniques can identify *Pseudomonas aeruginosa* DNA by amplification of genomic targets. The PCR assays use primers that target specific *Pseudomonas aeruginosa* DNA sequences, such as the 16S rRNA operon. This set of PCR primers targeting 16S rRNA gene sequences was designed. PCR parameters were optimized to develop a robust and reliable protocol for the selective amplification of bacterial pathogen 16S rRNA genes (Gholami *et al.,* 2016).

Other molecular methods for bacteria identification are isothermal amplification techniques, such as LAMP methods; these are novel gene amplification methods increasingly used in the specific detection of bacteria (Gerace *et al.,* 2022). These techniques present some advantages over PCR methods: shorter analysis time, ease of use, inexpensive running costs, higher specificity, and sensitivity, but they have a complicated design, requiring the use of at least

four primers, and it is also difficult to develop multiplex tests using these methods (Srivastava *et al.,* 2023).

Numerous biosensor-based methods have recently emerged for the rapid, sensitive, cost-effective, and easy detection of many bacteria. The biosensors have two main elements: a bio-receptor and a transducer (Castillo-Henríquez *et al.,* 2020). The bio-receptor can be a biological material, such as enzymes, antibodies, phages, nucleic acids, and cell receptors, biologically derived material, such as aptamers and recombinant antibodies, or biomimetic imprinted polymers and synthetic catalysts (Sande *et al.,* 2021).

The transducer can be optical, electrochemical, mass-based, thermometric, micromechanical, or magnetic. Among the essential advantages of these methods is the possibility of miniaturization and portability and the possibility of performing on-site and real-time analyses without the need for complex sample preparation, thus being preferred in routine laboratories for rapidly detecting bacteria. The possibility of using nanomaterials to increase the sensitivity of the detection method is another advantage of biosensors (Franco-Duarte *et al.,* 2019).

Immunological methods, such as Enzyme-linked Immunosorbent assay (ELISA) and immuno-chromatographic Assay (ICA), are based on the antigen-antibody specific interaction that leads to a visible reaction in the test medium if the antigen is present in the sample. These methods are widely used for detecting bacteria such as *Pseudomonas aeruginosa* due to their advantages, such as short analysis time, ease of use, high specificity, and relatively inexpensive equipment (Narayanasamy *et al.,* 2024). The advent of commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits and immunochromatographic assay (ICA strips has led to their widespread use for routine testing in developed countries (Ouyang *et al.,* 2022).

Conventional methods for detecting *Pseudomonas aeruginosa* are time-consuming. Therefore, a more rapid analytical gold nanoparticle-based immunochromatographic assay, using monoclonal antibodies (Mabs) against *Pseudomonas aeruginosa,* has been developed in

several laboratories (Zeng *et al.,* 2021). It has the advantage of portability and can be used to detect *Pseudomonas aeruginosa* in the field. However, these methods have lower sensitivity than other methods, such as molecular methods, which are sensitive to temperature and pH changes (Zeng *et al.,* 2021).

Identifying nosocomial infections caused by *Pseudomonas aeruginosa* can also be achieved based on detecting specific biomarkers, such as quorum sensing molecules, various virulence factors, or other specific metabolites of these bacteria (Miller *et al.,* 2020). Usually, the first step in detecting specific toxins or metabolites is detecting the toxin-producing gene using a molecular method, especially PCR (Priyanka *et al.,* 2016). Detecting bacteria, such as *Pseudomonas aeruginosa*, involved in nosocomial infection is of fundamental importance in the medical field due to the emergence of drug resistance pathogens, so researchers are still focused on developing new, improved methods that allow the early detection of infections with these pathogens (Diggle *et al.,* 2020).

2.5.2. Genetic diversity of *Pseudomonas aeruginosa*

The complete *Pseudomonas aeruginosa* genome was sequenced by Stover et al. (Stover *et al.,* 2000), suggesting that the *Pseudomonas aeruginosa* genome is the largest among bacteria. The genes encode bacteria proteins functionally involved in virulence factors, cell adhesion, and motility. However, lipopolysaccharide (LPS) synthesis enzymes and other secreted proteins are involved in the pathogenesis.

Other genes expressed in *Pseudomonas aeruginosa* are encoded for outer membrane proteins such as regulatory networks, the OprDporin family, and antibiotic resistance efflux pump systems. The size and complexity of the *Pseudomonas aeruginosa* genome are responsible for the capacity of this pathogen to adapt and grow in various environments (Lorusso *et al.,* 2022).

The genetic diversity of clinical *Pseudomonas aeruginosa* is assessed by the phylogenetic analysis of a concatenated tree. Gomila *et al.* (2013) observed that the population structure of clinical *Pseudomonas aeruginosa* in Spanish hospitals indicated the coexistence of non-resistant and resistant isolates with the same sequence type. Wiehlmann *et al.* (2015) investigated the population structure of *Pseudomonas aeruginosa* by genotyping 2921 isolates from 1448 independent habitats. Clinical isolates either belonged to ubiquitous *Pseudomonas aeruginosa* clones such as C and PA14 or clones uncommon in the environment. In a study by Cabot *et al.* (2012), Multilocus Sequence Typing (MLST) analysis revealed that most *Pseudomonas aeruginosa* XDR isolates belonged to sequence type 175 (ST175) or ST111, both recognized as international high-risk clones (Dufkova *et al.,* 2023).

Previously described *Pseudomonas aeruginosa* genome MLST analyses are available in gene databases and tools (https://pubmlst.org/paeruginosa/) and are used for comparison with new genetic diversity data analysis. The following phylogenetic tree represents the 20 most frequent *Pseudomonas aeruginosa* clones (Figure 3).

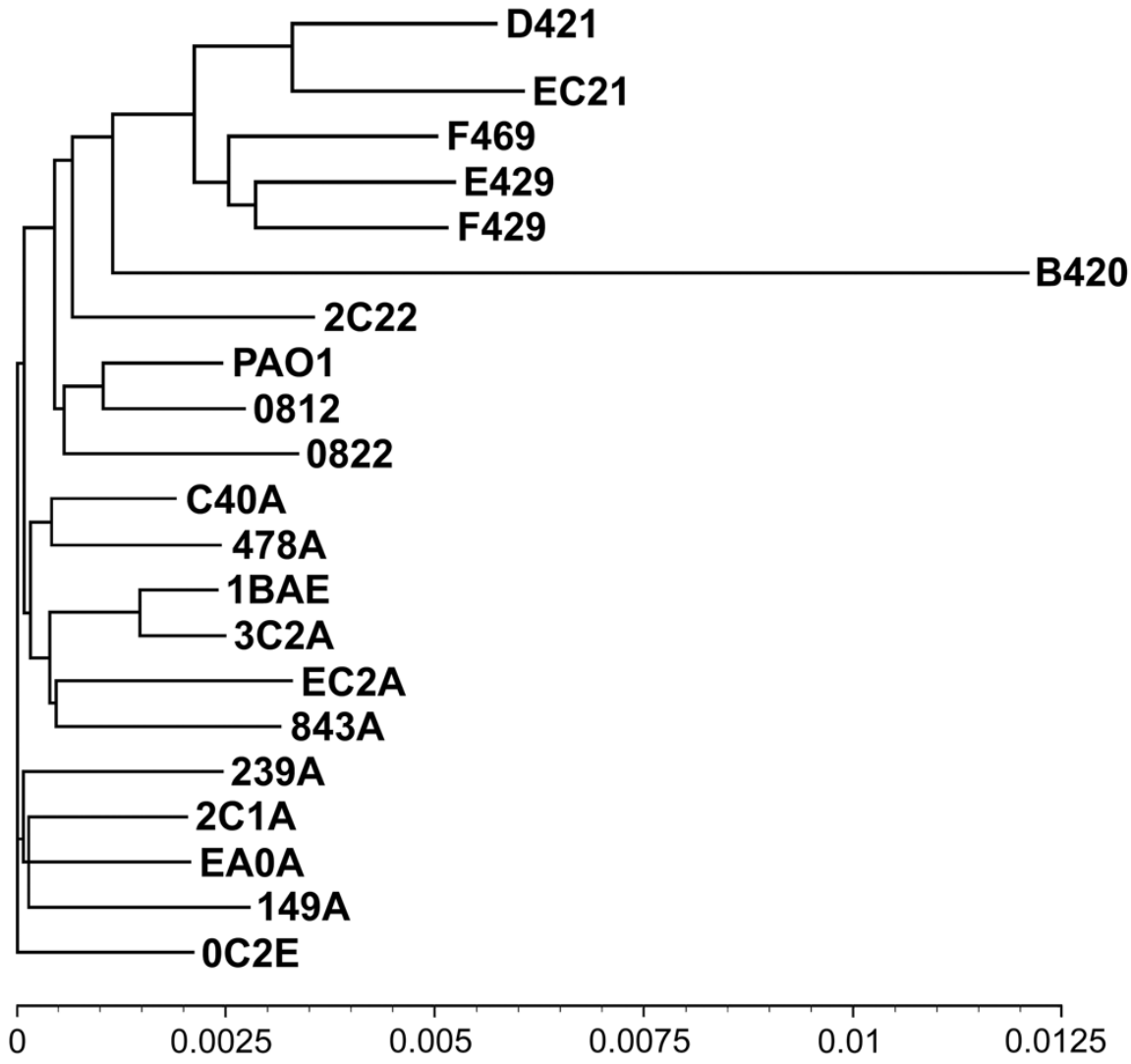


FIG 4. Phylogenetic tree of *Pseudomonas aeruginosa* strains representing the 20 most common clones in the global *P. aeruginosa* population (Wiehlmann *et al.,* 2015)

2.6. Management of *Pseudomonas* aeruginosa nosocomial infections

2.6.1. Appropriate antimicrobial use

The Centers for Disease Control and Prevention (CDC, 2021) estimates that each year, medical practitioners prescribe about 100 million courses of antibiotics; approximately 50% are unnecessary (Salam *et al.,* 2023).

Antimicrobial use should be justified by the proper clinical diagnosis or isolation of a bacterial pathogen causing the infection. However, the selection of antimicrobials should also be based on the patient's tolerance and the nature of the disease and pathogen (Aricò *et al.,* 2023). Antimicrobial therapy should aim to use a selectively active drug against *Pseudomonas aeruginosa* that is least likely to cause resistance and adverse effects (Mayegowda *et al.,* 2022).

2.6.2. Drug susceptible *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is intrinsically resistant to certain antibiotics and can potentially acquire resistance during treatment (Pachori *et al.,* 2019). Therefore, two agents from different classes should be used when the risk for antibiotic resistance is suspected. The treatment consists of two-drug combination therapy, such as an antipseudomonal beta-lactam and an aminoglycoside (Zheng_Pang *et al.,* 2019).

Pseudomonas aeruginosa infection can be treated with a combination of an antipseudomonal beta-lactam, such as penicillin or cephalosporin, and an aminoglycoside (Qureshi *et al.,* 2023). Carbapenems such as imipenem or meropenem combined with antipseudomonal quinolones may be used with an aminoglycoside. Except for cases involving febrile patients with neutropenia, monotherapy with ceftazidime or a carbapenem (e.g., imipenem, meropenem) may be used (Wang *et al.,* 2021).

In patients with *Pseudomonas aeruginosa* pneumonia, most experts recommend starting with two anti-pseudomonal antibiotics and then de-escalating to monotherapy, except in patients with cystic fibrosis where the role of an aerosolized ceftazidime or aminoglycoside is controversial (Ramsay *et al.,* 2023).

According to the American Thoracic Society and the American Infectious Diseases Society, guidelines for ventilator-assisted pneumonia treatment should start with a combination

including a beta-lactam and aminoglycoside for five days and de-escalate to monotherapy based on organism culture sensitivity (Torres *et al.,* 2018).

In patients with bacteremia, antibiotic therapy is instituted once pseudomonal sepsis is suspected. For neutropenic patients, presumptive therapy combines an aminoglycoside and a broad-spectrum antipseudomonal penicillin or cephalosporin. Fluoroquinolones provide an alternative for the beta-lactam–sensitive patient, and the addition of rifampin to the beta-lactam and aminoglycoside combination may improve bacteriologic cure (Qureshi *et al.,* 2023).

In a patient with catheter-associated urinary tract infections (CAUTI), parenteral aminoglycosides may remain the antibiotics of choice, although quinolones are often used (Werneburg *et al.,* 2022). Tobramycin is preferred to gentamicin in patients with renal dysfunction. UTI can be treated with a single agent, except in cases of bacteremia and upper tract infections with abscess formation (Qureshi *et al.,* 2023).

Alternative antibiotics include antipseudomonal penicillin and cephalosporins, carbapenems (e.g., imipenem, meropenem), and aztreonam. Ciprofloxacin continues to be the preferred oral agent (Zakhour *et al.,* 2022). Duration of therapy is 3-5 days for uncomplicated infections limited to the bladder, 7-10 days for complicated infections, especially with indwelling catheters, 10 days for urosepsis; and 2-3 weeks for pyelonephritis. A longer duration of treatment is necessary for those patients with perinephric or intrarenal abscesses (Qureshi *et al.,* 2023).

2.6.3. Drug resistant *Pseudomonas aeruginosa*

Patients with complicated *Pseudomonas aeruginosa* infections are treated with a combination of cephalosporin and a beta-lactamase inhibitor (Alnimr *et al.,* 2020). The ceftazidime component has activity against Gram-negative bacteria, including *Pseudomonas aeruginosa*. The addition of a beta-lactamase inhibitor improves the outcome. Ceftazidime/avibactam is

indicated for treating patients aged 18 years or older with complicated intra-abdominal infections and UTIs (Qureshi *et al.,* 2023).

Ceftolozane/tazobactam is a novel cephalosporin developed with a beta-lactamase inhibitor for treating complicated UTIs and other infections, including ventilator-associated bacterial pneumonia (Zhanel *et al.,* 2014). Ceftolozane has similar activity to ceftazidime, piperacillin/tazobactam, and the carbapenemase family of antibiotics. The tazobactam component allows the drug to act against extended-spectrum beta-lactamase (ESBL) bacteria (Lizza *et al.,* 2021).

Ceftolozane/tazobactam is a carbapenem-sparing alternative for treating complicated UTIs and infections, including those caused by ESBL-producing multidrug-resistant *Pseudomonas aeruginosa (*Bassetti *et al.,* 2022).

Aminoglycosides, Fosfomycin, and/or polymyxins (colistin or polymyxin B) therapy is used for multidrug-resistant isolates. In 2018, the concept of "difficult-to-treat" resistance (DTR) was proposed, and a guidance document was put in place to treat drug-resistant *Pseudomonas aeruginosa* (DTR). DTR was defined as *Pseudomonas aeruginosa* exhibiting non-susceptibility to all of the following antibiotics: piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin (Karruli *et al.,* 2023).

The latest evidence and recommendations support ceftolozane-tazobactam and ceftazidime-avibactam, characterized by targeted clinical activity against many *Pseudomonas aeruginosa* strains with limited treatment options (Karruli *et al.,* 2023).

In northwest Ethiopia, Asamenew *et al* (2023) reported a prevalence of 19.3% of *Pseudomonas aeruginosa* from different clinical samples in Debre Tabor Comprehensive Specialized Hospital. Asamenew *et al.* (2023) further described the antibiotic resistance profile of *Pseudomonas aeruginosa* isolates as alarming. *Pseudomonas aeruginosa* showed resistance

against gentamicin at 62.2%, ceftazidime at 51.4%, cefepime 50%, amikacin 29.7%, imipenem 28.4% and ciprofloxacin 14.9%. The level of multi-drug resistance (MDR) was 45.9%, and the suspicious extreme-drug resistance (XDR) rate was 9.5%. Being inpatient and wound swab samples were factors associated with detecting *Pseudomonas aeruginosa* from clinical samples.

Previously, Savas *et al* (2005) reported a high frequency of *Pseudomonas aeruginosa* in patients treated at ICUs in a university teaching hospital in Türkiye. The authors also reported an alarmingly high resistance rate to several antibiotics including carbapenems.

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains are increasing worldwide and further limiting therapeutic options. Understanding and continuously monitoring the prevalence and resistance mechanisms of MDR *Pseudomonas aeruginosa* will enable policymakers to formulate rational treatment strategies to combat nosocomial infections (Tam *et al.,* 2010).

In the Islamic Republic of Iran, Shahcheraghi *et al* (2009) conducted a study to investigate the drug susceptibility testing and PCR assay to determine the antibiotic susceptibility patterns and prevalence of genes encoding extended spectrum βetalactamases (ESβLs) among 600 isolates of *Pseudomonas aeruginosa* cultured from patients at two hospitals in Tehran. The frequency of bla(VEB), bla(SHV), bla(PER), bla(GES), and bla(TEM) among the ESβL isolates were 24%, 22%, 17%, 0%, and 9%, respectively. Isolates containing bla(VEB) were resistant to almost all tested antibiotics except Imipenem.

2.7. *Pseudomonas aeruginosa* nosocomial infections therapeutic options and outcomes

In-hospital all-cause mortality ranged from 25 to 60% in patients infected with the MDR *Pseudomonas aeruginosa* group, 15 to 59% in the resistant *Pseudomonas aeruginosa* group, and 7 to 50% in the susceptible *Pseudomonas aeruginosa* group (Fatima *et al.,* 2021). Mortality was 34% (95% CI: 27% – 41%) in patients with resistant and MDR *Pseudomonas aeruginosa* compared to 22% (95% CI: 14% – 29%) with susceptible *Pseudomonas aeruginosa (*Nathwani *et al.,* 2014). When comparing patients with resistant *Pseudomonas aeruginosa* infections versus those with susceptible *Pseudomonas aeruginosa* infections, resistance was associated with a 24% higher risk of in-hospital all-cause mortality (Nathwani *et al.,* 2014). The MDR *Pseudomonas aeruginosa* group had a greater than 2-fold increase in the risk of in-hospital all-cause mortality (Fatima *et al.,* 2020).

The need for mechanical ventilation was studied as an index of patient outcomes in one case-control study; patients with MDR *Pseudomonas aeruginosa* required more days of mechanical ventilation than patients with susceptible *Pseudomonas aeruginosa* (15 versus 11 days) (Gatti *et al.,* 2022). One study reported persistence of microbiological infection of 75% and clinical persistence or recurrence of 38% in patients with MDR *Pseudomonas aeruginosa* infection versus 61% and 39%, respectively, in the non-MDR *Pseudomonas aeruginosa* group (Horcajada *et al.,* 2019).

The mortality rate associated with nosocomial infections caused by *Pseudomonas aeruginosa* is higher than that of of *Staphylococcus aureus* in certain types of infections, such as bloodstream infections (Thaden *et al.,* 2017). Multidrug resistant *Pseudomonas aeruginosa* (MDRPA) infections are associated with higher mortality compared to non-multidrug resistant *Pseudomonas aeruginosa* (non-MDRPA) infections. According to Morata *et al.* (2012), inappropriate empiric treatment has been shown to increase mortality.

Quinolones are the only group of antibiotics that can be orally administered to treat *Pseudomonas aeruginosa* infections while Aminoglycosides are used intravenously in monotherapy only for uncomplicated urinary tract infections. Otherwise, they are administered in combination with other antibiotics to treat nosocomial infections. Colistin has considerable side effects and is therefore reserved as a last resort for the treatment of nosocomial infections. β-lactam antibiotics such as piperacillin-tazobactam, ceftazidime, and cefepime are available as the first level, followed by carbapenems in the second level for the treatment of *Pseudomonas aeruginosa* nosocomial infections. Novel β-lactams-β-lactamase inhibitors and cefiderocol are available in the third level and the highest levels respectively (Canton *et al.,* 2022).

Several types of β-lactamases, including class C, class A, and certain class D enzymes can be inhibited by Avibactam, a potent β-lactamase inhibitor. However, ceftazidime-avibactam has shown limitations, such as vulnerability to efflux pumps and lack of activity against metallo-β-lactamases. Another antibiotic, Ceftolozane is a stable antibiotic against AmpC-type β-lactamases and efflux pumps of *Pseudomonas aeruginosa.* It has less potential for resistance development compared to ceftazidime. However, it lacks activity against metallo-β-lactamases and class A carbapenemases (Canton *et al.,* 2022).

Relebactam restores imipenem activity against strains of *Pseudomonas aeruginosa* expressing AmpC overproduction and OprD deficiency, and it is stable against efflux pumps and some β-lactamases (class A and D). The *Pseudomonas aeruginosa* porin OprD, an outer membrane protein, is a substrate-specific porin that facilitates the diffusion of basic amino acids, small peptides, and carbapenems into the cell. Deficiency of OprD confers *Pseudomonas aeruginosa* a basal level of resistance to carbapenems, especially to imipenem (Li *et al.,* 2011). OprD-mediated resistance occurs as a result of decreased transcriptional expression of OprD and/or loss of function mutations that disrupt protein activity. Meropenem-vaborbactam is

weaker against strains with decreased expression of OprD porins and those with efflux pumps. It also has no activity against class B β-lactamases. It has increased stability against serine and metallo-β-lactamases and extended spectrum of activity against other non-fermentative Gram-negative bacilli. However, there is limited data on its activity against class D β-lactamases, such as *Pseudomonas aeruginosa* chromosomal OXA-50 (Canton et al., 2022).

Choosing appropriate empirical treatment for MDRPA infection requires consideration of various factors, such as clinical presentations, comorbidities, and the presence of risk factors for multidrug-resistant pathogens. Local epidemiological data should be used to ensure effective treatment (Montravers et al., 2018).

Identifying the causative microorganism by timely collecting the appropriate sample for culture is essential in facilitating the appropriate antibiotic de-escalation from empirical treatment (Garnacho-Montero *et al.,* 2015). In addition to selecting the appropriate antibiotic, optimizing pharmacokinetics is crucial for successful treatment (Lodise *et al.,* 2007).

In addition to optimal management with antibiotics, adequate control of the source of infection is key to overcoming the infection (Habboush *et al.,* 2023). The duration of treatment should not differ based on whether the infection is caused by MDRPA or a sensitive strain (Tamma *et al.,* 2022).

Successfully decolonizing a patient or preventing colonization altogether could potentially avoid future nosocomial infections in a patient. One strategy to achieve this is studying the microbiome and modifying it (Fan *et al.,* 2021).

Future perspectives on addressing MDRPA include antimicrobial peptides, the development of new antibiotics, and phage therapy. Other options are nanoparticles, gene editing tools, and development. These show great promise for the future successful management of *Pseudomonas aeruginosa* infections. These novel approaches offer a wide range of potential options to combat and control this pathogen (Qin *et al.,* 2022).

2.8. Conceptual framework of transmission and outcome of resistant *Pseudomonas aeruginosa nosocomial infections*

The conceptual framework for our study included the nosocomial infections dynamics transmission model and antimicrobial resistance model. The dynamic transmission model improved our understanding of events and risk factors associated with nosocomial infection (NI) transmission. Esposito *et al.* (2020) described the Multi-Agent Modelling and Simulation of Hospital Acquired Infection Propagation Dynamics by Contact Transmission in Hospital Ward Transmission. According to this model, patients are divided into five classes: uninfected patients (*not infected*), patients infected only by the non-resistant strain (*sensitive*), and three classes of patients infected by resistant bacteria (*XDR*), (*MDR*), and (*PDR*).

Environmental contamination may contribute to transmitting pathogens when healthcare workers contaminate their hands or gloves by touching contaminated surfaces or when patients come into direct contact with contaminated surfaces. Several investigators have documented the transmission of *Pseudomonas aeruginosa* from environmental surfaces to gloves or hands and dust coats of healthcare workers (HCWs) (Pham *et al.,* 2022; Mwamungule *et al.,* 2015).

Pham *et al.* (2019 and 2022) developed a mathematical model including background transmission, cross-transmission, and environmental contamination. According to this model, patients contribute to a pool of *Pseudomonas aeruginosa* pathogens by shedding bacteria into the environment. Natural decay and cleaning of the environment lead to a reduction of that pool. Environmental contamination during and after a patient's hospital stay was assessed by assigning the *Pseudomonas aeruginosa* bacterial load shed during an ICU stay to cross-transmission. The relative importance of the considered *Pseudomonas aeruginosa* acquisition route was determined for two intensive care units (ICUs) of the University Hospital in Besançon, France (Pham *et al.,* 2019). Based on a data-augmented Markov Chain Monte Carlo method, information about the ICU patients' admission and discharge days, screening days, and

screening results were used. This model demonstrated that background and cross-transmission played a significant role in the transmission process in both ICUs. In contrast, only about 1% of the transmissions were due to environmental contamination after discharge. The environment after discharge had only a limited impact on preventing *Pseudomonas aeruginosa* in the two considered ICUs of the University Hospital in Besançon (Pham *et al.,* 2019).

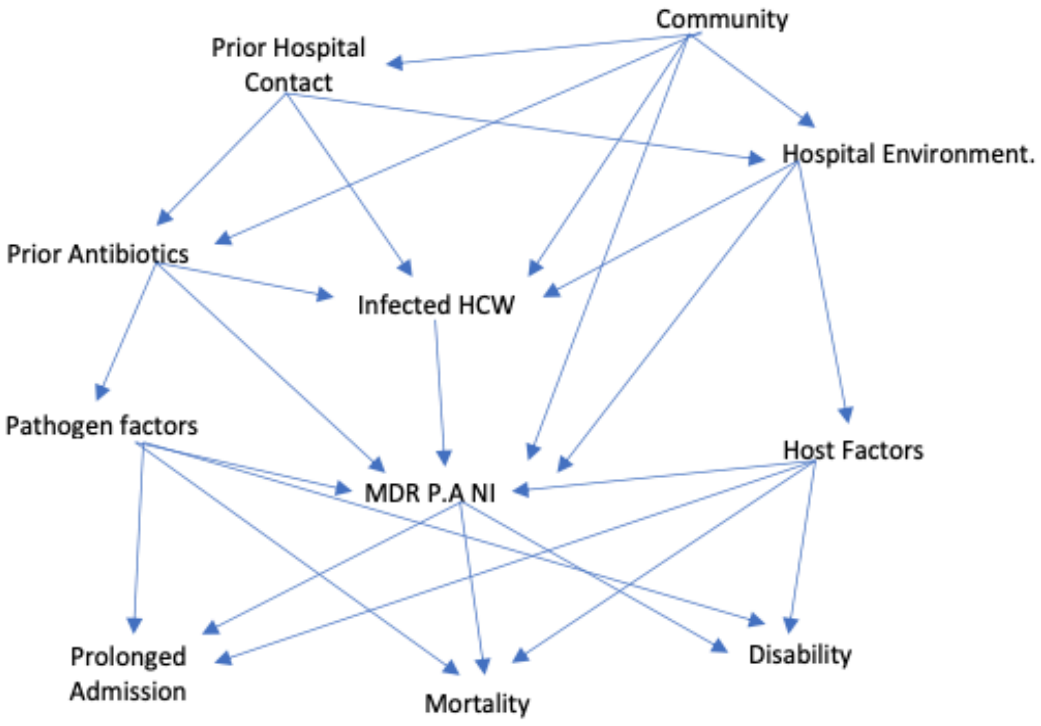


FIG 5. Graphical representation of the conceptual Framework for Risk of transmission dynamics of drug-resistant *Pseudomonas aeruginosa* and outcome among patients at UTH and NTH.

Antibiotic exposure, central to the emergence and spread of these resistant *Pseudomonas aeruginosa*, was incorporated with other factors, such as average length of stay, to fully understand the factors propagating the antimicrobial-resistant *Pseudomonas aeruginosa* at UTH and NTH. Patients admitted to healthcare institutions are the main reservoirs of antimicrobial-resistant bacteria (Pachori *et al.,* 2019). Infections acquired in hospitals are favored by a hospital environment and referred to by the technical term 'nosocomial.' Though

some studies are modeling the impact of hospital environmental contaminations on nosocomial infections (Wang *et al.,* 2022). Little is known about the determinants of these infections in our setting, especially in the transmission dynamics and outcomes of *Pseudomonas aeruginosa*, which motivated this research. The fundamental concepts in the framework are graphically presented in Figure 4. It allows for visualizing contamination propagation due to human spatial behavior and activities in a given environment at risk of *Pseudomonas aeruginosa* colonization. The conceptualization factors in host-related factors. These include susceptibility to infection (HIV, Neutropenia, Diabetes mellitus), extreme age (above 65 years), positive family history, and genetic factors (Duguma *et al.,* 2020). Pathogen-related factors were also included. We factored in the genetic mutations related to Bla OXA, AMP C, and IMP genes. Hospital and clinical-related factors were also included. These consisted of prolonged and inappropriate prescriptions of antibiotics, prolonged admissions, and the use of invasive medical devices. Community factors such as self-medication were taken into account, too. The risk factors assessment was done through a what-if scenario so that we help advise policymakers on the prevention and control measures for pathogen propagation and outcome.

2.9.Knowledge gap

Literature has revealed that drug-resistant *Pseudomonas aeruginosa* nosocomial infection has been investigated in Europe, Asia, and North America. Despite these studies, the World Health Organization (WHO) still identified *Pseudomonas aeruginosa* as a priority pathogen, especially in developing countries, and recommended further research to understand resistance dynamics and improve clinical outcomes (WHO, 2017; Reig *et al.,* 2022). Several authors still report alarming AMR *Pseudomonas aeruginosa* situations globally. AMR is expected to have a substantial financial cost, and we may soon be facing the end of the antibiotic era if drug-resistant pathogens such as *Pseudomonas aeruginosa*

are not researched enough (WHO, 2021). The literature review identified a more alarming situation regarding Africa's lack of antimicrobial resistance data, including that of *Pseudomonas aeruginosa* nosocomial infections, affecting almost half of African countries. Kariuki *et al.* (2022) reported a disproportionate burden of AMR in Sub-Saharan Africa, Zambia inclusive, owing to a high burden of infectious diseases, poor regulation of antimicrobial use, and a lack of alternatives to ineffective antimicrobial agents (Kariuki *et al.,* 2022).

In Zambia, a systematic review on antimicrobial resistance from a One Health perspective highlighted findings that AMR is understudied in the country. Secondly, the level of resistance to commonly prescribed antibiotics is significant across the human, animal, and environmental sectors in Zambia; hence, there is a need to investigate the clinical outcome of resistant pathogens such as *Pseudomonas aeruginosa* (Nowbuth *et al.,* 2023).

Pseudomonas aeruginosa AMR is understudied in Zambia, with data on clinically resistant *Pseudomonas aeruginosa* limited to a few isolates tested for sensitivity. This review highlights a gap in investigating *Pseudomonas aeruginosa* nosocomial infection and antimicrobial resistance in general. Overall, AMR data was only available for some of the ten provinces of Zambia. A systematic review of Zambian studies on AMR identified only a few bacterial isolates from the human, animal, and environmental health sectors tested against 36 antimicrobial agents across 13 classes of antibiotics (Nowbuth *et al.,* 2023).

Literature has also revealed that, from the Zambian perspective, one study reported the novel heavy metal-tolerant *Pseudomonas aeruginosa* strain Zambia SZK-17 Kabwe 1 (Mtengai *et al.,* 2022) isolated from the environment in Kabwe but no such study of the genetic determinant of clinical isolates of *Pseudomonas aeruginosa* outcome has been done before among patients at UTH and NTH. The evolutionary characterization of clinical isolates of *Pseudomonas aeruginosa* was also unknown in Zambia at the time of the study.

Therefore, in addition to the clinical and phenotypic evaluation, the point of departure for our research from previous studies, we conducted the molecular identification, evolutionary characterization, and determinants of the occurrence and outcome of *Pseudomonas aeruginosa* nosocomial infections at the University Teaching Hospital in Lusaka and the Ndola Teaching Hospital in Ndola, Zambia.

2.10. Conclusion

Pseudomonas aeruginosa is an important Gram-negative pathogen, particularly in patients with chronic lung disease and in patients at risk for nosocomial infections. It is a particularly important pathogen in hospital-acquired infections such as ventilator-associated nosocomial pneumonia.

Nosocomial infections are a major cause of morbidity and mortality in both developed and developing nations. *Pseudomonas aeruginosa* is an important Gram-negative pathogen, particularly in patients with chronic lung disease and in patients at risk for nosocomial infections. It is a particularly important pathogen in hospital-acquired and ventilator-associated nosocomial pneumonia. *Pseudomonas aeruginosa* possesses a number of virulence factors that enable it to attack respiratory epithelial cells and is adept at developing mechanisms for antimicrobial resistance. Treatment of *Pseudomonas aeruginosa* will often involve combination therapy with two antibiotics which are known to have activity against *pseudomonas aeruginosa* for better clinical outcomes. Newer antibiotics have been developed for the treatment of MDR *Pseudomonas aeruginosa*, such as ceftolozane-tazobactam, which is increasingly being used as therapy for patients with suspected MDR *P. aeruginosa*.

Prevention of *Pseudomonas aeruginosa* nosocomial infections include simple measures such as strict hand hygiene, isolation, sterility, and elevated head position to prevent

aspiration in unconscious patients. Other measures such as judicious use, and prompt removal of central catheters, urinary catheters, and endotracheal tubes can also dramatically affect the frequency of nosocomial infections.

The medical community must take steps to reduce and prevent nosocomial infections. Future efforts should be made to distinguish community-acquired infection from nosocomial infection, and to reduce the development of resistant organisms through prudent use of antibiotics and barrier nursing measures.

2.10.1. Future research directions.

Further evaluation of risk factors and *Pseudomonas aeruginosa* virulence factors is needed for better control of nosocomial infections due to this pathogen. *Pseudomonas aeruginosa* strains expressing virulence factors such as the ExoU exoenzyme effector are known to associate with the most adverse patient outcomes, likely owing to the ability of ExoU to subvert host innate immune responses and cause cellular damage. Virulence factors, such as the T3SS and ExoU, represent the next frontier of precision medicine therapeutic targets to combat the imminent threat of antibiotic-resistant infectious agents such the ESKAPE pathogens. ExoU is an especially attractive potential target, considering its contributions to *Pseudomonas aeruginosa* virulence and its requirement for a eukaryotic-specific protein, ubiquitin, to fully hydrolyze membrane substrates. Investigating the structural transitions between inactive and active states of these proteins may provide a better understanding of these virulence factors.

CHAPTER THREE

MATERIAL AND METHODS

3.1.Study Design

This study was an analytical cross-sectional facility-based study conducted between 2019 and 2021. It was conducted to undertake the characterization of *Pseudomonas aeruginosa* clinical isolates. Clinical assessment and in-hospital patient follow-up until discharge, 30 days, or death were also undertaken to establish the clinical outcomes of patients.

3.2.Study setting

The study was conducted in Lusaka district (Lusaka province) and Ndola district (Copperbelt province) in Zambia. According to the 2022 Zambia Central Statistics Office population census, Zambia had a total population of 19,610,769, with Lusaka's population at 3,042,000 in the metropolitan area. We selected the two densely populated provinces of Zambia for this study. The Central Statistics Office (CSO) report of 2022 indicated that Lusaka and Copperbelt provinces had the most significant number of households at 687,923 and 581,138, respectively. These two provinces also had the two largest teaching hospitals in the country.

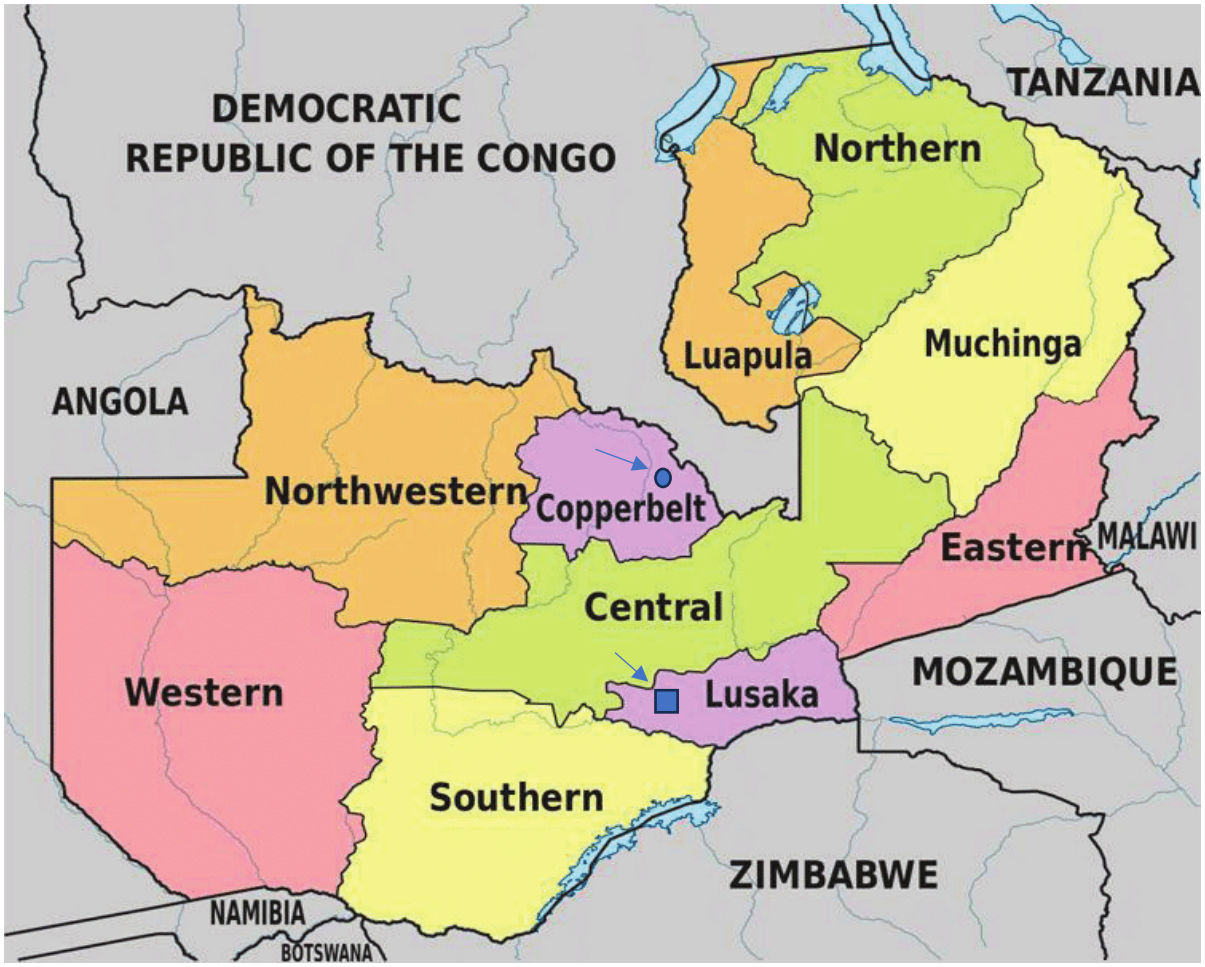


FIG 6. Map of Zambia, showing its ten provinces and the location of Lusaka and the Copperbelt provinces.

The University Teaching Hospital (UTH) in Lusaka is a 2000-bed tertiary hospital and Zambia's highest referral centre. It has fifty-six wards, of which nine are medical, with a standard internal medicine capacity of 224 beds.

UTH functions as a national referral hospital in Zambia. This teaching hospital's size and the pyramid-type referral system make it an ideal place to study nosocomial infections in Zambia. This hospital hosts the University of Zambia, the School of Medicine (UNZA/SOM), and nursing training institutions for training diploma and degree nurses.

The Zambian Health Care System, which has a pyramid-based referral structure, receives referrals from level one, general hospitals, and community clinics and directly from the community. Including such a facility in this study would help understand antimicrobial

resistance patterns in a broader context. Lusaka also has a network of clinics and five general hospitals providing referrals for tertiary care to the hospital. These are done in addition to referrals from provincial and private hospitals and the Levy Mwanawasa Teaching Hospital.

UTH samples were analyzed in the Microbiology Laboratory in the Department of Diseases Control, the School of Veterinary Medicine at the University of Zambia. Similarly, samples from NTH were processed at the Tropical Diseases Laboratory Centre (TDRC). These two laboratories had significant capacity to conduct phenotypic cultures and molecular work, including PCR and Sanger sequencing.

The Department of Disease Control was one of the four academic Departments in the School of Veterinary Medicine at UNZA. The Department has the following mainstream laboratories: Virology, Bacteriology, Public Health, Biochemistry, Pathology and Haematology/Parasitology. In addition, the Department has a Biohazard facility containment level 2 where infectious materials are handled. In 2008, a Biosafety Level-3 laboratory facility was constructed by the Centre for Zoonoses Control project of Hokkaido University, Japan.

This modern facility has dramatically improved the capacity of the Department to engage in research work involving various pathogens. Also, it provides service to stakeholders, including the Ministry of Health, the School of Biomedical Sciences, and the School of Medicine.

The other study site was the Ndola Teaching Hospital (NTH). This hospital is located in the Copperbelt province, 320 Km from Lusaka. The hospital is located in Ndola district, the provincial capital of the Copper-Belt province of Zambia. It had a capacity of 851 beds and 97 baby cots and was the second largest hospital in Zambia with over 1027 staff, of which over 75 are doctors and over 300 are nurses (WHO, 2020). Our study was on adult patients; hence, the study focused on adult wards of internal medicine and surgical wound infections in adult surgical wards. This research was conducted in the Out-Patient Department (OPD) and the in-

patient wards. Ndola Teaching Hospital was also a critical hospital outside of Lusaka with a referral system similar to UTH's. The hospital hosted the Copper-Belt University School of Medicine and three other medical training institutions. This hospital also hosts the Tropical Diseases Research Centre (TDRC) Laboratory. Samples collected from Ndola Teaching hospitals were analysed at TDRC.

The Tropical Diseases Research Centre (TDRC), located in Ndola, is a biomedical research centre established by the World Health Assembly in 1977. TDRC was initiated by the World Health Organisation (WHO) in collaboration with the Zambian Government in response to a resolution of the World Health Assembly of 1974, which called for the intensification of operational research into tropical diseases (WHA27.52, May 1974). This stipulated that, as far as possible, the work should be done in developing countries where these diseases are endemic. Activities of the institute include epidemiological and clinical research in infectious diseases, malaria, schistosomiasis, African trypanosomiasis, HIV/AIDS, micronutrient deficiencies, health systems research, health impact and evaluation, training and service. NTH represents an ideal study site that allowed us to avoid selecting all *Pseudomonas aeruginosa* from one clone in Lusaka.

3.3. Study population

The target population was adult patients from outpatients (those with prior contact with the hospital) and inpatient wards, and high dependency/Intensive Care Units at the Ndola Teaching Hospital (Tertiary) (NTH) in Ndola district and at the University Teaching Hospital (Tertiary) (UTH) in Lusaka district who presented with nosocomial infection. Patients with Chronic Obstructive Pulmonary Disease (COPD) and Tuberculosis (TB) presenting with acute exacerbation or secondary bacterial infections with persisting and worsening symptoms were also included after obtaining consent. Patients with medical devices such as urinary catheters,

central lines, and endotracheal tubes were also included. Patients with infected wounds (burn, pressure, and traumatic) were also assessed for *Pseudomonas aeruginosa.*

3.4. Inclusion criteria

Consenting patients aged at least 18 years who were admitted or presented to OPD as a returning patient presenting with features of the urinary tract, respiratory, skin, and invasive infections (sepsis) 48 hours after admission were screened for nosocomial infections and subsequently recruited into the study if culture positive for *Pseudomonas aeruginosa* infection.

3.5. Exclusion criteria

Adult patients with nosocomial infections not caused by *Pseudomonas aeruginosa* were excluded. Patients who refused to consent were also excluded from enrolling in the study; patients who presented with nosocomial infection symptoms and signs on admission or less than 48 hours after admission were equally excluded from the study.

3.6.Sample size estimation

Data on the prevalence of hospital-acquired infections in Zambian hospitals were scarce (Gosling *et al.,* 2013). Considering the estimated prevalence of 14 % (Raoofi *et al.,* 2023; Pachori *et al.,* 2019; Irek *et al.,* 2018) positivity to *Pseudomonas aeruginosa* among screened nosocomial infection patients, the sample size was calculated taking into account the following:

$n= Z^2xp(1-p)/e^2$

80% power

95% Confidence level

$e^2=0.0025$ Allowable error

Z (1-a/2) = 1.96 (1.96 for a 2-sided test at the 0.05 level)

Based on the above assumptions, the estimated sample size (n) (*Pseudomonas aeruginosa*) was 116 patients.

Considering the estimated 14% positivity rate of isolating *Pseudomonas aeruginosa* among nosocomial infection patients (Mekonnen *et al.,* 2021), we projected to screen a maximum of 961 in and out-patients to reach the sample size of n (116) *Pseudomonas aeruginosa*-infected patients. Further, considering the capacities of the two hospitals included in the study, we set to screen two-thirds of the patients at UTH and one-third at NTH.

3.7.Sampling

In this study, we applied consecutive sampling. We included patients who met the inclusion criteria as they attended the two hospitals (UTH and NTH) from April 2020 to April 2021. All consecutive (until meeting sample size) *Pseudomonas aeruginosa* infected patients meeting the eligibility criteria were identified and enrolled after informed consent. Potential bias related to convenient sampling such as lack of representativeness of certain subgroups were mitigated by avoiding generalizing the findings to the broader target population.

3.8.Data collection tools

The questionnaire was set to collect bio-data (such as age and gender), socio-demographic variables, and potential risk factors associated with nosocomial infections and antimicrobial resistance. Clinical outcomes data such as mortality and complications were also collected.

3.9.Clinical and molecular evaluation of nosocomial infections

3.9.1. Patient recruitment and data collection

Patients were recruited from the Out-Patient Department (OPD) (patients with a history of hospital contact within 30 days) and in-patients in medical and surgical wards. Participants were enrolled from consecutive patients aged 18 years and older after obtaining their consent or that of their proxy and screened for nosocomial infections. Eligible patients were de-identified using an allocated study number with records containing patients' identifiers locked and available only to the principal investigator. This was to ensure that the confidentiality of

patients' rule was adhered to. Nosocomial infections were defined according to the World Health Organization case definitions (Suetens *et al.,* 2018; Storr *et al.,* 2017).

Each participant's demographic variable information was collected by face-to-face interview using a structured questionnaire (Appendix 3). Questionnaire data was administered to enrolled participants using the Epi Collect Five electronic tools (https://five.epicollect.net/) (Gohil *et al.,* 2020). Additional data, such as collateral history and other clinical details, was obtained from attending internists and surgeons. Face-to-face interviews (Slade *et al.,* 2022) were used to collect information on each patient's socio-demographic variables and potential risk factors of NI. Clinical data on chronic diseases, hospitalization, admission ward type, and previous antimicrobial-taking history were collected by reviewing the patient's medical record and consulting the attending physician and surgeon. Clinical specimens such as urine, respiratory secretions, and wound swabs were collected as soon as NI was suspected.

Socio-demographic and background data on previous hospital contacts were documented by taking a complete medical history with an additional collateral history obtained from the patient's next of kin. The history of hospital contacts was used to classify infections into community and hospital-acquired infections (Irek *et al.,* 2018). Nosocomial infection also includes an infection acquired in the hospital by a patient admitted for a reason other than that infection and infections acquired in the hospital but appearing after discharge (Baggs *et al.,* 2018). The collected data included demographic characteristics, comorbidities, types of infections, infection acquisition site, signs and symptoms, laboratory and microbiology test results, and discharge and outcome details, including information on mortality. Information on drugs and other treatments the patient has been taking, such as corticosteroids and antibiotic therapy, was also documented. Risk factors such as anatomical urinary tract modification and obstructive uropathy were reported. Additionally, the presence of indwelling urinary catheters, urinary retention, obstructive uropathy, neurogenic bladder, renal dysfunction caused by

intrinsic renal disease (eGFR < 60 mL/min), and urinary tract and renal transplantation were documented.

Further, the presence of chronic respiratory conditions such as chronic obstructive respiratory diseases (COPD), lung malignancy, and cystic fibrosis was documented. Eligible patients admitted to the medical, surgical, and Intensive Care Unit (ICU) wards were carefully screened for nosocomial infection (NI). Patients with purulent drainage, pain, localized swelling, redness, or heat in the skin, subcutaneous tissue, deep soft tissue, organ or spaces, and positive culture for *Pseudomonas aeruginosa* after 48 h of admission or operation were considered as nosocomial surgical site infection.

Medical records and discussions with the attending physician or surgeon were also conducted to obtain additional information on comorbidities, hospitalization history, surgical and medical procedures, and antibiotics. Patients who had either fever (> 38 °C), urgency, frequency, dysuria, or suprapubic tenderness with no other recognized cause but had positive urine culture after 48 h of admission or catheterization were considered nosocomial urinary tract infections. On the other hand, patients with fever (> 38 °C), chills, hypotension, and respiratory symptoms with positive sputum or bronchial secretions culture for *Pseudomonas aeruginosa* after 48 h of admission or intubation were considered as nosocomial respiratory infection.

The study physician conducted a file review and further clinical evaluation to identify and document the admitting complaints or diagnosis before hospital admission, and a physical examination was conducted. After clinical assessment, laboratory tests can help determine the diagnosis and monitor nosocomial infection over time.

During data collection, the following variables were included for analysis: comorbidities such as the presence of neutropenia (absolute granulocyte count of <500/mL), the use of immunosuppressive therapy (chemotherapy, radiotherapy and immunosuppressive drugs

during the bacteremia presentation), and benign prostate enlargement (BPH) were documented. Nosocomial infection complications and all causes of mortality were recorded.

Medical records were reviewed for all enrolled patients to collect other relevant clinical and therapeutic data. After this clinical evaluation, baseline characteristics of patients with *Pseudomonas aeruginosa* infections were documented from history taking, physical examination, and medical records. *Pseudomonas aeruginosa* infection was recorded according to the patient's clinical characteristics such as sites of infections, patient's location in the hospital [Out-Patients Department (OPD), in-patient medical wards, renal units, ICU, burns units, surgical wards], presence of medical devices such as a urinary catheter, endotracheal intubations, and central lines. Local and invasive *Pseudomonas aeruginosa* infections determined the severity of infection. Clinical specimens were collected and processed for pathogen identification and molecular analysis.

3.9.2. Specimen Collection

After labeling sterile containers, clinical specimens were collected using the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2022). Clinical specimens (sputum, urine. and swabs) were compiled by the study team as soon as NI was reported. Hospital and wards of origin, date and time of collection, specimen type, and tests performed were carefully noted. All specimens were placed in tightly sealed, leak-proof containers, transported in sealable, leak-proof plastic bags in an insulated transport bag containing ice packs, and delivered to the laboratory within 30 minutes to 2 hours of collection for processing. Collected specimens were processed for pathogen identification and molecular analysis.

Wound sample collection and processing

Skin swabs were collected from patients with septic burns, pressure, or other wounds and at intravenous injection sites for those in a dialysis unit. Using Levine's technique, wound/pus

specimens were collected aseptically by sterile cotton swabs dipped in normal saline. This technique has been reported to detect more organisms in acute and chronic wounds than other techniques (Song et al., 2021). After labeling sterile containers, samples were collected by placing the swab into the sterile test tubes with 0.5 ml of the clean standard saline solution according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI et al., 2022). Collected clinical specimens were then transported to the University of Zambia (UNZA) (UTH specimens) or the Tropical Diseases Research Centre (TDRC) (NTH specimens) microbiology laboratory within 30 minutes.

At the laboratory, samples were inoculated onto blood agar (Oxoid Ltd, Basingstoke, UK) and MacConkey (Oxoid Ltd, Basingstoke, UK) and incubated aerobically at 37°C for 24 hours. Presumptive identification of Pseudomonas was made using biochemical tests. Non-lactose fermenting colonies from MacConkey plates were sub-cultured onto nutrient agar (Oxoid Ltd, Basingstoke, UK) and subjected to conventional biochemical tests: Oxidase, Simon's citrate, Urease, Sulphur Indole Motility testing (SIM), Lysine Iron Agar (LIA), Triple Sugar Iron (TSI) and Gram staining. The large, flat, dark greenish colonies from blood agar (after sub-culturing on nutrient agar) were also Gram-stained and tested for catalase and coagulase production. The isolates were further sub-cultured onto two nutrient agar (Oxoid) plates and incubated separately at 37°C for pigment production and growth at 42°C (CLSI, 2022).

Urine sample collection and processing

Non-catheterized patients with suspected urinary tract infections were instructed to collect 10 ml midstream clean-catch urine samples using a wide-mouth sterile container. For catheterized patients, the catheter was clamped below the port to allow for urine to collect in the tubing. The catheter was disinfected with 70% alcohol, and 10 ml of freshly voided urine was aseptically collected through the port using a syringe. While changing catheters, the medical

device tips were put in normal saline and taken for cultures. The urine sample was transferred to a sterile container and immediately transported to the UNZA or TDRC microbiology laboratory with collected medical device tips.

At the laboratory, urine was inoculated on blood and MacConkey agar plates using the calibrated loop that measures about 1µL. All inoculated agar plates were incubated aerobically at 35–37°C for 24–48 hrs. and inspected daily for bacterial growth. Colonies on blood agar were counted using a colony counter and checked for significant bacteriuria. Cultures from catheterized and non-catheterized patients that grew $\geq 10^2$ CFU/ml and 10^5 CFU/ ml were taken as significant bacteriuria and processed further (Armbruster et al., 2021).

For culture tips, a semiquantitative roll-plate technique was performed by transferring each catheter tip onto a plate with blood agar and rolling the tip back and forth across the surface at least 3 to 4 times, as previously described by Maki (Guembe *et al.,* 2012).

Respiratory sample collection and processing

Sputum samples were collected from patients with chest symptoms or a medical device such as an inserted endotracheal tube. Hospital and wards of origin, date and time of collection, specimen type, and tests performed were carefully noted. All specimens were placed in tightly sealed, leak-proof containers, transported in sealable, leak-proof plastic bags, and delivered to the laboratory within 30 min to 2 hours of collection for processing. The specimens were immediately transported to the Microbiology Laboratory at the University of Zambia, School of Veterinary Medicine in Lusaka, or the Tropical Diseases Research Centre Laboratory in Ndola for immediate processing. Samples were inoculated onto blood agar (Oxoid Ltd, Basingstoke, UK) and MacConkey (Oxoid Ltd, Basingstoke, UK) and incubated aerobically at

37°C for 24 hours and further processed. Phenotypic identification of Pseudomonas isolates was made as described above for the isolates from wounds to make a presumptive diagnosis.

3.9.3. Bacterial culturing and identification

Samples were inoculated onto blood agar (Oxoid Ltd, Basingstoke, UK) and MacConkey (Oxoid Ltd, Basingstoke, UK) and incubated aerobically at 37°C for 24 hours. Non-lactose fermenting colonies from MacConkey plates were sub-cultured onto nutrient agar (Oxoid Ltd, Basingstoke, UK) and subjected to conventional biochemical tests: Oxidase, Simon's citrate, Urease, Sulphur Indole Motility testing (SIM), Lysine Iron Agar (LIA), Triple Sugar Iron (TSI) and Gram staining. The large, flat, dark greenish colonies from blood agar (after sub-culturing on nutrient agar) were also Gram-stained and tested for catalase and coagulase production. The isolates were sub-cultured onto two nutrient agar (Oxoid) plates and incubated separately at 37°C for pigment production and growth at 42°C. *Pseudomonas aeruginosa* (ATCC 27853) standard strain was used as a reference strain.

3.9.4. Molecular detection of bacterial pathogens

In addition to the bacteriological analysis, molecular analysis was also conducted as another means of pathogen identification. DNA was extracted from purified presumptive positive bacterial cultures using commercial DNA extraction kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) amplification was used to amplify 16S rRNA gene fragments from purified genomic DNA of culture isolates. The PCR master mix contained 1 x PCR buffer [50 mM KCl, 1.5mM $MgCl_2$, 10mM Tris/HCl (pH9.0), ten µg gelatin ml^{1}], 200µM dNTPs, 0.3µM each of the 16S rRNA primers, 1.5 U *Taq* polymerase (Gibco BRL) and approximately 10-100ng DNA in a final reaction volume of 100µl. Polymerase chain reaction based on Extaq protocol according to the manufacturer's instructions (Takara Biotechnology (Dalian) Co., Ltd.) was used for species identification

using specific primers targeting the 16sRNA gene as previously prescribed (Sambo et al., 2018). The following thermal cycling conditions were used: 98˚C for 10s, 35 cycles of 98˚C for 30s, 54˚C for 30s, and 72˚C for 1min and final extension at 72˚C for 5min. The positive amplicons were purified using Wizard® SV Gel and Clean-Up System (Promega, Madison, WI, USA) according to the manufacturer's protocol. The purified DNA was later sequenced directly using a Big Dye terminator cycle sequencing ready reaction kit v3.1 and analyzed on a 3500 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

3.9.5. Antimicrobial Susceptibility Testing of *Pseudomonas aeruginosa*

The susceptibility of clinical isolates to different antibiotics was determined by Kirby-Bauer's disk diffusion agar method on cation-adjusted Mueller–Hinton agar (Merck, Darmstadt, Germany) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2022). Antibiotic disks (MAST Diagnostics, Merseyside, UK) tested were Ceftazidime (CAZ, 30 µg), piperacillin/tazobactam (PTZ, 100 µg/10 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GM, 10 µg), amikacin (AK, 30 µg), tobramycin (TOB, 10 µg), imipenem (IMI, 10 µg), aztreonam (30ug) and cefepime (30ug). The turbidity standard of a 0.5 McFarland (1.5×108 CFU/mL) of *Pseudomonas aeruginosa* was prepared and spread on Mueller-Hinton Agar as a lawn culture. *Pseudomonas aeruginosa* (ATCC 27853) standard strain was used for the quality control of antimicrobial susceptibility testing disks. *Pseudomonas aeruginosa* is intrinsically resistant to ceftriaxone and cefotaxime; therefore, clinical isolates AST to third-generation cephalosporin were based on susceptibility to Ceftazidime.

Molecular detection of Carbapenem-resistant Pseudomonas aeruginosa

Pseudomonas aeruginosa isolates were identified using conventional microbiological tests. The susceptibility of isolates to antibiotics was assessed using a disk diffusion test. The

imipenem/EDTA (IMP-EDTA) combination disk phenotypic was performed to detect MBL-producing strains. Then, a polymerase chain reaction (PCR) was performed to detect MBL genes, *bla*IMP, and an imipenem-resistant gene. The following primers were used in this study to amplify various genes:

Table 2. 16S rDNA-Based PCR primer set for identification of *Pseudomonas aeruginosa* (Adejobi *et al.,* 2021; Spilker *et al.,* 2004)

Primer	Sequence (5'- 3')	Target	Annealing temp (°C)	Location [a]	Product size (bp)
PA-SS-F	GGGGGATCTTCGGACCTCA	*Pseudomonas aeruginosa*	58	189–206	956
PA-SS-R	TCCTTAGAGTGCCCACCCG	*Pseudomonas aeruginosa*		1124–1144	
PA-GS-F	GACGGGTGAGTAATGCCTA	*Pseudomonas Species*	54	95-113	618
PA-GS-R	CACTGGTGTTCCTTCCTATA	*Pseudomonas Species*		693-712	

[a] Position and size relative to 16S rDNA sequence of *Pseudomonas aeruginosa* AT2 (AB091760).

Table 3. Primers for the detection of carbapenemase genes (blaIMP and blaVIM) in *Pseudomonas aeruginosa.*

Primer	Sequence (5′ → 3 ′)	Gene	Product length (bp)
***Bla*IMP**	GTTTGAAGAAGTTAACGGGTGG (F)	blaIMP	459
	ATAATTTGGCGGACTTTGGC (R)		
***Bla*VIM**	TGGTGTTTGGTCGCATATCG (F)	blaVIM	595
	GAGCAAGTCTAGACCGCCCG (R)		

Table 4. Primers for the detection of CEPHALOSPORINASE amp C resistance genes in *Pseudomonas aeruginosa.*

Primer	Sequence (5′ → 3 ′)	Gene	Product length (bp)
ampC	CGGCTCGGTGAGCAAGACCTTC (F)	ampC	218
	AGTCGCGGATCTGTGCCTGGTC (R)		

Table 5. This study evaluated primers for detecting Oxacillinase resistance genes in Pseudomonas aeruginosa isolates.

Target Gene	Primer Sequence (50–3)	Amplicon Size (pb)	Gene Product
blaOxa23	GATCGGATTGGAGAACCAGA (F)	501	Oxacilinase
	ATTTCTGACCGCATTTCCAT (R)		
blaOxa24	GGTTAGTTGGCCCCCTTAAA (F)	246	Oxacilinase
	AGTTGAGCGAAAAGGGGATT (R)		
blaOxa51	TAATGCTTTGATCGGCCTTG (F)	353	Oxacilinase
	TGGATTGCACTTCATCTTGG (R)		
blaOxa58	AAGTATTGGGGCTTGTGCTG (F)	599	Oxacilinase
	CCCCTCTGCGCTCTACATAC (R)		

3.9.6. Genomic DNA extraction using the QIAGEN kit

According to the manufacturer's protocol, genomic DNA was extracted from purified colonies using the QIAGEN DNA extraction kit (Hilden, Germany). Briefly, a loopful of culture colonies was suspended in 200µl phosphate buffer saline (PBS). Twenty microliters (20uL) of proteinase K were added to a 1.5 ml microcentrifuge tube, followed by a 200 µl Buffer AL. This was mixed thoroughly by vortexing and incubating at 56°C for 15 minutes. Two hundred microliters (200 µl) of 96 percent ethanol was added to the tube and mixed by vortexing. The mixture was pipetted into a DNeasy MiniSpin column, placed in 2ml collection tubes, and centrifuged at 6,000*xg* for one minute. The tube containing the filtrate was discarded, and the

mini spin column was placed in a new 2ml collection tube, to which 500µl Buffer AW1 was added.

The mixture was centrifuged at 6,000*xg* for one minute, and the filtrate tube was discarded. Five hundred microliter (500µl) Buffer AW2 was added to the Mini spin column and centrifuged at 20,000*xg*. The Mini spin column was placed in a new 2ml collection tube, the old collection tube was discarded, and one dry spin was run. After centrifuging at full speed for one minute, the Mini spin column was placed in a clean 1.5ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. 60µl Buffer AE was added to the Mini spin column and incubated at room temperature for one minute. After that, it was centrifuged at 6,000*xg* for one minute. The extracted DNA was stored at -80°C until further use. DNA quantity and purity were determined using a NanoDrop™ 1000 spectrophotometer (Thermo Fischer Scientific, Wilmington, USA).

3.9.7. PCR amplification of 16S rRNA genes

Identification of *Pseudomonas* spp. and *Pseudomonas aeruginosa* by PCR using Sanger sequencing

PCR was used to confirm the identities of the isolates. A 25 µL PCR mixture (12.5 µL one *Taq* Quick-Load 2X master mix with standard buffer, 0.5 µL of 10 µM each of forward primer and reverse primer, 3 µL template DNA, and 8.5 µL of nuclease-free water) was set up to amplify the genes of *Pseudomonas* spp. and *Pseudomonas aeruginosa* using the primers PAGS 618 bp (F: GGGGGATCTTCGGACCTCA, R: TCCTTAGAGTGCCCACCCG) and PASS 956 bp (F: GGGGGATCTTCGGACCTCA, R: TCCTTAGAGTGCCCACCCG) respectively.[12] PCR conditions were observed according to Ghosh et al.[13] Each amplicon (10 µL) was electrophoresed on a 1.5% agarose gel pre-stained with 0.5 µg/mL ethidium bromide

in 1X Tris-Acetate-EDTA buffer and viewed with a transilluminator (Avebury, UK). The positions of the PCR products were determined by the positions of the 100 bp molecular weight marker (Biolabs, UK).

PCR was done for all 138 *Pseudomonas* isolates and reference strain ATCC 27853. The *Pseudomonas aeruginosa* 16S rRNA gene fragment was amplified using the Ex-Taq HS PCR kit (Takara Bio USA, Inc.) and universal primers pairs P3mod-F (5'-ATT AGA TAC CCT GGT AGT CC-3') forward primer and P5-R (5'-GGT TAC CTT GTT ACG ACT TC-3') reverse primer designed by Tsen *et al.* (1998). According to the manufacturer's instructions, this was done using the Veriti 96 well Thermocycler AB (Applied Biosystems, Grand Island, NY).

Briefly, 2µl of DNA template was added to a final reaction volume of 20µl consisting of 2µl 10X Buffer, 1.6µl of dNTPs, 0.8 µl of each primer, 0.1 µl of Ex-Taq HS and 13.7µl of Nuclease free water. The PCR mixture contained positive (DNA isolated from the S19 vaccine) and negative controls (nuclease-free water). The PCR conditions were as follows: initial denaturation at 95ºC for five minutes, followed by thirty-five cycles of denaturation at 95ºC for thirty seconds; annealing at 54ºC for ninety seconds, extension at 72ºC for ninety seconds and a final extension at 72ºC for ten minutes. The PCR products were visualized on 1.5 percent agarose gel stained with ethidium bromide after electrophoresis at 100 volts for thirty minutes. This was done according to the manufacturer's instructions and as described by Unver *et al.* (2006). The amplicon's size was assessed based on the comigration of a standard DNA ladder of molecular weight in the 100-1000 bp range for the amplification of 16S rRNA.

3.9.8. 16S rRNA gene sequencing

DNA fragments for sequencing were prepared from PCR-positive samples using the QIA quick Gel Extraction Kit (Qiagen Inc. Valencia, CA, USA) according to the manufacturers' recommendations. The PCR products were first purified using the Wizard ® SV Gel and PCR Clean-Up System according to the manufacturer's instructions. Sequencing was carried out using the Brilliant Dye Terminator v3.1 cycle sequencing kit (Edge Biosystem™). The sequencing reactions had a final volume of 20µl: This consisted of 1µl of PCR product, 1µl BrilliantDye Terminator (Edge Biosystems™), 3.5µl of 5X~sequencing buffer (Applied Biosystems™, Foster City, CA, USA), 1µl of the appropriate sequencing primer and 13.5µl of deionized water. Sequencing primers were universal primer pairs P3mod-F (5'-ATT AGA TAC CCT GGT AGT CC-3') forward primer and P5-R (5'-GGT TAC CTT GTT ACG ACT TC-3') reverse primer. Sequencing was done in duplicate for each primer. The cycle conditions were 96°C for 45 seconds, followed by 25 cycles of 96°C for 10 seconds, 50°C for five seconds and 60°C for two minutes. Following the cycle-sequencing protocol, the reaction was purified using ethanol precipitation.

In the ethanol precipitation procedure, 2µl of 3M Sodium acetate (NaAOc) (Sigma-Aldrich) (pH 4.6), 2µl of 125mM EDTA, and 90µl of 100 percent ethanol were added to each reaction tube containing the sequencing products. After vortexing, the samples were centrifuged at 15,000 rpm for 20 minutes. The supernatant was aspirated, and 200µl of 70% ethanol was added. The samples were centrifuged at 15,000 rpm for five minutes. The supernatant was aspirated, and 200µl of 70% ethanol was added. The samples were centrifuged at 15,000 rpm for five minutes and vacuum-dried for 10 minutes. The tubes were covered with aluminum foil before adding 20µl of HIDI formamide and vortexing for 15 seconds. Denaturing was done at 95°C for two minutes using a heating block. The precipitated product was loaded in the

sequencer called Genetic Analyzer 3500 (Applied Biosystems 3,500 series genetic analyzer (Thermo Fisher Scientific). The results were analyzed using ATGC software, and the nucleotide sequences obtained were blasted on PubMed blast searched at http:// www.ncbi.nlm.nih.gov/BLAST.

3.10. Phylogenetic analysis

Evolution analysis was performed using gene nucleotide sequences for *Pseudomonas aeruginosa* 16S rRNA genes. The evolutionary tree was drawn based on the genetic sequences of the classification of *Pseudomonas aeruginosa*, compared to the analysis of the 16S rRNA genes. The phylogenetic tree was obtained by blasted sequences and alignment with objective sequences found in NCBI, and phylogenetic analysis of the 16SrRNA gene sequences was drawn in the MEGA6 program (Sato *et al.,* 2017). The nucleotide sequences were aligned using the CLUSTAL software (Chenna *et al.,* 2003). The UPGMA analysis method was used to reconstruct phylogenetic trees (Onodera *et al.,* 2023). Sequence analysis of 16S rRNA accurately identifies unknown bacteria to the genus level. *Pseudomonas aeruginosa* clinical isolates phylogenetic analysis was performed, and individual phylogenetic trees and concatenated analyses of the sequenced gene fragments were constructed. The allelic and nucleotide diversities were calculated from the gene sequences using the DnaSP package to establish genetic diversity (Gomila *et al.,* 2013).

3.11. Clinical outcome

Patients were clinically assessed as described in the clinical assessment and diagnosis previously described. Patient response to treatment was longitudinally recorded. The outcome was recorded for hospital stay duration, complications, morbidity, and mortality.

The outcome was analyzed in association with *Pseudomonas aeruginosa* phenotypes and genotypes. MDR was defined by the international expert proposals (Rafailidis *et al.,* 2022;

Magiorakos *et al.,* 2012) as non-susceptibility to at least one agent in three or more antimicrobial categories (extended-spectrum penicillin, carbapenems, cephalosporins, aminoglycosides and fluoroquinolones) (Rafailidis *et al.,* 2022; Magiorakos *et al.,* 2012). Extensive drug resistance (XDR) was considered non-susceptible to at least one agent in all but two or fewer antimicrobial categories.

Outcomes were classified as follows (Gomila *et al,* 2019): the primary outcome was all causes of mortality. Early mortality was defined as patients who die within the first five days of treatment, while late mortality was defined as death from any cause occurring within 30 days after the onset of *Pseudomonas aeruginosa* infections (the 30-day mortality in the multidrug resistance *Pseudomonas aeruginosa* group).

Secondary outcomes included several variables such as; "Prolonged overall length of hospital stay," "improvement of symptoms at 5–7 days of treatment", "recurrence of symptoms at 30 days", and "readmission within 60 days from discharge". The clinical and molecular factors predicting 30-day mortality among patients with MDR *Pseudomonas aeruginosa* infections were also identified.

3.12. Analytical plan

3.12.1. Data management and storage

The study questionnaire captured demographic and other background data about each research participant. Clinical details were extracted from the patient's case file and direct interviews with the patient. This information was transferred to the data extraction sheet using Epicollect5 (https://five.epicollect.net/). Projects were created using the web application five.epicollect.net (Gohil *et al.,* 2020). Forms (questionnaires) were generated using web and mobile applications for data collection at UTH and NTH. Data were collected (including GPS and media) using multiple devices, and all data was viewed on a central server under the supervision of the principal investigator. Patients enrolled were identified using an allocated study number with

records containing patients' identifiers secured in a locked cabinet accessible to only the principal investigator. The anonymized data extracted from the data collection tool was then transferred onto an Excel sheet on a password-protected central computer. Data was stored on a password-protected cloud and computer for data security to ensure confidentiality.

3.12.2. Clinical data

3.12.2.1. Baseline and clinical characteristics

Descriptive statistics and frequency tables were conducted to analyse common phenotypic and genotypic characteristics. The Fisher exact test was used to screen association categorical variables, while the student t-test was used for continuous variables by variables. All analyses were performed at a (5%) significance level.

3.12.2.2. Predictors of nosocomial infection and treatment outcomes

A stepwise logistic regression model was used to assess the relationship between the outcome variables (*Pseudomonas aeruginosa* nosocomial infection and AMR) and independent variables (clinical and molecular characteristics) to determine predictors of nosocomial infection and antimicrobial resistance.

3.12.2.3. Antimicrobial resistance

Multivariate logistic regression analysis was used to analyse the relationship between the dependent variable (antimicrobial susceptibility pattern) and the independent variables (phenotypic and genotypic data of *Pseudomonas aeruginosa*). Odds ratios derived from the regression analysis were then used to measure the strength of the association between the antimicrobial susceptibility pattern and selected study variables.

The following baseline predictors were considered: age, site of infections, *Pseudomonas aeruginosa* phenotypic and molecular characteristics, sex, race, ethnicity, comorbidities, use

of antimicrobials in the preceding month, number of previous UTIs, diagnostic delay at admission, and symptom duration (0 days, 1–2 days, 3–4 days, 5+ days, unknown).

3.12.2.4. Clinical outcome

Logistic regression was used to identify variables independently associated with 30-day mortality. All variables associated with 30-day mortality in the bivariate analysis were included at model entry, and a stepwise approach was used to identify independent predictors of 30-day mortality. Variables were retained in the final model if the P value was ≤ 0.05. All calculations were performed with Stata version 14 software.

The COX regression (proportional hazards regression) analysis was used to analyse the longitudinal outcome study data. This method was used to analyse the association between Pseudomonas aeruginosa clinical, phenotypic, and genotypic factors with treatment outcomes because it is the most commonly used approach to the regression analysis of survival data.

Using the proportional hazards assumption with the following mathematical model:

$$\text{Log}\,(h/(t)) = \log(ho(t)) + \beta 1X1 + \beta 2X2 + \ldots\ldots\ldots\ldots\ldots + \beta pXp$$

In this equation, $h(t)$ was the hazard at time t, $ho(t)$ was the baseline hazard (the hazard for an individual in whom all exposure variables were 0) at time t, and $X1$ to Xp was the p exposure variables. We compared different exposure groups with an assumption that the ratio of hazards remained constant over time (Proportional hazards assumption).

This was done by considering the risk sets of subjects still being followed up each time an event occurred. At the time of event occurrence, the values of the exposure variables for the participants who experienced the event being assessed were compared to those of the exposure variables for all the other subjects still being followed and who did not yet experience the disease event being assessed. In our study, all participants were grouped into constructed risk sets consisting of all subjects followed when events occur, assuming the hazard ratio is the same across risk sets. Data on survival followed a defined event (outcomes) starting at time 0.

For the time axis, we will consider the time since the participant recruitment. The survival group was estimated using the Kaplan-Meier curve.

3.12.3. Laboratory data

3.12.3.1. Bacteriology

The bacteriology results and MIC values for the antimicrobial susceptibility tests were recorded in the Microsoft Excel spreadsheet WHONET 2023 version. WHO (2021) identifies misuse and overuse of antimicrobials as the main drivers in developing drug-resistant pathogens. So, we documented previous exposure to antibiotics as a variable. The clinical isolates were classified according to the sensitivity to antimicrobials for better resistance evaluation. Sensitivity, or lack of it, was documented. Lack of sensitivity to at least one agent in three or more classes of antibiotic (MDR), non-susceptibility to at least one agent in all but one or two antimicrobial categories (XDR), non-susceptibility to all agents in all antimicrobial categories (PDR) (Magiorakos *et al.,* 2012). Risk factors were identified using Odds ratios for each of these four categories.

3.12.3.2. Molecular typing (16SRNA)

The obtained sequences were verified using BLAST analysis on the NCBI website (http:// www.ncbi.nlm.nih.gov/BLAST), followed by assembly and editing using the ATGC plug-in the Genetyx Ver. 12. Multiple sequence alignments of the obtained sequences and the downloaded reference sequences from the Genbank were generated using CLUSTALW1.6. The multiple sequence alignment file was then utilized to generate a phylogenetic tree using MEGA ver. 6 with 1,000 bootstrap replicates as a confidence level. All the sequences generated in this study have been deposited in the NCBI DNA database.

3.12.3.3. Phylogenetic analysis

Individual phylogenetic trees and concatenated analyses of the sequenced gene fragments were constructed (Gomila *et al.,* 2013). The allelic and nucleotide diversities were calculated from the gene sequences using the DnaSP package, version 6 (Rozas *et al,* 2017). The sequence type (ST) and allele assignment were performed at the *Pseudomonas aeruginosa* MLST website (http://pubmlst.org/paeruginosa/).

The nucleotide sequences of each allele determined in this study were sent to the curator for introduction into the *Pseudomonas aeruginosa* Gene bank at NCBI. For phylogenetic analysis**,** a similarity search was conducted using the BLAST software by NCBI (National Center Biotechnology Information, www.ncbi.nml.nih.gov). The tree was generated by neighbour joining using the maximum composite likelihood model (Tamura *et al.,* 2007). The evolutionary distance was calculated with different software programs. Gene distance matrices were calculated from nucleotide sequences by the Jukes-Cantor method. Mann-Whitney's U test was employed for within-group and between-group mean distances, and significance levels were determined. Dendrograms were generated by neighbour-joining (NJ), maximum parsimony (MP), minimum evolution, the unweighted pair group method with arithmetic means, and split decomposition (SD) with bootstrap analysis (1,000 replications) using the Molecular Evolutionary Genetics Analysis (MEGA), computer software for conducting statistical analysis of molecular evolution and for constructing phylogenetic trees. Analyses were done on each gene sequence and the concatenated data set.

3.13. Innovation and Potential Impact of the Study

This study provided insight into *Pseudomonas aeruginosa* nosocomial infections in Zambia. It evaluated the clinical and molecular characteristics of *Pseudomonas aeruginosa* in Zambia's University Teaching Hospital in Lusaka province and Ndola Teaching Hospital in the Copperbelt province. Additionally, the study contributed to a better understanding of local antimicrobial resistance patterns and appropriate recommendations to guide the local antibiotic policy to counter the threat that antimicrobial resistance poses. This study provided results for phenotypic and genotypic resistance patterns of clinical isolates in Zambia.

Additionally, this study was the:

- First comprehensive evaluation of *Pseudomonas aeruginosa* nosocomial infections in Zambia
- First report of the prevalence of resistance genes such as *Bla Oxa 51* in *Pseudomonas aeruginosa* in Zambia in the context of Nosocomial Infections
- First report of the association of resistance genes with *Pseudomonas aeruginosa MDR and XDR phenotypes* in the context of Nosocomial Infections in Zambia

Therefore, this study generated knowledge needed for policy formulation and intervention strategies for Pseudomonas aeruginosa Nosocomial Infections in Zambia.

3.14. Ethical consideration

Ethical clearance was sought from the University of Zambia Biomedical Research Ethic Committee (UNZA REC) [REF. No. 671-2019]. Participants provided informed written consent to collect anonymized clinical and demographic data. The consent form was verbally explained to all participants in their local language. For illiterate participants, a witnessed fingerprint was obtained. All obtained information was confidential and only used for research

or academic purposes. Electronic devices used to collect and store the data were password protected.

The study was also approved by the University Teaching Hospital (UTH), the Lusaka District of Health, the Ndola Teaching Hospital (NTH) management, and the various Department where research was conducted taking into account the Helsinki Declaration (World Medical Association (WMA), 2003).

Several ethical issues were addressed during the study. These had to do with observing patients' privacy and confidentiality. During the interview and examination, well-ventilated, private, and secured rooms with adequate light were used. Patient interviews, physical examinations, and investigation data were kept confidential by researchers and were not disclosed without consent from the participants. Only participants giving informed consent were enrolled in the study. Before enrolment, the study was explained to patients; the benefits and risks of participating were clearly explained to participants in a language they understood.

Information was stored in both soft and hard copies. Soft data was held on a password-protected computer accessible only to the principal investigator. Physical records were kept in lockable cabinets with keys accessible only to the principal investigator.

The processing of patients' data was anonymized in compliance with existing local and international data protection legislation. The patient's identity was coded using a unique study number to ensure confidentiality. The unique identification number was attributed using the initials from the Clinical (C) and Molecular (M) Pseudomonas (P) Study (S). Numbers ranged from CMPS001 to CMPS 861. The key to this code was only known to the principal investigator.

During this study, possible adverse effects were discussed with participants. It was explained to patients that possible psychological distress could arise from the lengthy interview, which lasted close to 30 minutes per patient, to gather adequate disease history. Stress during history

taking was minimized by proper counseling and showing psychological support to the patient and the affected family, stopping the interview in case of undue distress and continuing later.

If the study interview time coincided with ward rounds, investigations, medications, or any procedures, the risk of interrupting clinical care was addressed by postponing our interview. However, in this situation, it was made clear that clinical care took precedence, and the interview was to be deferred.

It was also explained to the patient that there was minimal risk of pain and possible minor bleeding from phlebotomy sites during the collection of clinical samples, but this was minimized by using proper technique and qualified personnel. The explanation was given to patients that such investigations were part of routine clinical investigations for clinicians to diagnose patients with nosocomial infections.

There were neither monetary benefits nor preferential clinical treatment for patients enrolled in the study at the expense of patients declining to participate. The only indirect benefit was that, after examining and collecting patient culture samples, the study's findings were availed to attending physicians to manage the participants efficiently as a direct benefit of the study.

Furthermore, it was hoped that, among other benefits, this study may provide a greater understanding of the factors associated with nosocomial infections caused by *Pseudomonas aeruginosa* and the predictors of antimicrobial resistance in patients attending UTH and NTH.

Participants answered questions freely and were not compelled to answer questions they were uncomfortable with forcefully.

CHAPTER FOUR

RESULTS

4.1.**Participant's enrolment per study sites**

Eight hundred and forty-one patients were screened: 640 from the University Teaching Hospital (UTH) and 201 from the Ndola Teaching Hospital (NTH). Table 6 displays the distribution of patients according to the various wards.

Table 6. Nosocomial infection types and specimens collected from UTH and NTH

Study Sites	**Wards**	**Specimen types**				**Total**
		UTI (MSU)	*Infected wound (Swab)*	*Pneumonia (Sputum)*	*Infected Medical devices*	
UTH (76%)	Medical OPD	22	18	7	1	48
	Medical IPD	45	85	21	7	158
	Surgical OPD	176	48	10	78	312
	Surgical IPD	25	79	4	14	122
NTH (23.9%)	Medical OPD	9	23	3	18	53
	Medical IPD	26	28	20	1	75
	Surgical IPD	10	23	2	9	44
	Surgical OPD	1	22	2	4	29
Total		314	326	69	132	841

UTH=University Teaching Hospital, NTH=Ndola Teaching Hospital, MSU=Midstream Urine, OPD=Out-Patient Department, IPD=In-Patient Department

4.2.Socio-demographic characteristics of participants

In this study, the prevalence of *Pseudomonas aeruginosa* nosocomial infection was 13.7%. The majority of participants were male (81.2%). Study participants ranged from 15 to 98 years, with a mean age of 51 (SD ± 18). There were more males than females in the sampled population; the majority of participants (71%) were married, only 75 screened patients (9%) completed tertiary and 150 (18%) secondary education, while 563 achieved at least primary

education. Around 6% (53) of participants reported no formal education. Only 10% (85) of participants reported being in formal employment, while the majority (75%) were either in the informal sector or unemployed. The most frequently collected specimen types and isolated pathogens per ward are highlighted in Table 7

Table 7. Mapping of isolated nosocomial infection Pathogens per wards at UTH & NTH

Study Sites	Department	Bacterial Specimen Identified					Total
		P.aeruginosa	*E. coli*	*Proteus spp.*	*K. pneumonia*	Others	
UTH (640)	Medical OPD	15	28	12	3	10	68
	Medical IPD	25	28	14	18	29	114
	Surgical OPD	41	78	29	22	39	209
	Surgical IPD	25	29	10	6	9	79
NTH (201)	Medical OPD	0	18	8	8	2	36
	Medical IPD	4	14	7	8	10	43
	Surgical IPD	4	2	3	7	5	21
	Surgical OPD	2	2	4	7	3	18
Total (841)		116 (13.7%)	199 (17.8%)	87 (5.6%)	79 (5.5%)	107 (11.4%)	588 (69.9%)

UTH (University Teaching Hospital), NTH (Ndola Teaching Hospital), OPD (Out-patient department), IPD (In-patient department). Med (Medical wards) and Sgy (Surgical wards)

4.3.Clinical features and Risk factors of nosocomial infections at UTH and NTH

Invasive medical device-related nosocomial infection was expected, as most participants (78.5%) had one inserted. The urinary catheter was the most commonly inserted invasive medical device in most screened patients. Hence, Catheter-Associated Urinary Tract Infection (CAUTI) was the most common NI type found (57%). Among surgical patients, a urinary catheter was frequently inserted in patients being managed for benign prostate enlargement (BPH).

The other common diagnosis was infected pressure sores (38.7%). These were common in patients being managed for paralysis, such as stroke and traumatic spinal cord injuries, and chronically ill bedridden patients. Table 8 highlights the factors associated with nosocomial infections. These were medical device insertion ($p=0.0000$), age ≥ 65 years ($p=0.0003$), male gender ($p=0.000001$), prior admission and prolonged hospital admission ($p=0.0000$), co-morbidities included hypertension (8%) and diabetes mellitus (4%).

Nine (N=9) participants were psychiatric patients presenting with infected wounds. More than half (62%) of participants reported a history of previous hospital exposure within the past 30 days, which were prior medical visits or being ex-bed-siders taking care of patients.

Table 8. Analysis of the association between clinical characteristics, risk factors, and positive culture

Age category			
≥ *65 years*	174	1.94 (1.34-2.81)	0.0003440*
≤ *65 years*	433		
Gender			
Male	599	6.10 (1.45-9.01)	<0.0000001*
Female	85		
Department			
Medical	261	1.10 (0.80-1.51)	0.539
Surgery	330		
Ward			
OPD	355	1.95 (1.43-2.66)	<0.0001*
IPD	248		
Cough	40	0.43 (0 .26-0.72)	0.0009*
No cough	36		
Dysuria	116	1.56 (1.02-2.44)	0.0384*
No dysuria	34		
Fever	430	8.43 (5.92-2.06)	0.0000*
No fever	61		
Pyuria	54	1.58 (0.85-3.08)	0.1330
No pyuria	15		
Wound			0.0005*
Positive	346	0.86 (0.62- 2.12)	
Negative	156		
Suprapubic Pain			
Positive	52	0.55 (0.34-0.90)	0.0139*
Negative	37		
Medical device ≥ 48			
hrs.	462	2.06 (1.47- 2.88)	0.0000*
Positive	160		
Negative			
Specimen (MSU)			
Positive	228	1.20 (0.87-1.67)	0.2427
Negative	86		
Specimen (sputum)			
Positive	32	0.50 (0.28-0.89)	0.0129*
Negative	27		
Specimen (swab)			
Positive	289	0.79 (0.58-1.08)	0.1513
Negative	137		
BPH			
Positive	82	1.76 (1.05-3.08)	0.0285*
Negative	21		
Prolonged admission			
Positive	568	24.19 (14.27-	0.0000*
Negative	135	42.39)	
Low systolic BP			
Positive	568	24.19(14.27-	0.0000*
Negative	135	42.39)	

BPH=Benign prostate enlargement, BP=Blood pressure, MSU=Mid stream Urine, OPD=Out-patient department, IPD=In-patient department (*=statistically significant)

4.4.Clinical specimens and bacteriological profiles

Out of 841 screened patients, *Pseudomonas aeruginosa* was isolated in 116 participants, giving a prevalence of 13.7%. The most frequently collected specimen types were skin swabs (38.7%) and midstream urine (37.3%). Of the 326 swabs, 103 were collected from the UTH medical department, 127 in UTH surgical wards while 51 were from the NTH medical department and 45 from the NTH surgical department.

The majority (69.9%) of clinical specimens had positive bacterial cultures. More pathogens were isolated from surgical (55.6%) compared to medical wards; returning OPD patients had relatively more pathogens isolated (56.2%) compared to admitted patients. The most frequently observed pathogens included *Escherichia coli* (17.8%), *Pseudomonas aeruginosa* (13.7%), *Klebsiella pneumonia* (5.6%) and *Proteus vulgaris* (5.5%).

4.5.Molecular identification of clinical isolates of *Pseudomonas aeruginosa*

PCR identification using *Pseudomonas aeruginosa* genus specific (PA-GS) and species specific (PA-SS) Primers (Adejobi *et al.,* 2021; Choi *et al.,* 2013; Spilker *et al.,* 2004).

Hundred and twelve (96.5%) clinical isolates were PCR positive for the *PA-SS* gene out of 116 tested, while 86 (74.1%) clinical isolates were positive for the *PA-GS* gene on PCR (Adejobi *et al.,* 2021; Choi *et al.,* 2013; Spilker *et al.,* 2004).

Figure 7 shows the PCR amplicons of *Pseudomonas aeruginosa on gel electrophoresis laboratory methods***.** PASS gene was positive for the clinical isolates 1-23, 26, 28-37, 39-48, 49-63, 65-72, 73-96, 97-115. PASS gene was negative in the clinical isolates-24, 27, 38, and 64. The negative control is represented in well *25*, while the positive control (*Pseudomonas aeruginosa* ATCC27853) is represented in well 116.

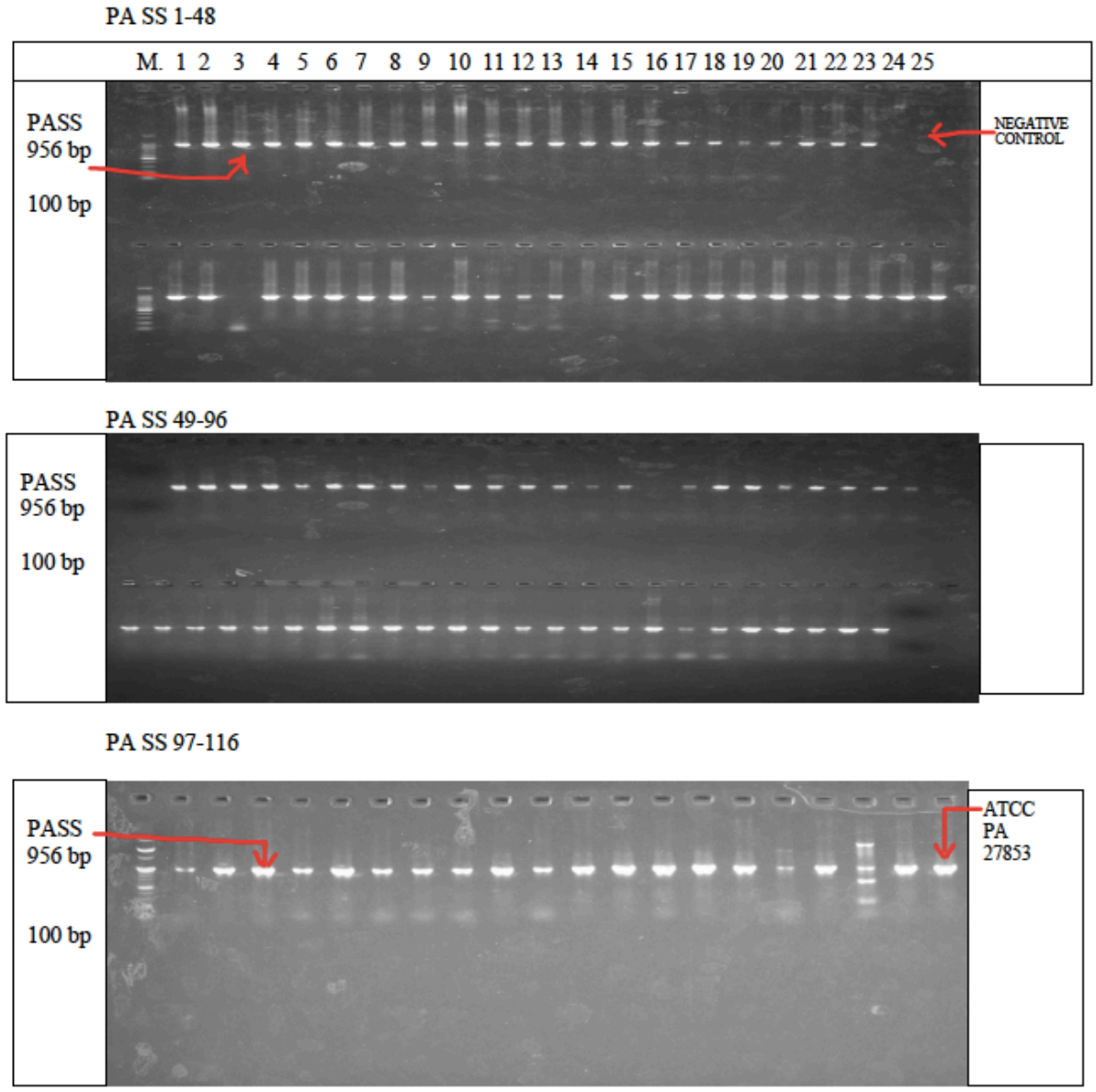


Figure 7:
Electrophoretogram showing amplicons of *Pseudomonas aeruginosa* specific primers *(*PA-SS). Lane 1-23, 26, 28-37, 39-48, 49-63, 65-72, 73-96, 97-115 positive for PA-SS. PA-SS negative isolates- 24, 27, 38, and 64. Ladder (Lane M): 100 bp molecular maker, P: positive control, N: negative control, 25. Positive control *Pseudomonas aeruginosa* ATCC27853 (116) (New England Biolabs Inc.).

16S rDNA sequencing

The 16S rRNA gene of the nine samples out of the total 116 clinical isolates' extracted DNA was successfully sequenced, and a *blast* n search gave a nucleotide identity ranging between 90 and 97 percent, and the sequences were identified as belonging to *Pseudomonas aeruginosa*. The following *Pseudomonas aeruginosa* strains were identified after 16S rRNA sequencing:

1. KF031123.1 182-1032
2. SeqID2
3. >MZ618953.1:201-1052 *Pseudomonas aeruginosa* strain MDM1 16S ribosomal RNA gene, partial sequence
4. >MG966347.1:219-1070 *Pseudomonas aeruginosa* strain P2 16S ribosomal RNA gene, partial sequence
5. >KF031123.1:182-1032 *Pseudomonas aeruginosa* strain SN3 16S ribosomal RNA gene, partial sequence
6. >CP097557.1:713091-713942 *Pseudomonas aeruginosa* strain C1.3 chromosome, complete genome
7. >CP036492.1:221946-222797 *Pseudomonas aeruginosa* strain Paer4 chromosome, complete genome
8. >JQ659969.1:211-1065 *Pseudomonas aeruginosa* strain R8-590-1 16S ribosomal RNA gene, partial sequence
9. >JQ659968.1:211-1065 *Pseudomonas aeruginosa strain* R8-590 16S ribosomal RNA gene, partial sequence

Details of the National Centre of Biotechnology Information (NCBI) alignment identity of the sequenced 16 S rRNA are shown in Table 9.

Table 9. *Pseudomonas aeruginosa* 16 S rRNA blast alignment and isolates details

Pathogen	Partial Sequence	% Identity Alignment	Site	Dpt	wards	Specimen	Genes
Pseudomonas aeruginosa	16 S rRNA	98%	UTH	OPD	SGY	Urine	AMPC
Pseudomonas aeruginosa	16 S rRNA	90%	NTH	IPD	MED	Swab (Pressure sore)	AMPC, OXA23
Pseudomonas aeruginosa	16 S rRNA	96.76%	UTH	IPD	SGY	Swab (Infected wound)	AMPC, OXA23, OXA51,
Pseudomonas aeruginosa	16 S rRNA	92.59	UTH			Urine	AMPC,OXA51,
Pseudomonas aeruginosa	16 S rRNA	97.5	UTH	IPD	MED	Bronchial secretion	OXA51
Pseudomonas aeruginosa	16 S rRNA	94.76	UTH	IPD	SGY	Swab (Pressure sore)	AMPC, OXA51,
Pseudomonas aeruginosa	16 S rRNA	91.48	NTH	IPD	MED	Swab (Pressure sore)	AMPC, OXA51
Pseudomonas aeruginosa	16 S rRNA	99.4	UTH	OPD	SGY	Urine	AMPC, OXA23, OXA51, IMP
Pseudomonas aeruginosa	16 S rRNA	97.81	UTH	OPD	SGY	Swab (Infected wound)	OXA51, IMP

4.6.Evaluation of antimicrobial profiles

Drug susceptibility to common anti-pseudomonal drugs

The antibiotic sensitivity of *Pseudomonas aeruginosa* was best with Amikacin (81%) and Tobramycin (77.5%). It was worse with Aztreonam (27.5%) and Cefepime (37.9%).

We observed a high prevalence of drug resistance (73.6 %) with MDR (60 %) and possible XDR (37.2%) resistance patterns, respectively. Tables 10, 11 and Figure 8 highlight the antimicrobial resistance pattern of the clinical isolates to various antipseudomonal drugs. Isolates were resistant to Cefepime (>60%), Aztreonam (40%), Ceftazidime (> 40%) and ciprofloxacin (>20%). The antibiotic resistance patterns for all 116 *Pseudomonas aeruginosa* clinical isolates are shown in Table 9, ranging from 14.7 % for Imipenem to 40 % for

Aztreonam and above 60% for cefepime. The sensitivity pattern per ward is further described in Tables 10, 11 and 12.

Table 10. Antibiotic resistance patterns from the surgery department (n=65)

Antibiotic Name	Break points	R%	I%	S%	R % 95%C.I.	S % 95%C.I.
Piperacillin/ Tazobactam	18 – 20	14.035	21.05	64.912	6.7-26.3	51.1-76.8
Ceftazidime	18 – 20	20.33	13.56	66.10	11.4-33.2	52.5-77.6
Cefepime	19 – 24	70.17	26.31	3.50	56.4-81.2	0.6-13.2
Aztreonam	18 – 20	28.07	28.07	43.86	17.4-41.7	31.0-57.6
Imipenem	20 – 22	8.77	36.84	54.38	3.3-20.0	40.8-67.4
Amikacin	15 – 16	1.69	10.17	88.13	0.1-10.3	76.5-94.7
Gentamicin	13 – 14	8.77	0	91.23	3.3-20.0	80.0-96.7
Tobramycin	13 – 14	5.26	0	94.74	1.4-15.5	84.5-98.6
Ciprofloxacin	22 – 25	14.03	3.50	82.46	6.7-26.3	69.6-90.8

Table 11. Antibiotic resistance patterns from the medical department (n=51)

Antibiotic name	%R	%I	%S	%R 95%C.I.	%S 95% C.I.
Piperacillin/ Tazobactam	48.72	2.56	48.72	32.7-65.0	32.7-65.0
Ceftazidime	48.84	6.97	44.19	33.6-64.3	29.4-60.0
Cefepime	67.5	27.50	5	50.8-80.9	0.9-18.2
Aztreonam	51.28	25.64	23.07	35.0-67.3	11.7-39.7
Imipenem	5.13	30.77	64.10	0.9-18.6	47.2-78.3
Amikacin	2.32	11.63	86.05	0.1-13.8	71.4-94.2
Gentamicin	28.20	2.56	69.23	15.5-45.1	52.3-82.5
Tobramycin	12.82	5.13	82.05	4.8-28.2	65.9-91.9
Ciprofloxacin	30.77	7.69	61.54	17.5-47.7	44.7-76.2

Table 10-12 highlights the antimicrobial sensitivity patterns of *Pseudomonas aeruginosa* clinical isolates from UTH and NTH.

Antipseudomonal antimicrobial resistance is shown in Figure 8 with high resistance recorded to Cefepime (FEP), Aztreonam (ATM), Ceftazidime (CAZ), Tazobactan & Piperacillin (TZP) and Ciprofloxacin (CIP).

Table 12. Overall (for medical and surgical) Antibiotic resistance patterns (n=116)

Antibiotic name	%R	%I	%S	%R 95%C.I.	%S 95%C.I.
Piperacillin/Tazobactam	30.0	13.59	56.31	21.7-40.0	46.2-65.9
Ceftazidime	34.5	10	55.45	25.9-44.3	45.7-64.8
Cefepime	69.2	26.92	3.84	59.3-77.7	1.2-10.1
Aztreonam	39.8	25.24	34.95	30.4-49.9	26.0-45.0
Imipenem	6.7	34.95	58.25	3.0-14.0	48.1-67.8
Amikacin	2.72	10	87.27	0.7-8.4	79.2-92.6
Gentamicin	19.41	0.97	79.61	12.5-28.6	70.3-86.7
Tobramycin	8.73	4.85	86.40	4.3-16.4	77.9-92.1
Ciprofloxacin	24.27	4.85	70.87	16.6-33.9	61.0-79.2

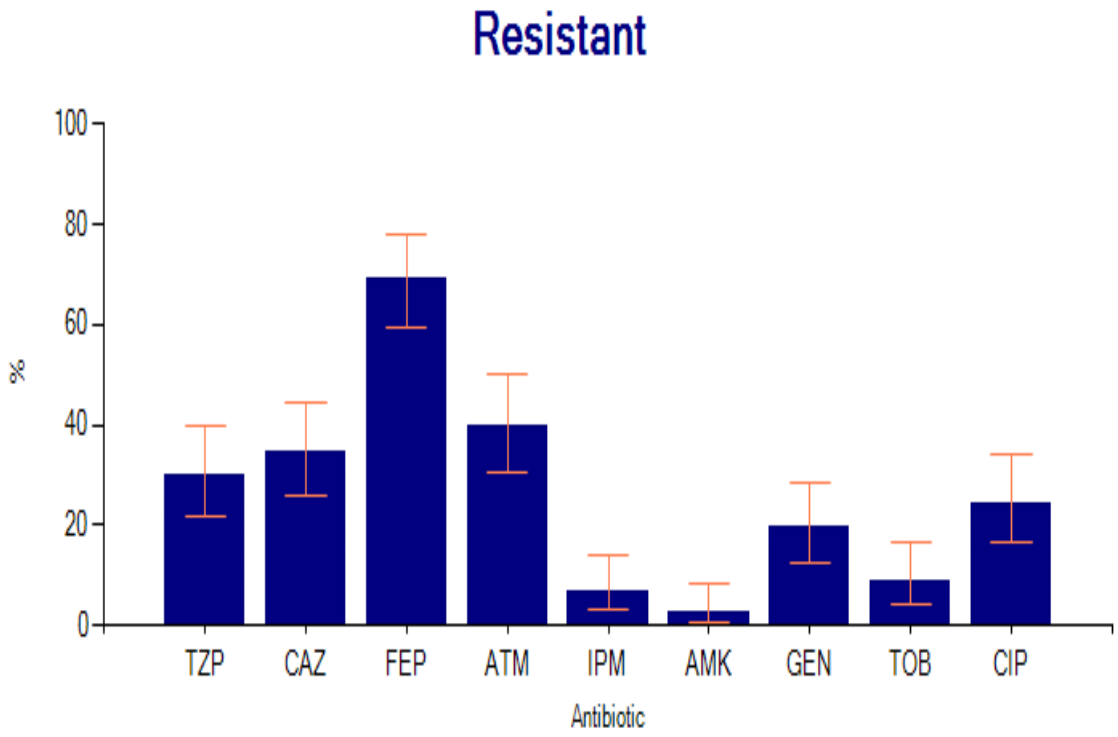


Figure. 8 Graphical representations of anti-pseudomonal resistance in clinical isolates

TZP (Piperacillin & Tazobactam), CAZ (Ceftazidime), FEP (Cefepime), ATM (Aztreonam), IPM (Imipenem), AMK (Amikacin), GEN (Gentamycin), TOB (Tobramycin and CIP (Ciprofloxacin).

UTH had more resistant cases than NTH as shown in (Table 14); while resistance of clinical isolates to antibiotics was observed to be different according to wards (exact = 0.024) with isolates from the surgical wards showing more resistance than medical wards as shown in Table 13.

Table 13. AMR patterns of clinical isolates of *Pseudomonas aeruginosa* according to departments

AMR Patterns/wards	Medical	Surgical	Total
Sensitive	9	21	30
Resistant	42	44	86
Total	51	65	116

Table 14. Distribution of MDR and XDR *Pseudomonas aeruginosa* per hospital

AMR Pattern	Frequency		Total	Associated resistance genes
	UTH	NTH		
MDR	76 (20.5%)	5 (27%)	81 (73.6%)	*AMP C* *Bla OXA 23* *Bla OXA 51*
XDR	40 (15.3%)	1	41 (37.2%)	*AMP C* *Bla OXA 23* *Bla OXA 51* *Bla IMP*

AMR (Antimicrobial resistance), UTH (University Teaching Hospital), NTH (Ndola Teaching Hospital)

4.7.Determinants of nosocomial infections with MDR *Pseudomonas aeruginosa*

Invasive medical devices-related nosocomial infection was a common presentation (78.5%) among participants. However, the presence of the medical device was not associated with AMR (p-value 0.4581). A urinary catheter was frequently inserted among surgical patients being managed for BPH. Table 15 highlights the factors associated with antimicrobial resistance in univariate analysis. Antimicrobial resistance was associated with carbapenem-hydrolysing b-lactamases gene *blaOXA-51* (p=0.001), literacy level (p=0.033) and surgical ward attendance (*0.014).

Age ≥ 65 years, male gender, prior admission and prolonged hospital admission were not associated with antimicrobial resistance. More than half (62%) of participants reported a history of previous hospital exposure within the past 30 days, which were prior medical visits or being ex-bed-siders taking care of patients.

Table 15. Association of clinical and molecular characteristics with Antimicrobial Resistance to *Pseudomonas aeruginosa* among patients treated for nosocomial infection at two tertiary hospitals, Lusaka and Copperbelt, Zambia.

Independent variables		AMR		OR	CI	p. value
		YES	NO			
Gender	Male	50	46	1.49	0.55-4.04	0.4415
	Female	8	11			
Age (Years)	15-65	49	50	0.84	0.35- 2.00	0.8261
	> 65	14	12			
Education status	Illiterate	27	16	2.28	1.05-5.07	0.0335*
	Literate	30	41			
Residence	High density	36	41	0.68	0.31- 1.47	0.4320
	Low density	22	17			
Occupation	Employed	14	13	0.98	0.41-2.32	1.0000
	Unemployed	48	44			
Previous antimicrobial use	Yes	13	17	0.73	0.31-1.69	0.5226
	No	40	38			
Hospital	UTH	53	48	2.57	0.63-10.52	0.2027
	NTH	3	7			
Wards	Medical	27	21	1.84	0.84-4.02	0.1681
	Surgical	23	33			
Department	In-patient	31	27	1.37	0.65- 2.88	0.4536
	Out-patient	25	30			
Wards	Surgical	48	17	4.16	1.30- 13.29	0.014*
	Medical	47	4			
History of the previous admission (30 days)	Yes	41	47	0.48	0.16-1.46	0.273
	No	12	7			
Duration of hospital stay (days)	≤ 2	25	33	0.86	0.39-1.86	0.8437
	≥3	25	25			
Prior medication (Antibiotics or other)	Yes	26	28	0.90	0.45-1.79	0.8616
	No	42	41			
Invasive Medical Devices Presence	Yes	42	46	0.66	0.24-1.80	0.4581
	No	11	8			
Urinary retention	Yes	3	92	0.19	0.03-1.24	0.081
	No	3	18			
Co-morbidity	HIV+ve	10	3	0.7082	0.1834-3.478	0.615
	HIV-ve	85	18			
	BPH +	9	10	2.25	0.784-6.451	0.1654
	BPH -	18	45			
	CKD +	17	14	1.661	0.660-4.178	0.3517
	CKD -	19	26			
Resistance genes	Amp C +ve	43	20	0.806	0.274-2.369	0.7924
	Amp C –ve	16	6			
	BLA OXA 23 +ve	7	1	3.365	0.392-28.859	0.4252
	BLA OXA 23 - ve	52	25			
	BLA OXA 51 +ve	65	6	5.331	1.915-16.3	0.0010*
	BLA OXA 51 – ve	30	15			
	BLA IMP +ve	5	1	1.165	0.1294-10.52	0.4817
	BLA IMP –ve	90	21			

OPD: Out-patient Department, IPD: In-patient Department, UTI: Urinary Tract Infection, MSU: Midstream urine, RTI: Respiratory Tract Infection, NTH: Ndola Teaching Hospital, UTH: University Teaching Hospital

The results of the multivariate logistic regression model showed that *Pseudomonas aeruginosa* antimicrobial resistance was associated to high-density residence, surgical ward attendance, current antibiotics therapy and presence of *Bla OXA* 51 gene as shown in Table 16 and 17.

Table 16. Multivariate Analysis of the factors associated with *Pseudomonas aeruginosa* nosocomial infection and multi-drug resistance (MDR) at UTH and NTH (Logistic Regression)

Variables	*Odds ratio*	*P>[z]*	*95% Conf. interval*
District Lusaka (UTH) (1) Ndola (NTH) (0)	*0.11*	*0.032*	*0.01 -0.83*
Department Surgical (1) Medical (0)	*0.15*	*0.006*	*0.03- 0.58*
Current antibiotics treatment Yes (1) No (0)	*4.36*	*0.039*	*1.08- 17.58*
Bla oxa51gene1 Present (1) Absent (0)	*8.80*	*0.000*	*2.6- 29.39*
Self-medication Yes (1) No (0)	*2.05*	*0.260*	*0.58- 7.20*
Cons	*180.97*	*0.005*	*4.99-6561.30*

Note: cons estimate baseline odds.

Table 17. Results of the logistic regression analysis of the factors associated with *Pseudomonas aeruginosa* nosocomial infection and extensive drug resistance (XDR) at UTH and NTH.

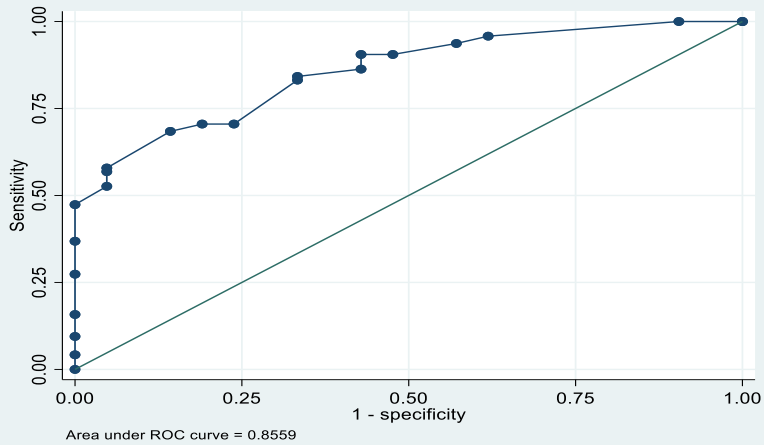
Variables	*Odds ratio*	*P>[z]*	*95% conf. interval*
Age group	*1.92*	*0.033*	*1.05- 3.51*
oxa23gene	*12.52*	*0.007*	*2.02- 77.62*
oxa51gene1	*4.67*	*0.003*	*1.70- 12.7*
Cons	*0.02*	*0.000*	*0.003- 0.1*

Note: _cons estimate baseline odds.

4.8.The Roc Curve Analysis

The area under ROC curve = 0.8559 (Figure 9), indicated a good model predictability. Since the AUC was approximately 0.85, the variables surgical department, self-medication of antibiotics, and Bla OXA 51 gene predicted resistance among MDR *Pseudomonas aeruginosa* nosocomial infection patients with reasonable accuracy. The Receiver Operating Characteristics were evaluated to check the performance of the determinants assessed.

Result: Goodness-of-fit test after logistic model (Variable: mdr) (Objective 3)



Number of observations = 116

Hosmer and Lemeshow

chi2(8) = 5.41
Prob > chi2 = 0.7131

lroc

Logistic model for mdr
Number of observations = 116

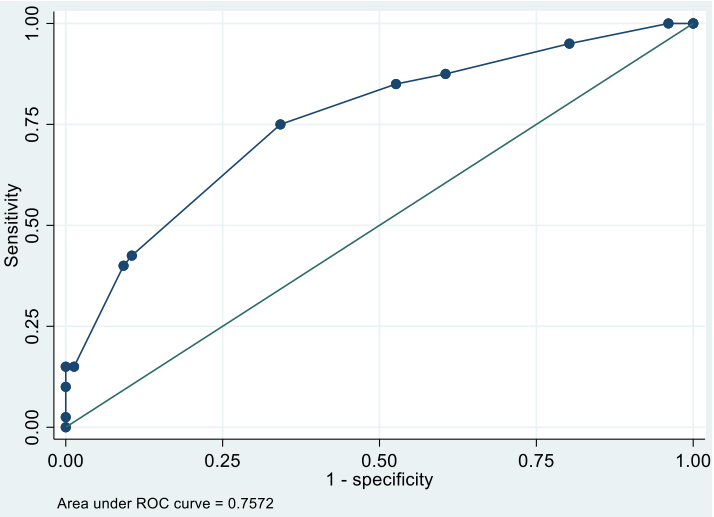
Area under ROC curve = 0.8559

11/27/23 29

Figure 9. *The ROC* ***(Receiver Operating Characteristics)*** *curve (MDR) was drawn to determine the AUC* ***(Area Under the Curve)***

For the second model, with XDR as an outcome variable, the area under the ROC curve = 0.7572 (Figure 9), which indicated good model predictability. Since the AUC was approximately 0.75, hence age, *Bla* OXA 51 and *Bla* OXA 23 genes were statistically significant in influencing resistance among XDR *Pseudomonas aeruginosa* nosocomial infection patients. The Receiver Operating Characteristics were evaluated to check the performance of the determinants assessed.

Goodness-of-fit test after logistic model (Variable: xdr) (Objective 3)



Number of observations = 116

Hosmer and Lemeshow

chi2(5) = 1.37

Prob > chi2 = 0.9272

lroc

Logistic model for mdr

Number of observations = 116

Area under ROC curve = 0.7572

11/27/23 31

Figure 10. *The ROC (Receiver Operating Characteristics) curve (XDR) was drawn to determine the AUC (Area Under the Curve)*

4.9.Genomic analysis.

Table 18 highlights the *Pseudomonas aeruginosa* analysis of targeted resistance genes**.** The results of amplified genes by PCR showed that 6 (5.1%) clinical isolates contained *bla* IMP. These six isolates all belonged to the University Teaching Hospital (UTH) in Lusaka and were cultured from urinary tract infection (n=4) and skin swabs from patients with infected decubitus ulcers (n=2). All these six patients were from the surgical out-patient department (50%) and the medical out-patient department (50%) with a history of prior medical contact within 90 days and self-medication. These clinical isolates were multi-drug resistant and harboured AMP C and *Bla OXA* 51 genes.

The results of the PCR assay for 116 clinical isolates showed that the majority (99) (85.3 %) harbored the *AMPC gene.* These 99 isolates were recovered from UTH (87) and NTH (9) in patients presenting with infected wounds (37) (37.3%), urinary tract infection (49) (49%), and

respiratory tract infection (5%). Figure 9 shows agarose gel electrophoresis of PCR product for the presence of amp C gene in *Pseudomonas aeruginosa* isolates.

The results of amplified genes by PCR showed that only 8 (6%) of clinical isolates contained *Bla OXA 23*. At NTH, *Bla OXA 23* was found in 10% of isolates from infected wounds and 6% of isolates from UTH from patients with infected wounds (n=2), urinary infection (n=3), and respiratory tract infection (n=2).

The results of amplified genes by PCR showed that 72 (62%) clinical isolates contained *Bla OXA 51*. At the Ndola Teaching Hospital, 40% of *Pseudomonas aeruginosa* recovered from infected wounds (n=4) harbored *Bla* OXA 51. In comparison, UTH 64.1 % of *Pseudomonas aeruginosa* recovered from infected wounds (n=21), urinary tract infection (n=40) and respiratory tract infection (n=5) harbored *Bla OXA* 51 gene.

Table 18. *Pseudomonas aeruginosa* analysis of targeted resistance genes

Genomic analysis				
Resistance Genes & Primers used		*+ve*	*-ve*	*%*
Primers	*PA-SS* *GGGGGATCTTCGGACCTCA (F)* *TCCTTAGAGTGCCCACCCG (R)*	*87*	*25*	*112 (96%)*
	PA-GS GACGGGTGAGTAATGCCTA (F) CACTGGTGTTCCTTCCTATA (R)	70	16	86 (74%)
	AMP-C CGGCTCGGTGAGCAAGACCTTC (F) AGTCGCGGATCTGTGCCTGGTC (R)	79	20	99 (85.3%)
	*Bla*Oxa23 GATCGGATTGGAGAACCAGA (F) ATTTCTGACCGCATTTCCAT (R)	7	1	8 (6%)
	*Bla*Oxa51 TAATGCTTTGATCGGCCTTG (F) TGGATTGCACTTCATCTTGG (R)	65	7	72 (62%)
	*Bla*IMP GTTTGAAGAAGTTAACGGGTGG (F) ATAATTTGGCGGACTTTGGC (R)	6	0	6 (5%)

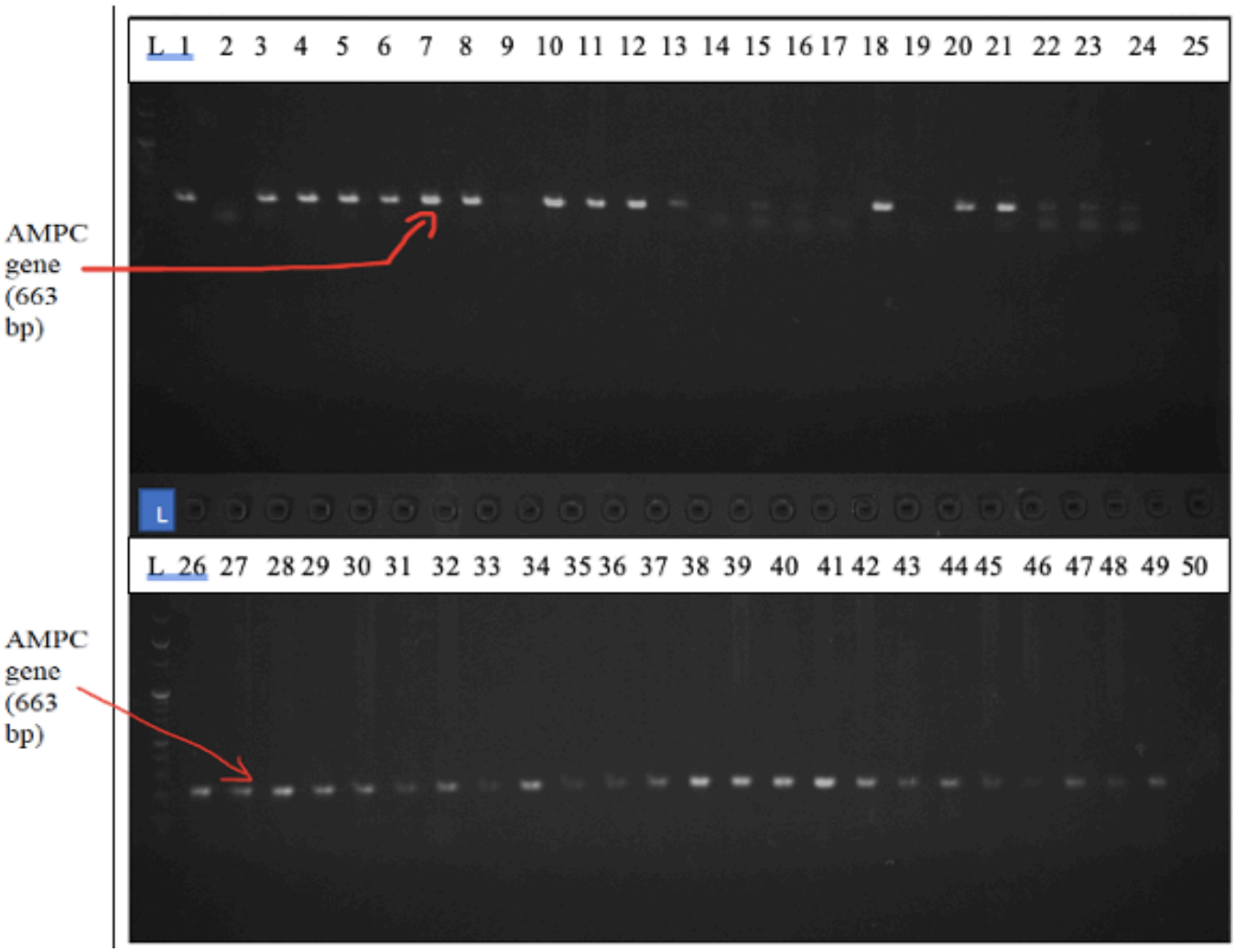


Figure 11. Agarose gel electrophoresis of PCR product for the presence of ampC gene in *Pseudomonas aeruginosa* clinical isolates. Lane L represents the 100pb DNA ladder. Lanes 1, 3-8, 10-13, 18, 20-24, and 26-49 are positive for Amp C. Lanes 2, 9, 14-17, and 19 are Amp C negative samples. Negative control: 25 & 50.

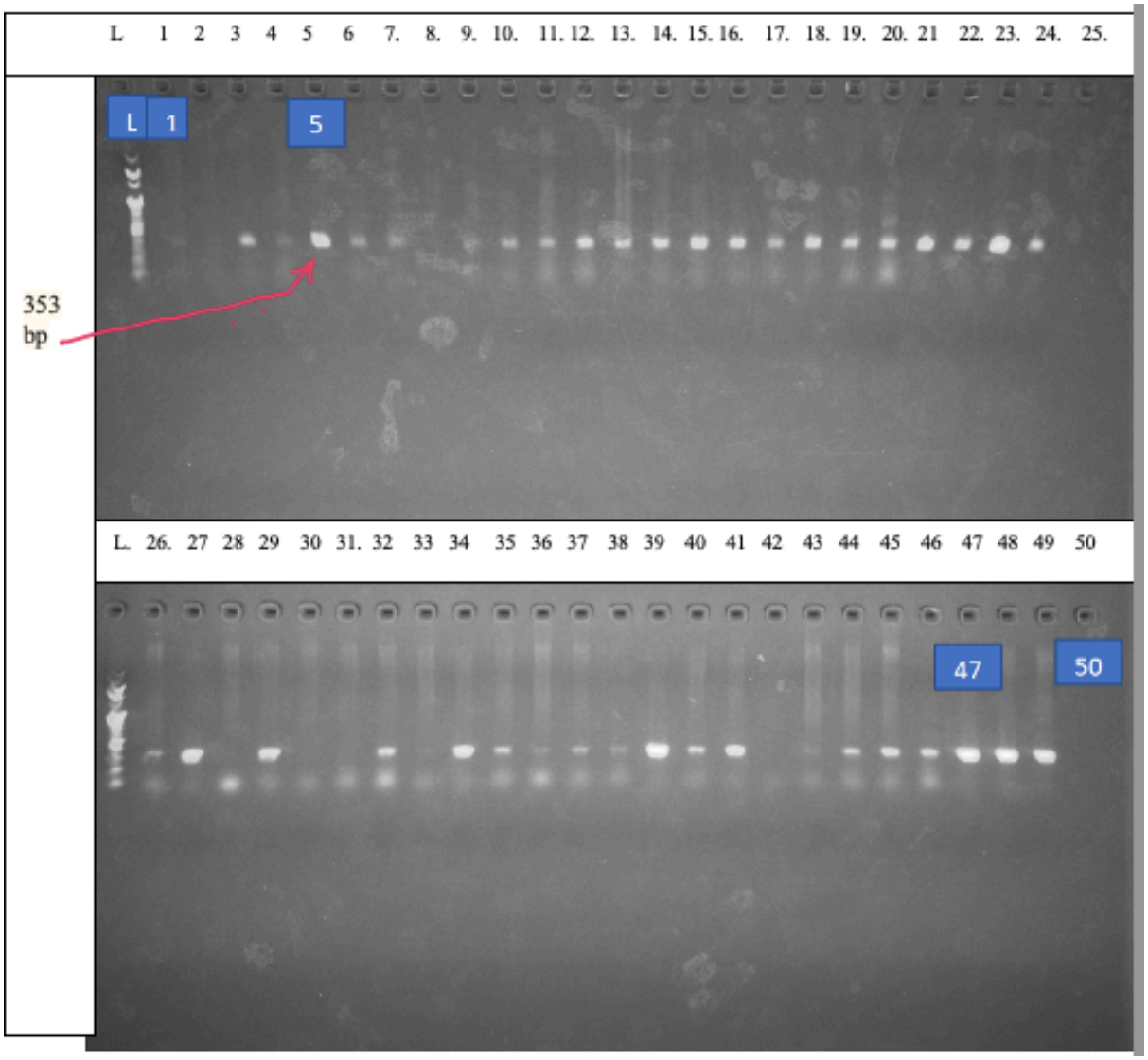


Figure 12. Agarose gel electrophoresis of PCR product for the presence of *Bla OXA* 51 gene in *Pseudomonas aeruginosa* isolates. Lane L represents the 100pb DNA ladder. Lanes 3, 5-7, 9-24, 26, 27, 29, 32, 34-41, and 44-49 are positive for *Bla* OXA 51. Lanes 4, 8, 25, 28, 30, 31, 33, and 42 are *Bla* OXA 51 negative samples. Negative control: 25 & 50.

4.10. Phylogenetic analysis

Pseudomonas aeruginosa from UTH and NTH clinical isolates clustered into various lineages corresponding to different sequence types (STs), also belonging to different clonal complexes (Figure 13). The phylogenetic tree of *Pseudomonas aeruginosa* isolates pointed to a distant relationship between strains from the NTH and UTH (Figure 13). The isolates from NTH shared a phylogenetic affiliation with members of bacteria within cystic fibrosis lung bacterial

communities, such as the clone 16sps15-2f04.p1k (FM995853) Submitted from King's College London, London, SE1 9NH, UNITED KINGDOM (Accession number FM995853).

The isolates from UTH shared a phylogenetic affiliation with members of bacteria obtained from clone libraries prepared from human tracheal aspirates of patients colonized with *Pseudomonas aeruginosa*, such as the clone P7D82-578 (EF509464). The isolates from UTH also shared a phylogenetic affiliation with environmental isolates such as the *Pseudomonas aeruginosa* strain P2 (MG966347) from rhizopheric soil (Shisham, India) and *Pseudomonas aeruginosa* strain alkanany (OK576381) isolated from oil polluted soil in Basra, Irak.

Among the isolates from UTH, SeqID4 and SeqID6 isolated from surgical in-patients from infected wounds shared a close phylogenetic affiliation and common resistance genes AMPC and *Bla OXA* 51. However, these were closely related and were distant from SeqID1, SeqID3, SeqID5 and MZ618953. These were isolated at UTH from surgical OPD (Urine isolates), Surgical IPD (Infected wound isolates), and medical IPD (bronchial secretions). Resistance genes in this group included AMPC (seqID1, seqID3), *Bla OXA* 23 (SeqID3, MZ618953), *Bla OXA* 51 (SeqID3, SeqID5 and MZ618953).

Phylogenetic analysis of the clinical isolates and 357 publicly available *Pseudomonas aeruginosa* strains further demonstrated that the clinical isolates STs had large phylogenetic distances despite clustering in three related clusters, one at Ndola Teaching Hospital and two at the University Teaching Hospital.

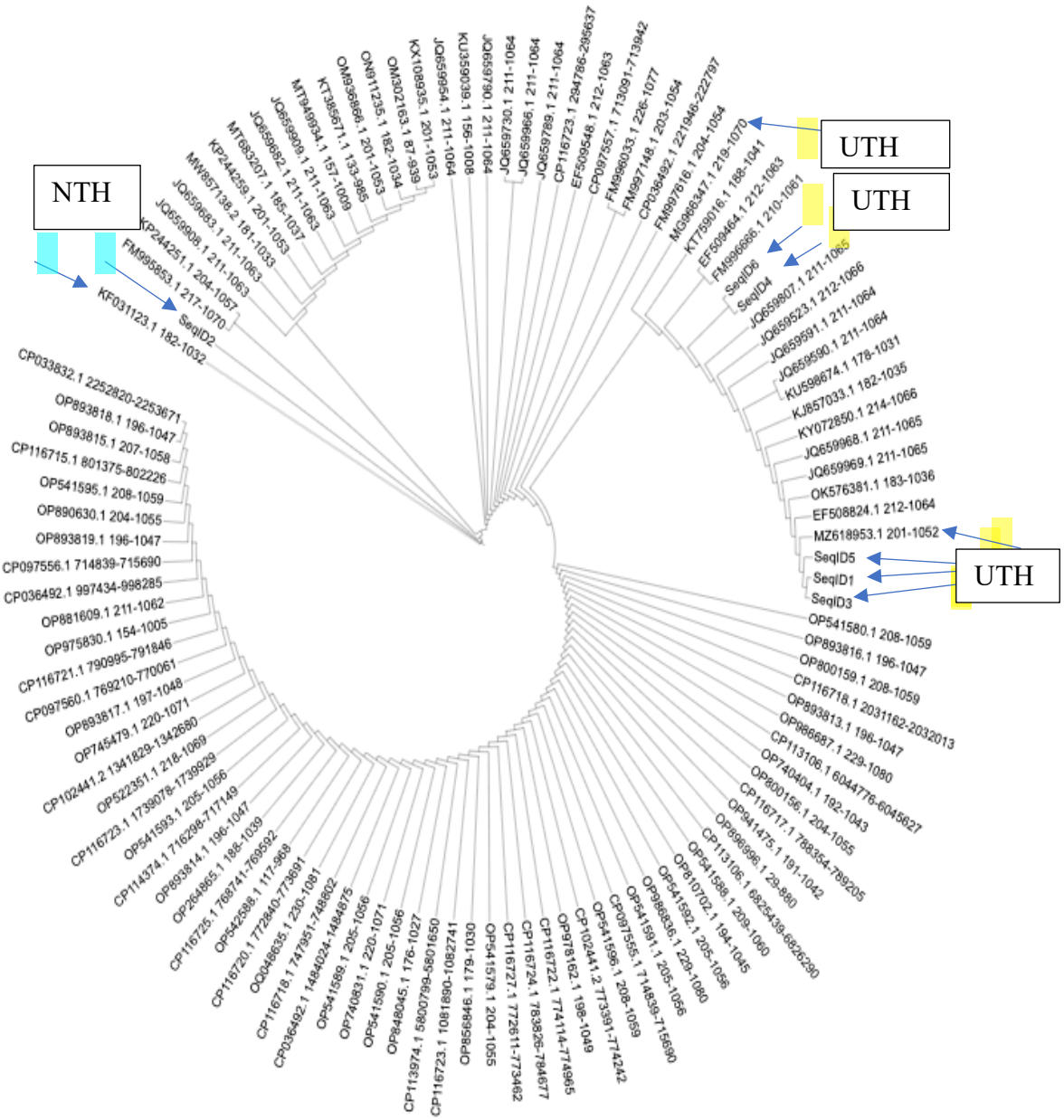


Figure 13: Phylogenetic tree of *Pseudomonas aeruginosa* from the study (UTH and NTH) and the National Centre for Biotechnology Information public database.

4.11. Analysis of the outcomes of *Pseudomonas aeruginosa* nosocomial infections at UTH and NTH

Though mortality among patients was low (5%) during the follow-up period, adverse outcomes occurred in up to 45.6%. Most of the cases of complications occurred in patients with prolonged

admission with pressure sores (36.2%) and catheterization (56.8%). All causes of mortality occurred in patients presenting with septic wounds (Pressure or diabetic ulcers and burns) with fever. *Pseudomonas aeruginosa* infected cesarean section wounds with sepsis was responsible for 34% of mortality cases.

All causes of mortality in this study occurred among Lusaka (UTH) participants, and all were female (p=0.000) from high-density areas of Lusaka (p=0.001)—other factors associated with mortality are provided in Table 19.

The Human Immune Deficiency Virus (HIV) infection (p=0.511), education status (p=0.227), profession (p=0.702), and wards (medical or surgical, p=0.539) were not associated with mortality. Similarly, Amp C (p=0.106), *Bla* OXA 23, *Bla* OXA 51(p=0.570), and *Bla* IMP (p=0.763) genes were not associated with all causes of mortality in this study.

All *Pseudomonas aeruginosa* isolated in the mortality cases were sensitive to aminoglycosides (Amikacin, Gentamycin, and Tobramycin) and Carbapenems' (Imipenem). In these patients, mortality may not be associated with *Pseudomonas aeruginosa* AMR. Though the prevalence of other genes was high (*AMP* C and *OXA*s), it was high, too, in the group of survivors. Other factors associated with mortality are analysed in Table 19.

Table 19. Association of independent clinical and molecular variables with all causes of mortality among *Pseudomonas aeruginosa* infected patients at two tertiary hospitals, Lusaka and Copperbelt, Zambia.

Independent variables		Mortality		p. value
		Yes	No	
Gender	Male	0	15	0.000
	Female	6	95	
Hospital	UTH	6	95	0.000
	NTH	1	10	
Wards	Medical	3	43	0.7357
	Surgical	3	58	
Department	In-patient	6	36	0.021*
	Outpatient	1	65	
History of the previous admission (30 days)	Yes	6	93	0.023*
	No	1	17	
Duration of hospital stay (days)	≥ 8	6	19	0.000347*
	≤ 7	1	91	
Fever	Yes	6	35	0.014*
	No	1	75	
Medical Devices Presence	Yes	6	63	0.072*
	No	1	47	
Infected wound	Yes	6	57	0.009*
	No	1	43	
Aminoglycosides AMR	Yes	1	9	0.5840
	No	6	101	
Quinolones AMR	Yes	2	16	0.2803
	No	4	94	
Carbapenems AMR	Yes	1	6	0.4144
	No	6	104	
Co-morbidity	HIV +ve	1	22	0.511
	HIV -ve	6	88	
	DM +ve	1	6	0.4144
	DM -ve	6	104	
Resistance genes	Amp C +ve	5	70	0.3742
	Amp C –ve	1	40	
	BLA $_{OXA23}$ +ve	1	5	0.3088
	BLA $_{OXA23}$ -ve	5	105	
	BLA $_{OXA51}$ +ve	4	54	0.4428
	BLA $_{OXA51}$ –ve	2	56	
	BLA $_{IMP}$ +ve	1	4	0.2978
	BLA $_{IMP}$ –ve	6	106	

CHAPTER FIVE

DISCUSSION

This study aimed to evaluate *Pseudomonas aeruginosa* nosocomial infections in patients at UTH in Lusaka province and at NTH in Copperbelt province of Zambia. It also aimed to analyse antimicrobial profiles, evaluate selected AMR genes and assess the clinical outcomes of patients admitted at UTH and NTH. But also aimed to analyse the relatedness of clinical isolates from different clinical specimens.

5.1. Clinical characteristics of *Pseudomonas aeruginosa* NIs

The hospital environment is known to select for and promote the spread of nosocomial pathogens. Therefore, clinical characteristics, causes, and risk factors of nosocomial infections at two urban tertiary-level hospitals in Zambia were evaluated.

Most participants were enrolled from UTH compared to NTH. At NTH, the data was collected during the third wave of the COVID-19 pandemic, hence the low enrolment (23.9 %) of participants at this site due to a decrease in the number of patients attending hospital for non-COVID 19 related illnesses during the study period.

These observations agree with Quadros *et al.* (2021), who linked the decrease in the number of patients with non-COVID19 related diseases seeking medical attention at health facilities to public anxiety about acquiring the viral infection in the hospital and the subsequent risk of mortality and lockdown. Nosocomial infection pathogens were isolated in most (71.1%) patients with inserted medical devices. The prior hospital visit was significantly associated with the risk of nosocomial infection, as previously observed by Baggs *et al.* (2018) in a retrospectively identified cohort of hospitalized patients from the Truven Health MarketScan Hospital Drug Database in the United States of America (USA).

In the present study, the prevalence of *Pseudomonas aeruginosa* infection was 13.7% (116/841), consistent with a previous report from Ethiopia (Mekonnen *et al.,* 2021). However, it was relatively higher than that of other reports from Ethiopia (1.6–8.4%) (Gashaw *et al.,* 2018), Iran (0.4–0.7%) (Davoudi *et al.,* 2012), Italy (9.3%) (De Francesco *et al.,* 2013) and Uganda (8.2%) (Kateete *et al.,* 2016). The possible explanation for the observed difference might be due to the variations in sample size, the clinical site of nosocomial infection and the severity of underlying diseases. But also differences in the hospital settings, patients' exposure to different invasive medical devices, standards of infection prevention practice, and the length of hospitalization.

The present study found that most (69.9%) of clinical specimens had positive bacterial cultures, of which most were isolated form the surgical ward. The rate of positivity in our study was high compared to other studies, such as the descriptive cross-sectional study conducted at Upendra Devkota Memorial National Institute of Neurological and Allied Sciences in Nepal (Sharma *et al.,* 2023), which reported a prevalence of 4% (36 *Pseudomonas aeruginosa* for 770 processed blood, sputum, and urine samples).

In the present study, more pathogens were isolated from surgical (55.6%) compared to medical wards. Further, UTH and NTH returning out-patients (OPD) patients had relatively more isolated pathogens (56.2%) than admitted patients. In the surgical UTH and NTH OPDs, some patients diagnosed with BPH were discharged with a urinary catheter in situ while awaiting surgery, some for several months. These were reviewed in OPD and had high prevalence of infections due to the presence of a medical device for a long period of time. Similarly, Bennett *et al.* (2018) observed that patients with both a central venous catheter and urinary catheter had over 2.5-fold increased daily risk of nosocomial infections.

Contrary to our findings, medical wards had more isolated pathogens such as in southern Thailand (Saengsuwan *et al.,* 2022) where *Pseudomonas aeruginosa* infection was found to be a major cause of complications, particularly pneumonia, in the extreme age (children and older patients above 65 years). However, no skin and soft tissue samples were included in the Thai study.

In the present study, more (71.2%) male patients (599/841) were diagnosed with nosocomial infections than female patients. Our findings are consistent with those of another study at Single University Hospital Center in Germany (Yayan *et al.,* 2015). According to this previous study, *Pseudomonas aeruginosa* caused most infections in males (67.3%) than females (32.7%), with a mean patient age of 68.1±12.8 years though the mean age in our study was 51 years (SD ± 18). Although Adejobi *et al.* (2021) recovered slightly more isolates of pseudomonads from female patients (51%) compared to males (49%) in his study, arguing that the anatomical structure of the female reproductive system makes the invasiveness of *Pseudomonas* spp. easier in females when immunity is compromised. However, according to our study, the high prevalence of catheterisation in male patients with enlarged prostates could have contributed to an increase of *Pseudomonas aeruginosa* infection in males compared to females.

Therefore, we observed catheter-associated UTI (57%) as the most common nosocomial infection. Our findings corroborate the finding by Gad *et al.* (2007) who recorded the highest yield of *Pseudomonas aeruginosa* in urine (22.5%).

Contrary to our findings, Adejobi *et al.* (2021) recovered the majority of the *Pseudomonas aeruginosa* clinical isolates from wound swabs (44%), while Pezhman *et al*. (2021) observed that the most common sites of nosocomial infection were the respiratory tract (39.4%). The findings by Pezhman *et al* (2021) reported the intensive care unit (ICU) as the most affected ward involved in recorded nosocomial infection. This could explain why most common site of

nosocomial infection in this study was the respiratory system due to high rate of intubation among patients admitted in the intensive care (Divatia *et al.,* 2011).

This present study found the highest prevalence of nosocomial infection in elderly patients with a mean age of 51 years (+- 18). This is contrary to findings in South-West Nigeria where the highest frequency (28.7%) was reported in patients between 30-39 years (Adejobi *et al.,* 2021); North-Eastern Nigeria where high prevalence was reported even in lower age groups of 20-29 years by Okon *et al*. (2011) in a study predominantly investigating *Pseudomonas aeruginosa* superficial infections.

The low frequency of respiratory nosocomial infection recorded in this study could be due to infection prevention measures scaled up during the COVID19 waves. The other reason could be that cases of respiratory infections were considered suspected Covid 19, since this study was conducted during the pandemic period. People with suspected cases were told to go into self-quarantine and would not go to the hospital as the case was prior to the pandemic. Contrary to our findings, a study by Fazeli *et al.* (2012) isolated more clinical *Pseudomonas aeruginosa* strains from infected wounds (33.3%), followed by tracheal secretions (31.2%) and infected urine (16%). Ford *et al.* (2022) also highlighted the central role of *Pseudomonas aeruginosa* as a common pathogen isolated in combat-related infections in Iraq and other battlefields; however, reassuringly, isolation of MDR *Pseudomonas aeruginosa* in this acute traumatic infected wound was uncommon.

Previous studies on traumatic wound infections demonstrated variable infection rates, ranging from 3.9% in Vietnam to 8.2% of non-combat trauma patients admitted to a contemporary military facility and as high as 37% in a civilian trauma population (Ford *et al.,* 2022). The study by Ford *et al.* (2022) showed that 32% of patients developed at least one infection, and 17% were *Pseudomonas aeruginosa*.

We did observe a high prevalence of NI at the two tertiary hospitals in Zambia. According to reports before the COVID 19 pandemic, the prevalence of *Pseudomonas aeruginosa* was on an uptrend (Xiang *et al.,* 2023). However, the incidence rates for *Pseudomonas aeruginosa* infection during the COVID 19 pandemic remained contested, with four reports showing a higher incidence of *Pseudomonas aeruginosa* infections during the pandemic period (as compared to non-pandemic periods) (Amarsy *et al.,* 2022; Meschiari *et al.,* 2022), others reporting no change in trend (İpek *et al.,* 2022; Despotovic *et al.,* 2021), and two reporting decreases in the incidence of *Pseudomonas aeruginosa* infections (Yardimci *et al.,* 2022; Hirabayashi *et al.,* 2021). Su *et al.* (2021) observed that infection prevention and control measures for the COVID 19 pandemic reduced nosocomial infection in almost all departments in a Chinese tertiary hospital during the waves. However, they found that catheter-related infections did not differ over time; which agreed with the current study.

During the COVID 19 pandemic, infection control measures in hospitals were thought to be enhanced, which should have theoretically reduced the incidence of hospital-acquired infections by pathogens such as *Pseudomonas aeruginosa* infections (Xiang *et al.,* 2023). Ipek *et al.* (2022) noted a decline in the incidence of pneumonia in their pediatric ICU and did not see cases of *Pseudomonas aeruginosa* infection, but these findings were in contrast with the present study. They attributed this remarkable finding to a rise in the hand hygiene rate above 99% during the pandemic's peak. However, these findings were in contrast with this present study despite *Pseudomonas aeruginosa* infection being preventable through good hygiene practices.

During the COVID 19 pandemic, improvements in infection control measures included hand hygiene, appropriate use of personal protection equipment (PPE), and an increased focus on environmental decontamination, all aimed at reducing the possibility of contact transmission

and other nosocomial spread (Barrera-Cancedda *et al.,* 2019). However, it is also possible that prioritizing respiratory infections may have had unintended effects, as per the experiences of other centers (Meda *et al.,* 2020), and other infection control measures may have been compromised during the COVID-19 pandemic. Furthermore, they were recent reports of decreased compliance with hand hygiene during the COVID19 pandemic (Meda *et al.,* 2020; Fakih *et al.,* 2019). This effect could have potentially contributed to the observed rise in the incidence of NEC in this study.

An Ethiopian study found a high frequency of nosocomial infection caused by *Pseudomonas aeruginosa* (13%) (Mekonnen *et al.,* 2021). This was in agreement with our findings. The observed difference with other studies could be explained by factors such as co-morbidities in participants, such as benign prostate enlargement, presence or frequency of medical device insertion, hygiene in different hospital settings, standards of infection prevention practice, and length of hospitalisation. We observed that the rate of nosocomial infection was significantly higher among patients with benign prostate hypertrophy (BPH) due to an inserted medical device such as a urinary catheter.

We further observed that outpatient attendance was associated with a high risk of nosocomial infection at UTH and NTH. Most of these were outpatients who frequented the hospital for complications related to long term urinary catheterization while awaiting surgery for benign prostate enlargement. These patients were catheterised and then discharged with the urinary catheter in-situ with options for self-catheterisation while awaiting prostate surgery due to shortage of urologist. This potentially puts them at risk of being infected with either community or hospital-acquired bacteria, including *Pseudomonas aeruginosa,* leading to nosocomial infection. These infections were commonly observed in the urology department at the

university teaching hospital in Lusaka. This finding was consistent with observation that aged males were more likely to be catheterised for reasons such as BPH (Dougherty *et al.,* 2024).

In addition, Mekonnen *et al.* (2021) reported that factors contributing to the high prevalence of nosocomial infection could be due to the commonly observed overcrowding of patients in various wards, the hospital environment, and poor implementation of infection control measures, particularly hand hygiene practices and decontamination of the hospital environment.

Surprisingly, the Acquired Immune Deficiency Syndrome secondary to Human Immune Deficiency Virus (HIV/AIDS) was not significantly associated with an increased risk of nosocomial infection. This is because most HIV patients in Zambia are on anti-retroviral treatment. According to data from UN/AIDS, the Zambian Ministry of Health (MoH) and the Centre for Infectious Diseases Research in Zambia (CIRDZ) indicated that out of 1,300,000 Zambians estimated to be living with HIV/AIDS, 1,176,000 (90.5%) were on treatment (CIDRZ, 2020; UNAIDS, 2018) in compliance with the 90, 90 and 90 UN AIDS strategy. According to this strategy, 90% of all people living with HIV must know their HIV status; 90% of all people with diagnosed HIV infection must receive sustained antiretroviral therapy. And 90% of all people receiving antiretroviral therapy must achieve HIV viral suppression.

A meta-analysis of 144 published studies from 2005 to 2016 (Xiang *et al.,* 2023; Schreiber *et al.,* 2018) found that irrespective of a country's income level, a significant proportion of healthcare-associated infections (35 to 55%) were, in fact, preventable. The meta-analysis further observed that the increasing incidence of nosocomial infection represents existing gaps in implementing infection control practices (Xiang *et al.,* 2023; Schreiber *et al.,* 2018). Although effectively washing or sanitizing one's hands is thought to be a cornerstone of infection control and prevention, data collected using an electronic hand hygiene monitoring

system in two Danish hospitals found that hand hygiene compliance was lower during the COVID-19 pandemic as compared to pre-pandemic periods (Sandbol *et al.,* 2022). This observation implied great potential to reduce the burden of nosocomial infection further by enhancing good hand hygiene practices (Mathur *et al.,* 2011).

5.2. Prevalence of *Pseudomonas aeruginosa*

The present study found that most (69.9%) of clinical specimens had positive bacterial cultures, of which 13% were presumptive *Pseudomonas aeruginosa*. Out of the 138 presumptive *Pseudomonas aeruginosa* isolates, 116 were confirmed on 16 sRNA.

The rate of positivity in our study was high compared to other studies, such as the descriptive cross-sectional study conducted at Upendra Devkota Memorial National Institute of Neurological and Allied Sciences in Nepal (Sharma *et al.,* 2023), which reported a prevalence of 4% (36 *Pseudomonas aeruginosa* for 770 processed blood, sputum, and urine samples). In the present study, more pathogens were isolated from surgical compared to medical wards; returning OPD patients had more pathogens isolated compared to admitted patients. *Pseudomonas aeruginosa* catheter-associated urinary tract infection was the most common nosocomial infection in this study; the majority of patients included in this study were catheterized for BPH, hence the high number of infections among surgical patients.

5.3. *Pseudomonas aeruginosa* strains

In the present study, the 16S rRNA gene sequenced identified 84% of presumptive clinical isolates DNA samples as *Pseudomonas aeruginosa*. After 16S rRNA sequencing, the following *Pseudomonas aeruginosa* strains were among those identified: strain MDM1, strain P2, strain SN3, strain C1.3, strain Paer4, strain R8-590-1, and strain R8-590.

The current study isolated *Pseudomonas aeruginosa* strain C1.3 from a UTH surgical catheterized patient with an infected traumatic wound. This strain harbored *AMP* C and *Bla*

OXA 51 genes. However, Kiel *et al.* (2022), in a study at Bielefeld University in Germany, isolated in biofilm this same strain and described it as "Novel *Pseudomonas aeruginosa* Strains Isolated from Household Appliances" and uploaded on NCBI under access number CP097557 in December 2023.

In the present study, the strain SN3, MCBI access number KF031123, was isolated from the urine sample of a UTH patient with an enlarged prostate and long-term Foley catheter management. The isolated strain harbored the *Amp* C gene and was sensitive to common antipseudomonal antibiotics. Nath *et al.* (2014) isolated this *Pseudomonas aeruginosa* strain SN3 and SN1 from contaminated crop fields near industrial sites, garages, and petrol pumps in the Cachar District of Assam, India. They described these strains as Cadmium and Lead Tolerant *Pseudomonas aeruginosa* in Seedling Germination of Rice (Oryza sativa L.).

Pseudomonas aeruginosa strain P2, NCBI accession number MG966347, was isolated from UTH in Lusaka, Zambia. This *Pseudomonas aeruginosa* strain was also isolated from patients undergoing hemodialysis in Iraq at the College of Medicine, Wasit University, Wasit Center for Dialysis (Mohammed & Shehab,2018).

5.4. Antimicrobial susceptibility and determinants of AMR

Globally, several studies have reported the increased resistance of *Pseudomonas aeruginosa* clinical isolates to a combined range of antibiotics. Studies from Turkey, Bangladesh, Iran, and Saudi Arabia showed high drug resistance (>50% – 98%) (Khan *et al.,* 2016).

The current study showed some resistance of *Pseudomonas aeruginosa* to carbapenems (41.65% resistance to Imipenem) in agreement with resistance ranges found by Kamali *et al.* (2020) who reported a high rate of drug resistance of *Pseudomonas aeruginosa* to carbapenems (30.6%).

Similarly, some studies reported a low resistance to carbapenems (5% and 18%) (Banar *et al.,* 2016; Gill *et al.,* 2016). The geographical variation in sensitivity pattern to carbapenems is influenced by the everyday use or not of these groups of antibiotics. The prevalence of MDR *Pseudomonas aeruginosa* in Iran was estimated at 58%, with a variation in geographical areas; the highest and lowest rates were observed in Tehran (100%) and Zahedan (16%), respectively (Vaez *et al.,* 2018). However, in Saudi Arabia, low-to-moderate rates of drug resistance among *Pseudomonas aeruginosa* isolates were observed, with multidrug resistance reported in 10.7% (Khan *et al.,* 2016). In Europe, comprehensive surveillance of antimicrobial resistance demonstrated a prevalence ranging from 0% (Iceland) to 59.1% (Romania) (European Antibiotic Awareness Day, 2021; WHO, 2023).

Isolates from critical care units were significantly resistant, particularly from certain countries such as Egypt and Libya, with high-level resistance to cephalosporins, carbapenems, and aminoglycosides (Al-Orphaly *et al.,* 2021). The reported prevalence of MDR *Pseudomonas aeruginosa* from general clinical samples had the highest prevalence in Egypt (75.6%) and the lowest prevalence in Morocco (0%) (Al-Orphaly *et al.,* 2021). In South-West Nigeria, Adejobi *et al*. (2021) reported *Pseudomonas aeruginosa* as a highly multidrug-resistant pathogen with a high multiple antibiotic resistance index in hospital settings. A study in Kenya on antimicrobial susceptibility testing found that 79% of the isolates were non-susceptible to piperacillin, 57% were non-susceptible to ticarcillin/clavulanic acid, 70% were non-susceptible to levofloxacin. The resistance rate to carbapenems was high then what was observed in the current study (Kiyaga *et al.,* 2022).

The difference in the rate of ciprofloxacin resistance is usually related to the frequency of use of fluoroquinolones and the availability of oral doses (Kanafani *et al.,* 2023). Resistance of *P. aeruginosa* to ciprofloxacin is a rising problem in many parts of the world. In our study, the

resistance rate to ciprofloxacin was 42.3%, much higher than that reported in earlier studies from Saudi Arabia (Khan *et al.,* 2016) and other parts of the world (Momenah *et al.,* 2023; Patel *et al.,* 2008). However, the resistance rate to ciprofloxacin was relatively higher (75.5%) than the current study reported from Bangladesh (Rashid *et al.,* 2007). A lower rate of *Pseudomonas aeruginosa* resistance to ciprofloxacin was reported by Orrett *et al.* in Trinidad and Tobago (2004).

Data from this study showed that from all anti-pseudomonal antibiotics tested, high antibiotic resistance to Cefepime, Ceftazidime (CAZ), and Aztreonam but low resistance to aminoglycosides, especially Amikacin in both UTH and NTH clinical isolates. In Zambia, Amikacin is normally reserved to treatment for resistant tuberculosis and is not routinely used. This could have contributed to the observed very low resistance. This observation was also made in other studies where a high antibiotic resistance rate to CAZ and PIP was reported in both clinical and environmental *Pseudomonas aeruginosa* isolates (Fazeli *et al.,* 2012). According to the study performed in Iran (Taheri *et al.,* 2008), the antibiotic resistance rate of *Pseudomonas aeruginosa* isolated from clinical and environmental specimens to CAZ was high (77%). Clinical isolates were found to be more resistant to antimicrobial agents than environmental isolates, possibly because clinical strains had been subjected to the selective action of antibiotics (Bischofberger *et al.,* 2020).

5.5. Genes encoding for antimicrobial resistance

In multi-drug resistant *Pseudomonas aeruginosa*, in particular strains showing multiple resistance to *β*-lactam antibiotics, the wide range of resistance is dependent upon the hospital setting, extensive use of antibiotics as well as dissemination of resistance genes among these bacterial pathogens (Pachori *et al.,* 2019).

This study investigated the prevalence of *AMP*C, *BLAOXA*23, *BLAOXA*51, and *BLA IMP* genetic mutations as antimicrobial resistance determinants. *Pseudomonas aeruginosa*

multidrug resistance (MDR) was observed to be associated with the presence of *AMP*C, *Bla OXA*23, and *BLAOXA* 51 mutations. At the same time, XDR and PDR were associated with *BLA IMP*, *AMP*C, and *OXA* (23 and 51) mutations.

***AMP*C Gene**

Plasmid-mediated *Amp* C was first described in the 1980s in Gram-negative bacteria (Fallah *et al.,* 2013). In *Pseudomonas aeruginosa,* the overexpression of the naturally occurring *Amp* C is associated with a decreased susceptibility or resistance to expanded spectrum cephalosporins, such as ceftazidime (Mikhail *et al.,* 2019).

Given the high level of drug resistance in the strains evaluated in this study, the occurrence of genes related to antimicrobial resistance was investigated. In the current study, it was observed that majority of *Pseudomonas aeruginosa* clinical isolates carried the ampC gene (85.3%), related to cephalosporins resistance. This alarming incidence of ampC (or its overexpression) in clinical isolates of *Pseudomonas aeruginosa* was also reported by other authors (Tam *et al.,* 2007). Studies have shown that the rates of molecular detection of AmpC has varied among clinical isolates of *Pseudomonas aeruginosa* (Mirsalehian *et al.,* 2014). These differences between detection rates may be related to mutations affecting the *Amp* C gene or specificity of the different primers or methodologies that have been used for their detection in *Pseudomonas aeruginosa* (Nitz *et al.,* 2021).

In the current study, 65% of the clinical isolates were resistant to aztreonam, suggesting coexistence of resistance mechanisms including AmpC type β-lactamases or MexAB-OprM Efflux Pumps (Linares *et al.,* 2005; Bhalerao *et al.,* 2010).The same finding was reported by Shahcheraghi *et al.* (2010) where all metallo β-lactamase producing isolates were resistant to Aztreonam.

*Amp*C β-lactamase production which is not inhibited by Clavulanic acid, may cause false ESBL production results. Increasing occurrence of multiple β-lactamases in these clinical isolates could lead to therapeutic failure. Hence, early detection of β-lactamase production can benefit implementation of proper antibiotic therapy and infection control policies.

Mutation-dependent overproduction of intrinsic-lactamase *Amp*C is considered the leading cause of resistance of clinical strains of *Pseudomonas aeruginosa* to antipseudomonal penicillins and cephalosporins. Berrazeg *et al.* (2015) analyzed 31 Amp C-overproducing clinical isolates exhibiting more excellent resistance to ceftazidime than to piperacillin-tazobactam. Observations were made that the genes coding for *Amp*C confer a higher (2-fold to >64-fold) resistance to ceftazidime and ceftolozane-tazobactam than the gene from reference strain PAO1 (Deroche *et al.,* 2023).

***Bla OXA 23 and Bla OXa 51* genes**

Globally; in the last decade, strains that revealed resistance to carbapenem were mainly among Gram negative bacteria including *Acinetobacter baumannii* and *Pseudomonas aeruginosa.* The resistance mechanisms include decreased membrane permeability, efflux pumps and an enzymatic resistance to carbapenems. Carbapenemase is enzymatically classified into Ambler class A, B and D based on amino acid homology (Queenan *et al.,* 2007). Acquired class D *β*-lactamases, also known as oxacillin hydrolyzing enzymes (*OXA*) genes, structurally are different in amino acid from class A and C enzymes and are widely distributed among Gram negatives rods.

This gene encoding Ambler class D oxacillinase (OXA)-like carbapenemase (Bla*OXa* 51), a chromosomally encoded *β*-lactamase, has been demonstrated to be universally present in all *Acinetobacter baumannii* strains, and carbapenems AMR have been associated with the expression of blaOXA-51 gene (Glen *et al.,* 2021). In a study conducted in Brazil, Nitz *et al.*

(2021) reported the presence of the genes that conferred resistance to oxacillin (*bla*OXA-23 and *bla*OXA-51) in clinical isolates of *Pseudomonas aeruginosa* as unprecedented from the literature. Nitz *et al.* (2021) report of the first presence of *bla*OXA-23 and *bla*OXA-51 genes in *Pseudomonas aeruginosa* was from clinical isolates provided by routine services of two private laboratories of clinical analysis. The samples were from patients attending different hospitals in São Luís, Maranhão, Brazil (Nitz *et al.,* 2021). The genes confer resistance to oxacillin (*bla*OXA-23 and *bla*OXA-51) were present only in three of the 99 clinical isolates. Only one of these isolates carried *bla* OXA 51 and *bla* OXa 23 genes. They described this as the first report of blaOXA-23 and blaOXA-51 genes in *Pseudomonas aeruginosa*.

Despite *bla*OXA genes being reported more frequently in *Acinetobacter baumannii*, the current study reported for the first time in Zambia, a high prevalence of genes that confer resistance to oxacillin, *bla*OXa 51-like gene (62%) and *bla*OXA 23 (6%) in *Pseudomonas aeruginosa* clinical isolates from UTH and NTH, higher than that reported for the first time in literature (Nitz *et al.,* 2021). Reports from literature indicates that genes that confer resistance to oxacillin such as *blaOXa* 51-like gene naturally occurs in *Acinetobacter baumannii* (Kateete *et al.,* 2016). Carbapenem resistance in clinical isolates of *Acinetobactor baumannii* is mediated by overexpression of either OXA-23 or OXA-51 through insertion of ISAba1 in their promoter region (Wong *et al.,* 2019). However, several variants of *OXA* enzymes with extended spectrum have been identified in Pseudomonas aeruginosa (Shaikh *et al.,* 2015).

Pseudomonas aeruginosa resistance can be acquired through horizontal gene transfer from *Acinetorbacter baumanii* strains or mutations because adaptive immunity involves biofilm development, which serves as a diffusion obstacle, limiting antibiotic access to the bacterial cell (Michaelis *et al.,* 2023). Furthermore, a study at Mulago hospital in Uganda provided evidence suggestive of mobility for carbapenem resistance genes in the hospital environment

via horizontal gene transfer processes (Okoche *et al.,* 2015). A study in Sudan found that carbapenem resistant gene (*bla*OXA-51, *bla*OXA-23) were found in *Pseudomonas aeruginosa* (Hou *et al.,* 2015).

Metallo-beta-lactamase (class D MBLs) and carbapenem-hydrolyzing oxacillinase are the primary sources of carbapenem resistance (Queenan *et al.,* 2007). However, the rates of resistance to antibiotics in research vary depending on factors such as antibiotic type, genetic variance in bacteria and strains, and the variability in antibiotic usage habits among different countries (Muteeb *et al.,* 2015).

Overuse of antibiotics and the development of antibiotic resistance genes may lead to resistance strains because *Pseudomonas aeruginosa* is well known for its high inherent and acquired susceptibility to a wide spectrum of antibiotics (Pachori *et al.,* 2019). Antimicrobial resistance is a public health concern because it alters the natural bacterial community and increases resistance levels (WHO, 2023).

A study carried out in Taiwan revealed that 87.6% of Acinetobacter species had the *bla*OXA-51-like gene, 4.5% of the isolates harbored the *bla*OXA-23-like gene, and 3.2% of the isolates carried the *bla*OXA-58-like gene. Numerous recent studies have reported that most *Acinetobacter baumannii* isolates contain the *bla*OXA-51-like gene (Santajit *et al.,* 2022). Furthermore, this gene is commonly identified in nosocomial isolates from Asia and Europe, frequently coexisting with the *bla*OXA-23-like gene (Su *et al.,* 2023).

Pseudomonas aeruginosa clinical isolates needs to be further evaluated at UTH and NTH for potential horizontal gene transfer of these elements from *Acinetobacter baumanii* to *Pseudomonas aeruginosa* in our hospital settings. In this context, the emergence of antibiotic resistance is related to the ease of mutation, the extent of exchange of genetic information in bacteria by conjugation, transformation, and transduction, and the large-scale use of antimicrobial agents in the biosphere (Michaelis *et al.,* 2023). The *Pseudomonas*

aeruginosa accessory genome holds significant therapeutic interest due to its ability to acquire genes that improve virulence, antibiotic resistance, and general bacterial fitness through horizontal gene transfer (Grace *et al.,* 2022).

***IMP* Gene**

The first metallo-β-lactamase (IMP-1) in *Pseudomonas aeruginosa* was detected in Japan and since then the incidence of these agents has been increasing (Watanabe *et al.,* 1991). Other mobile genetic elements (MGE)-associated metallo-*β*-lactamase have also been identified in *Pseudomonas aeruginosa* and their prevalence rates are gradually increasing (Queenan and Bush, 2007; Ghamgosha *et al.,* 2015).

In the current study, only 5.1% of *Pseudomonas aeruginosa* clinical isolates contained *bla*IMP. These were six clinical isolates all belonging to patients with history of prior hospital contact and self-medication being treated at the University Teaching Hospital (UTH) in Lusaka, Zambia. Self-medication of antibiotics may have played a role in resistance acquisition. These clinical isolates were all multi-drug resistant and also harboured *AMP*C and *Bla OXA* 51 genes.

In Southern Thailand (Saengsuwan *et al.,* 2022), the most common carbapenemase gene in all *Pseudomonas aeruginosa* isolates was *bla*VIM (27.7%), followed by *bla*IMP (69, 23.9%) and others-*bla*OXA (11.8%). In this Thai study, all isolates collected from the hospital showed high genetic variation; two were MDR-PA. Other isolates were found from different sites and belonged to five genotypes. All 17 *bla*IMP-positive isolates belonged to 11 clusters, but one cluster, linked to two isolates from ICU-obtained sputum samples, had *bla*NDM, *bla*OXA 48, *bla*DIM, and *bla*GIM.

In Iran, a study aimed to detect *bla*IMP, *bla*VIM and other resistance genes among imipenem-resistant *Pseudomonas aeruginosa* clinical isolates in burn wounds at Velayat Hospital in Rasht, Iran (Zolfaghari *et al.,* 2021). *Pseudomonas aeruginosa* isolated from burn wounds were

assessed for antibiotic resistance patterns, and imipenem-resistant strains were selected for more phenotypic and genotypic investigation. The highest and lowest resistance levels were found in Tobramycin (59%) and Imipenem (24%).

This study determined the prevalence rate of imipenem-resistant *Pseudomonas aeruginosa* carrying the metallo-ß-lactamase (MBL) gene. The prevalence of *bla* IMP was low (5%) in agreement with findings in the current study which found the MBL *bla* IMP gene in 5% of clinical isolates (6/116) from UTH.

Metallo beta-lactamases (MBLs) exhibit the broadest spectrum and can hydrolyze penicillins, cephalosporins, and even carbapenems, and they are not inhibited by clavulanic acid or tazobactam (Bush *et al.,* 2007). Metallo beta-lactamases, which can hydrolyze otherwise stable carbapenems, have recently spread. They are now found in various species, such as *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Klebsiella* spp., etc. MBLs are a group of *β*-lactamase enzymes that need one or two zinc in their active site to cleave the amide bond of the *β*-lactam ring to inactive *β*-lactam antibiotics (Bebrone *et al.,* 2013).

Contrary to our findings, other studies found the prevalence of MBL genes to be high. In 2012, Plotto *et al.* investigated 56 *Pseudomonas aeruginosa* clinical isolates and showed that 17/56 (30.3%) of imipenem-resistant strains were positive for producing MBL (Polotto *et al.,* 2012). Furthermore, other studies also found a higher rate than our findings. Franco *et al.* (2010) found that 76.8% of strains were MBL-positive in a Brazilian study, and 90% of imipenem-resistant isolates were positive for the production of MBL in Iran.

5.6. Factors associated with antimicrobial resistance

The increasing rate of multidrug-resistant (MDR) strains has complicated medical therapy against *Pseudomonas aeruginosa* globally (Hirsch *et al.,* 2010). In addition, the ability of *Pseudomonas aeruginosa* to produce biofilm is thought to be a primary factor involved in chronic infections. Biofilms are complex microbial cells embedded in an extracellular matrix composed of proteins, extracellular DNA, and exopolysaccharides, providing a protective lifestyle for bacteria and are highly challenging and costly to treat with antimicrobial compounds (Kamali *et al.,* 2020).

The tendency of *Pseudomonas aeruginosa* to cause persistent infections with increased mortality rates is partly due to its ability to form biofilms (Yin *et al.,* 2022) readily. Biofilm formation allows the bacterium to circumvent host immunity and increase antibiotic resistance (Thi *et al.,* 2020). The biofilm components of *Pseudomonas aeruginosa* are composed of at least three distinct exopolysaccharides, including alginate (Chung *et al.,* 2023).

Association of self-medication and drug resistance

Globally, several studies have reported the increased resistance of *Pseudomonas aeruginosa* clinical isolates to a combined range of antibiotics. The level of resistance was reported to be associated with the common usage of antibiotics (Olesen *et al.,* 2018). Similarly, studies (Leopold *et al.,* 2014; Tadesse *et al.,* 2017) reported a high resistance level to commonly used antibiotics compared to less prescribed antibiotics in Sub-Saharan Africa. This could explain the low resistance rate to amikacin observed in the current study.

The development by the World Health Organisation (WHO) of the Access, Watch, and Reserve (AWaRe) classification system of antibiotics was a significant milestone in the fight against antimicrobial resistance (AMR) because this is a more objective, user-friendly tool to organize

the antibiotics as part of the antimicrobial stewardship (AMS) programs (Mudenda *et al.,* 2022).

The multivariate logistic regression model in the present study showed that *Pseudomonas aeruginosa* antimicrobial resistance was associated with self-medication, high-density residence, and surgical ward attendance. Antimicrobial resistance was also related to carbapenem-hydrolyzing β-lactamases gene Bla OXA-51 and Metallo-β-Lactamase Gene (*bla* IMP).

A study (Sachdev *et al.,* 2022) shared concerns similar to that of the current research regarding self-medication, which he referred to as the consumption of drugs such as antibiotics by individuals based on their own experience and knowledge without consulting a doctor for diagnosis or prescription.

There is an increase in self-medication at an alarming rate globally, particularly in low- and middle-income countries (LMICs) (Aslam *et al.,* 2020). This was similar to our findings, as these authors reported antibiotic misuse as the primary cause of the development of antibiotic resistance in microorganisms. Antibiotic misuse puts patients at risk for adverse drug reactions, false symptom relief, and the rise of drug-resistant organisms.

A study (Ahmad *et al.,* 2014) reported similar concerns about self-medication, particularly in underprivileged communities in Uttar Pradesh, India. The World Health Organization (WHO) has estimated that 10 million deaths could be attributed to an increase in AMR by 2050 if this problem is not addressed now (de Kraker *et al.,* 2016).

The association between self-medication and antimicrobial resistance observed in our study could result from repeated exposure to antimicrobial agents through over-the-counter access to

these drugs. For example, studies have reported a strong correlation between increased uses of ciprofloxacin and increased prevalence of ciprofloxacin-resistant strains (OKO *et al.,* 2022).

Hence, another factor associated with the increase in MDR-*Pseudomonas aeruginosa* is due to the frequent use of antimicrobial agents. This acquired resistance may be due to the consequences of mutational events or the acquisition of resistance genes through horizontal gene transfer and can occur during antibiotic therapy (Pachori *et al.,* 2022).

We found the high-density residence to be associated with antimicrobial resistance. This was in agreement with the finding of a study (Bruinsma *et al.,* 2003) which investigated the influence of population density on antibiotic resistance and observed a strong correlation that population density was an essential factor in the development of antibiotic resistance.

The current study also observed an association between antibiotic resistance and literacy status. This observation agreed with Muflih *et al.* (2021), who conducted a cross-sectional study utilizing the WHO multi-country public awareness survey to assess general knowledge of awareness of antibiotics and antibiotic resistance during the COVID-19 pandemic. Their findings were that education, health literacy, and antibiotic knowledge were substantially associated with greater awareness of antibiotic resistance. However, findings in the current study are at variance with theirs concerning the association with age.

5.7. Treatment outcomes

This study observed a low mortality rate of 5% during the follow-up period. During the study period, all mortality occurred among female participants. Nevertheless, the adverse outcome occurred in nearly half of participants who had complications and were bedridden. The majority of this group had pressure sores with long-term catheterization (30%). *Pseudomonas aeruginosa* infected caesarean section wound with sepsis was responsible for 34% of mortality cases.

Ferreiro *et al.* (2017) reported mortality of patients admitted with *Pseudomonas aeruginosa* urinary tract infections evaluated, regardless of the presence or absence of associated bacteremia, showing high mortality at 30 and 90 days of diagnosis (17.7% and 33.9%, respectively). This difference could be explained by the fact that in the current study, participants included stable patients with BPH awaiting surgery while a urinary catheter was inserted to facilitate urine drainage.

All mortality occurred in patients presenting with septic wounds (Pressure or diabetic ulcers and burns) with fever and hypotension. This could be related to *Pseudomonas aeruginosa* sepsis, spreading from the skin and soft tissue infection being the likely explanation. Prior hospital exposure and invasive medical devices (IV access and urinary catheter) were reported in all mortality cases, but these were not associated with AMR.

All cases of mortality were HIV-negative and reported no history of diabetes mellitus. Co-morbidities recorded included burn, malnutrition and sepsis. All patients who died were in-patients, but medical and surgical wards had equal mortality rates.

All *Pseudomonas aeruginosa* isolated in the mortality cases were sensitive to aminoglycosides (Amikacin, Gentamycin, and Tobramycin) and Carbapenems' (Imipenem), while no *Pseudomonas aeruginosa* clinical isolate isolated among mortality cases was positive to MBL (BLA IMP) gene. So, in these patients, mortality may not be associated with *Pseudomonas aeruginosa* AMR. It seems related to prolonged illness with complications such as infected pressure ulcers, sepsis, and multiple organ failure. However, further studies, including blood cultures, must be carried out to clarify this. Though the prevalence of other genes was high (AMPC and OXAs), it was high, too, in the group of survivors.

A study by Ferreiro *et al.* (2017) found the frequency of severe sepsis or septic shock of 8% at the onset in patients with *Pseudomonas aeruginosa* urosepsis, a low prevalence by the current

study findings. They further observed that the association of two empirically antipseudomonal drugs was not associated with improved survival. Still, the number of patients treated with combination empiric therapy was so small that this limited the validity of this conclusion. As observed in the current study, although the empirical antibiotic treatment did not demonstrate a relationship with mortality, Ferreiro *et al.* (20217) observed that inadequate antibiotic therapy was an independent risk factor for mortality at 30 days. Furthermore, Chamot *et al.* (2003) analysed the antimicrobial treatment performed in 115 episodes of *Pseudomonas aeruginosa* sepsis associated with clinical features of systemic inflammatory response syndrome. They observed that in 19.4% of cases, the final antibiotic treatment performed was inappropriate after susceptibility testing was available, with a mortality rate greater than 50% in the group of patients with inadequate treatment, being statistically significantly higher compared to the group of patients appropriately treated (Chamot *et al.,* 2003). In another study, Kim *et al.(*2003) presented a set of 136 patients with *Pseudomonas aeruginosa* bacteremia, of which 20% received inadequate definitive antimicrobial therapy, with a 75% mortality rate at 30 days in those with inappropriate therapies (compared with the mortality of 29% in those treated with appropriate antibiotics; P <0.001) (Kang *et al.,* 2003). Therefore, it is essential to stress the importance of constantly adjusting the prescribed treatment according to the antibiogram, regardless of the initial evolution that the patient had presented with.

5.8. Phylogenetic analysis of *Pseudomonas aeruginosa*

The Quantitative Insights Into Microbial Ecology (QIIME), a Pipeline to process data from high-throughput 16S rRNA sequencing studies, is a bioinformatic tool (Estaki *et al.,* 2019) used to analyse the 16s RNA gene sequences for phylogenetic analysis.

In the current study, *Pseudomonas aeruginosa* clinical isolates clustered into ten lineages corresponding to 10 different sequence types (STs), also belonging to other clonal complexes. In the current study, phylogenetic analysis demonstrated large phylogenetic distances.

Bacterial species are considered groups of strains that are characterized by a certain degree of phenotypic consistency, by a significant degree (70%) of DNA hybridization (Goris *et al.,* 2007; Wayne et al., 1987), and by over 98.7–99% of 16S ribosomal RNA gene sequence similarity (Stackebrandt and Ebers, 2006).

16S rRNA gene sequences are highly conserved among strains of the same bacterial species and are frequently used to identify and classify microorganisms. Although several *Pseudomonas aeruginosa* genomes have been completely sequenced and annotated as a single circular chromosome (Roy *et al.,* 2010; Mathee *et al.,* 2008; Stover *et al.,* 2000). The species' phylogeny has been determined only recently. Knowledge of this species' diversity and evolution is essential in developing tools for predicting antimicrobial resistance (AMR) and other factors. Knowledge about diversity could be closely linked to the source of AMR, crucial information for treatment, surveillance, and epidemiology.

Topological evaluations showed common origin for native strains with other known strains with available sequences at the GenBank database. A threshold of 97% similarity in the MLSA study of four housekeeping genes (16S rRNA, gyrB, rpoD, and rpoB genes) has been proposed by Mulet *et al.* (2010) for species differentiation in the genus *Pseudomonas*. A comparative study of their genomes, which has been performed for the *Pseudomonas aeruginosa* strain PA7, must be conducted to confirm this possibility. This strain is considered an outlier within the species, and its genome contains a similar number of genes as the other *Pseudomonas aeruginosa* strains, but more than 1000 exclusive genes were found in PA7 compared with the *Pseudomonas aeruginosa* strains PAO1 and PA14 (Roy *et al.,* 2010).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.0.Conclusion

This study estimated the prevalence of Pseudomonas aeruginosa nosocomial infection at 13.7%. Invasive medical device-related (urinary catheter) nosocomial infection was the most common presentation. *Pseudomonas aeruginosa* isolated from patients at UTH and NTH had the lowest resistance rate to Aminoglycoside antibiotics (Amikacin, Tobramycin, and Gentamycin), with the best sensitivity observed with Amikacin (87.2%).

This study has further demonstrated that multidrug-resistant *Pseudomonas aeruginosa* is highly prevalent in the hospital settings in Lusaka and Ndola districts of Zambia and possibly countrywide. The study found a high prevalence of drug resistance (73.6 %) with MDR (60 %) and possible XDR (37.2%) resistance patterns, respectively. The highest rate of resistance was found with fourth-generation (cefepime), third-generation (Ceftazidime) cephalosporins and monobactams (Aztreonam) antibiotics.

Antimicrobial resistance in clinical isolates of *Pseudomonas aeruginosa* at UTH and NTH was surprisingly associated with carbapenem-hydrolysing β-lactamase genes *bla*OXA-51 and *bla*OXA-23 commonly found in *Acinetobacter baumannii*. Other associations were with province of origin and surgical ward attendance. Most clinical isolates harbored the chromosomally encoded AmpC β-lactamase (85.3 %) and the carbapenem-hydrolysing β-lactamases *Bla OXA 51* (62%) genes.

Furthermore, the predictors of antimicrobial resistance were self-medication, high-density residence and surgical ward attendance. Other factors, such as self-medication, hospital exposure within 90 days, and literacy status, were also associated with MDR.

The mortality among patients with *Pseudomonas aeruginosa* nosocomial infection was low during the study period but adverse outcomes occurred in nearly half of the participants. Most of these complications occurred in patients who had prolonged admission with pressure sores and prolonged catheterization with sepsis.

Pseudomonas aeruginosa infected post-operation wound with sepsis was responsible for a third of mortality cases. Predictors of mortality were female gender, the presence of fever, and old age. In-patient status, history of prior medical visits, and self-medication were also associated with mortality.

All mortality cases occurred in patients presenting with septic wounds (Post-operative, pressure or diabetic ulcers and burns) with fever. Therefore, mortality seems associated with prolonged illness with complications such as infected pressure ulcers, sepsis, and multiple organ failure.

The phylogenetic tree showed that *Pseudomonas aeruginosa* from UTH and NTH clinical isolates clustered into various lineages corresponding to different sequence types (STs), also belonging to different clonal complexes.

6.1. STUDY LIMITATIONS

This study was conducted at two large teaching hospitals in Zambia. The use of convenient sampling (non-probability) had a potential for under-representing certain subgroups, therefore results cannot be generalized to the broader target population. This limits the ability to extrapolate our findings to the general population.

Further, the data was collected during the third wave of the COVID-19 pandemic. During this period, the Ministry of Health scaled up infection prevention control measures with regular spraying of the wards, contributing to reduced isolates from the environment. Strict quarantine measures for patients with respiratory symptoms limited the number of clinical isolates from the respiratory system since most of such patients were restricted from coming to the hospital but rather quarantine from their home.

6.2. Conflict of Interest

There is no conflict of interest.

6.3. RECOMMENDATIONS

Based on the findings of this study, the following are the recommendations:

a) The Ministry of Health, through various hospital infection prevention committees, needs to review the current antibiotic prescription policy and infection control program (ICPs) to control the spread of MDR-*Pseudomonas aeruginosa* in this environment.

b) Furthermore, the Government, through the ministry of health, should implement antimicrobial stewardship programs (ASPs) to address the growing problem of *Pseudomonas aeruginosa* antibacterial resistance as a critical strategy for controlling bacterial resistance and improving clinical outcomes.

c) Further research, including blood culture and virulence genes, is required to expand this study to regional hospitals to obtain the national burden and AMR profiles of *Pseudomonas aeruginosa* nosocomial infections to implement evidence-based infection prevention measures based on identified predictors of *Pseudomonas aeruginosa* AMR and mortality.

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APPENDICES

Appendix 1

Patient's Information sheet

Title: Information sheet for the study on Clinical and Molecular Evaluation of *Pseudomonas aeruginosa* Infections in adult patients. In Zambia

You are invited to participate in this study which is looking at the Clinical and Molecular Characterization of *Pseudomonas aeruginosa* Infections in adult patients in Zambia. The study is being conducted as the requirement in fulfillment of PhD of Infectious Diseases.

This document will provide you with information about the study and one of the investigators will be available to explain the contents and answer any questions you may have. Please be sure that you understand the contents of the document and seek clarification where you are unclear.

Should you decide to participate in the study, you will be asked to give consent before you take part.

Participating in the study is purely voluntary; you are under no obligation to take part. You are also free to withdraw from the study even after giving consent. Should you decide not to participate or indeed decide to withdraw from the study, it will have no impact on your medical care. You are also not obliged to answer questions you are not comfortable with.

There will be no financial rewards for participating in the study

Study Title

Clinical and Molecular Characterization of *Pseudomonas aeruginosa* Infections in adult patients in Zambia.

Who is doing the study?

The doctor conducting this research is Dr Patrice Mukomena who is being supervised by Professor Muma and Dr Soddy Munsaka. Dr Mukomena will be responsible for the daily

activities involved in running of the study. The named doctors can be contacted through the African Center of Excellence in Infectious Diseases (ACEIDHA), University of Zambia.

Dr Patrice Mukomena Ntanda can also be contacted on Tel +260 979 832265, email patricemuko@gmail.com or through the department of Medicine at Eden University School of Medicine and Health Sciences Great East Campus opposite the University of Zambia Great East Campus.

My supervisors are **Professor John Bwalya Muma** and Dr Sody Mweetwa Munsaka. Professor John Bwalya Muma can be contacted on Tel +260 966744355 or jmuma@unza.zm and at the Department of Diseases Control, School of Veterinary Medicine, University of Zambia, Great East Campus in Lusaka.

Dr Sody Mweetwa Munsaka can be contacted at the School of Health Sciences at the University of Zambia or be contacted at +260977925304 and s.munsaka@unza.zm.

This study has been approved by the Biomedical Research Ethics Committee of the University of Zambia. The Biomedical Research Ethical Committee of the University of Zambia office is at Ridge Way Campus along Nationalist Road opposite UTH Paediatric Hospital in Lusaka Zambia and can be contacted at +260-1-256067. Fax: +260-1-250753 and E-mail: unzarec@unza.zm. It **Federal Assurance No. FWA00000338**

What is the purpose of the study?

The purpose of the study is to identify the reasons why people who are admitted to hospitals for other illnesses sometimes minor end up getting infected while in hospital with germs which most of the time are found in hospitals. Some of these germs infecting people in hospitals can be dangerous because they are difficult to treat. One of them is called *Pseudomonas aeruginosa*, and it is a very dangerous germ. It is for this reason that me and my supervisors want to know more about what makes it difficult to treat this germ. We are hoping that if we know that, people who are infected with this germ can be helped.

These infections which people get while in hospitals are known as hospital acquired infections or nosocomial infections and people critically ill such as those in intensive care unit (ICU) and people who have medical devices inserted in them are more vulnerable to such infections. Some of the antibiotics doctors commonly use in hospital cannot be used to treat the patients because such germs resist commonly used antibiotics.

Therefore, it is very important that doctors do research to find out why these antibiotics have become resistant so that we could help out ~~HIV~~ patients who present to the hospital with such infections.

This study is being conducted to identify the factors associated with *Pseudomonas aeruginosa* resistance. In addition, the study will identify the characteristics of patients who come in with particular conditions in order to identify factors associated with those conditions. This is information which can provide an understanding of reasons why *Pseudomonas aeruginosa* particularly in patients in ICU and with medical devices. This study will also determine the outcome of Zambian patients admitted or developing such infections while in hospitals.

Procedure of the study

Should you agree to participate in the study, you will be given a consent form and asked to sign it. A copy of this information sheet and of the consent form will be given to you; please ensure you keep it safely.

You will then be asked for information about your background such as your age, marital status, place of residence, occupation and level of education. Further information on your past medical history and the history of the current symptoms will then be obtained. During history taking, the doctor will ask you questions and collect answers about your symptoms and risk factors of diseases. During this period, you will be free to skip questions you are not comfortable answering. You will also undergo a physical examination.

Depending on which part of your body the doctor suspect that you might be having infections in, sample of either urine, sputum, pus or blood will then be taken to identify the germ making you sick and the antimicrobial resistance pattern.

Following this, the study doctor will monitor your progress for as long as you remain in the hospital unless this period goes beyond 30 days from the time you were admitted. This will be done in order to document any other test results that may be conducted during your hospital stay by the doctors in the unit that is looking after you in arriving at your final diagnosis.

Are there any risks involved from taking part in this study?

There is potential risk that possibly you get stressed with answering many questions but this issue can be addressed by you feeling free to skip questions you may find to personal or those you are not comfortable to answer. Feel free to answer when you are comfortable to do so.

There is also the potential risk of pain or discomfort in case doctors establish that blood might be drawn to find out if the germs entered your systems. There is also the potential risk of bleeding from the blood drawing. However, these will be minimized by ensuring that only qualified people collect these samples.

Benefits of this study

The findings of the study after examining and collecting samples from patients will be used by attending physicians to manage the participants as a direct benefit of the study. ~~There are no direct benefits for you as a participant.~~ Furthermore ~~However, the~~ information which will be gathered from this study can be used for the development of protocols or guidelines towards improving the medical care of people admitted at hospitals at UTH, NTH and Matero Level one.

Confidentiality

All information collected from you is strictly confidential. There is no point in the study at which your identity will be disclosed. The study will use number codes as identity for the data collection and information will be securely kept. Information will only be accessible to the medical staff involved with the research. However, clinical information and test results obtained as part of this study can be shared with your attending doctors in order to improve your clinical care.

If you have any grievances or complaints during the study, you may contact the biomedical research ethics committee on the contact details you will be given before assessing you. If you have any questions concerning the study you are free to ask now and at any time point; you can direct these questions to Dr Mukomena, or professor Muma or Dr Soddy Munsaka on the contacts given above.

Appendix 2

CERTIFICATE OF CONSENT

Informed consent form for the study on Clinical and Molecular Evaluation of *Pseudomonas aeruginosa* nosocomial infections in adult patients.

I have been invited to take part in a study being conducted at (tick when appropriate)

- at the University Teaching Hospital (UTH), or
- The Ndola Teaching Hospital (NTH)

The study is being conducted by Dr Patrice Mukomena Ntanda, professor Muma and Dr Soddy Munsaka as part of PhD study at the University of Zambia.

Contact number +260 979832265, +260966744355

Email patricemuko@gmail.com, jmuma@unza.zm

The study is being supervised by prof Muma and Dr Sody Munsaka Contact numbers +260 966744355

I have been told the purposes of the study. I understand the processes involved including any potential distresses.

I have been given the list of names of people as well as institutions I may contact relating to this research.

I have read information in the Clinical and Molecular Characterization of Pseudomonas aeruginosa Infections in adult Zambian patient's information sheet

.

I have had the opportunity to ask questions and have had them satisfactorily answered

I understand that I have the right to refuse to take part in the study, or to skip a question I am not comfortable with and that I have the right to withdraw from the study at any time without

compromising the quality of care. I understand that should I refuse to take part in the study or withdraw at any time, it will not affect my clinical care in any way.

I agree to take part in the study

PARTICIPANT'S INFORMATION

SIGNATURE_____or FINGERPRINT _____

SURNAME_____

NAME_____

DATE_____

THE PERSON WHO CONDUCTS THE INFORMED CONSENT DISCUSSION MUST ALSO SIGN AND DATE THIS FORM

SIGNATURE_____

DATE_____

SURNAME_____

NAME_____

SIGNATURE OF WITNESS IF APPLICABLE_____

WITNESSED BY_____

SIGNATURE OF WITNESS _____

DATE_____

FORMU YA KUSUMININAPO UKUBA MU KUSAMBILILA (KU BAFYASHI NELYO KU BASUNGA ABANA)

UMUTWE WA KUSAMBILILA:

Ubulwele bwa tushishi twa *Pseudomonas aeruginosa* twikata balya ba ikala sana pa chipatala Cha University Teaching Hospital na cha Matero Level One na pa Ndola Teaching Hospital mu Zambia

Abalefwailisha: Ba Patrice Mukomena Ntanda (Dr)

Bambi Abalefwailisha: John Bwalya Muma (Prof.), Ba Sody Mweetwa Munsaka (Dr

Mulishani ninebo Patrice Mukomena nafuma ku Schoolu I kalamba ya Zambia. Tulefwaya ukusambilila pa tushishi tusangwa mu bantu mu Zambia. Bantu nga baikala san aba chipatala utu tushishi twa baingila.

naisa mungafwilishe ine nakulamupusha elo imwe mwakula njasuka. Nga mulesuminisha, natotela sana. Nde mita ukungafwilishako pakufwilisha ichishinka pali u bulwele bwikata abantu ba chipatala. Ichi ichisambililo chilechitwa pantu efilefwaika ukungafwa muli ayaamasambililo yabu doctor of philosophy. Fyonse efyo mulefwaika ukwishiba fili muchi paper ichichine. Kwalaba umuntu umo uwalalondolola fyo mulingile ukuchita no kwishiba nga namukwata amepusho twalomba kutimwipushe. Twalomba ukuti mwishibe fyonse ifili muchipepa umu. Nga mwasumina ukutwafwa twalamilomba noku signer pachipepala.

Ubulondoloshi bunono ubwa Masambililo

Limbi mwalishiba ukuti amalwele ayengi mu bantu yaletwako no tushishi kabili icilenga fya kulya na menshi umuli utushishi. Pa mulandu wa kubomfya imiti ya maka kufuma mu

market ne kutwala mumayanda ishapusanapusana na mu bantu, utushishi tumo tulatwalilila ukubako nangu kube ukundapwa kwa musango yu. E mulandu tulandila ukuti utushishi tatufwa ku miti imo.

Muli uku kusambilila tulefwaisha ukusambilila pa tushishi utushifwa utuleta ubulwele bwa mubili ku kaba. Ifyebo fyalasendwa muli uku kusambilila fikabomfiwa ku kwishiba nga ca kuti abalwele balekwata utushishi twakosa ukufuma mu fipatala ne kulwalika a bantu. Ifyebo twalasenda muli kuno kusambilila fyalaba ni nkama kabili fikabomfiwa fye mu cinkumbawile kabili tafyakeshibikwe umwine wa fiko.

Nga twapwisha ukupima muli uku kusambilila limbi kuti twasunga fyonse ifyo twasendele kuli imwe nangu kuba lwele pa kuti tukafipime ku ntanshi. Uku kupima kukabako nga ca kuti utushishi utupya twaishibikwa nelyo nga ca kuti kwaba ukupima ukupya. Fyonse ifyo bakasenda kuli imwe tabakalembepo ishina pa kuti tafishibikwe umwine.

Nga ca kuti namukwata amepusho, ifya kuilishanya, nelyo amafya ayali yonse pa mulandu wa kuba muli uku kusambilila, kuti mwatumina ba Mercy Mukuma, aba ku Dipartmenti Icingilila Amalwele, ku cipani ca *School of Veterinary Medicine*, pe sukulu likalamba ilya *University of Zambia* (pa namba +26 096 7401451 nelyo pa patricemuko@gmail.com . Ukufwailisha konse pa kuitemenwa kwa bantu kupitulukwamo na komiti iibomba umulimo wa kucingilila insambu shenu no bumi bwenu. Nga ca kuti namukwata amepusho nelyo ifili fyonse ifilemusamika pa lwa nsambu shenu ifikumine ilyashi balefwailisha kuti mwalanda na ba, nga tamulefwaya ukwishibikwa landeni, kuti mwalanda na ba mwi ofesi lya University of Zambia Biomedical Research Ethic Committee pa Ridgeway Campus mu musebo wa Nationalist Road mu Burma, mu Lusaka ne mu Zambia. Amanamba ya foni aye ofesi lya ba UNZA Biomedical ni +260977925304 na keyala ka pa intaneti ni smunsaka1@gmail.com

Bushe ukusaina kwenu (nelyo icifwati) pali iyi formu kulepilibula cinshi?

Ukusaina kwenu (**nelyo icifwati)** pali iyi formu kulepilila:

- Ukuti nabamweba pa bufwayo bwa uku kusambilila, ifyo kukaba, ubusuma bulimo no bubi bulimo.
- Ukuti nabamupeela inshita ya kwipusha amepusho ilyo mushilasaina.
- Ukuti namuitemenwa ukusumina ukubamo muli uku kusambilila.

Mukwai lembeni nga ni Ee nelyo Iyo

- Nasumina ukwipushiwa kabili nasuminisha ukuti ifipepala fya fyebo fyandi ifya ku cipatala fisendwe pa kwasuka amepusho muli ukusambilila ☐Ee ☐Iyo
- Nasuminisha ukuti basende, mulopa, fikolala na misu pa kuti bayabomfye muli uku kusambilila ☐Ee ☐Iyo
- Nasumina ukuti mulopa, fikolala na misu yandi yasungwe pa kuti bakayapime ku ntanshi. ☐Ee ☐Iyo

Ishina lya mufyashi nelyo abasunga umwana:

_____ 

_____

_____

Ukusaina kwa mufyashi nelyo abasunga umwaice nelyo Icifwati Ubushikiu

Pa kusaina pano, nasumina ukuti nimbelenga no kumfwikisha ifyebo fili pali formu wa kusuminishanyapo kabili nasumina ukuti umwana wandi abemo muli ukusambilila.

Ishina lya kwa Kambone: _____

_____ _____

Ukusaina kwa kwa Kambone Ubushiku

_____ _____

Ukusaina kwa bapoka formu Ubushiku

_____ _____

Ukusaina kwa kwa Kambone Ubushiku

Ukusaina kwa kwa kambone pa mulu kulelangilila ukuti umuntu umbi namonako ukusuminisha kwa mufyashi nelyo ukwa basunga umulwele. Kambone tafwile ukuba na kapatulula kabili tafwile ukuba mu bantu balesambilila.

Appendix 3

Study Questionnaire

Questionnaire for the study on Clinical and Molecular Characterization of *Pseudomonas aeruginosa* nosocomial Infections in adult patients in Zambia.

a. **Hospital/Site 1. UTH**_____**2.** Ndola Teaching Hospital_____

b. **In-Patient?** _____**Out-patient?** _____

c. **Ward** 1. Medical_____2. Surgical _____

d. **City? Province?** 1. Lusaka_____2. Copperbelt_____

e. **Date?** _____/_____/_____

f. **Review? Visit #?** _____ Date_____/_____/_____

g. **Investigator Name** _____**Sign**_____

I. DEMOGRAPHIC CHARACTERISTICS

Note that personal information such as names, phone and ID numbers will only be accessible to the principal investigator (PI) as additional identifiers and will be used after decoding from the study number by the PI for long term follow-up while upholding confidentiality. The principal investigator will then assign a study number ranging from Clinical & Molecular Pseudomonas Study (CMPS) CMPS 001 to CMPS 265 to all study participants after de-identifying them.

1. What is your Name?

_____

2. What is the Study CMPS Number?

_____

3. What is the File Number from hospital records?

_____

4. What is your NRC or other ID?

_____

5. What is the Patient or Next of Kin Phone or contact?

_____

6. How old were you at your last birthday? (or What is your year of birth?)

_____

7. When were you born?

_____

8. What is the sex recorded on your official identification document? Sex: 1. M_____2. F_____

9. What do you do for a living (Profession)?

_____

10. Which of the following level of education did you achieve? 1. None _____ 2. Primary__ 3. Secondary _____ 4. College _____ 5.University _____

11. How would you describe your residence from the following?

1. Urban? _____2. Rural? _____

City/town/village Name _____ Province _____ Country_____

12. Which ethnic group or tribe or clan do you belong to?

_____

13. Which of the following ward are you receiving treatment from now or in the recent past? 1. Medical Ward? _____ 2. Surgical Ward? _____

14. Have you frequented for any reasons (e.g. Medicals, visiting patients or other) any clinic, hospital or home based care institutions in a recent past (e.g. within past 90 days)?

15. If the answer to the above question is yes, kindly state the reason for the visit? 1. Medical____ 2. Surgical _____

16. Are you currently or past 90 days been admitted to the Intensive Care Unit/ High Dependency Unit (s)?

17. If the answer to the above question is yes, kindly state the reason for the visit? _____

18. How would you describe your Marital status from the following? 1. Married __ 2. Single ___ 3. Divorced ___ 4. Widow ___ 5. Complicated situation (describe)_____

19. What religious group from the following to you best associate with? 1. Christian-Catholic_____ 2. Christian-Other Denominations_____ 3. Muslims _____ 4. Hindu _____ 5. Buddhism/Shintoism _____5. None_____

II. HISTORY TAKING

1. What are your current complaints or symptoms?

_____

_____

___

2. For how long have you been admitted to the hospital?

_____

3. Did you present with current symptoms since hospital admission?

_____

4. What was the duration of hospital stay before you started presenting with current complaints?

_____

__

5. What was the duration of intensive care unit (ICU) stay before you started presenting with current complaints?

_____

6. When did you start getting sick of these symptoms?

7. How did you get sick and symptoms progress until now?

8. Have you observed any change in the body temperature or abnormally felling cold?

9. Have you recently developed a sore on the skin?

10. Have you sustained recently any burn injury?

11. Have you, in recent past been inserted with medical devices (e.g. urinary catheter)?

12. Do you experience pain or itchiness when passing urine?

13. Have you been, prior to this illness, admitted or attended to by a medical personnel for any illness?

14. What did the doctor or the health worker say that you were suffering from?

15. Have you recently been taking care of a relative or friend sick of fever?

16. Have you ever been told by a doctor that you were suffering from a medical condition in the Lung called Chronic Obstructive Pulmonary Disease (COPD)?

17. Have you ever been told by a doctor that you were suffering from a genetic medical condition in the Lung called Cystic Fibrosis?

18. Have you ever been told by a doctor that you were suffering from a medical condition of the blood called Neutropenia?

19. Have you ever been told by a doctor that you were suffering from a medical condition of the kidney called acute kidney failure?

20. Have you ever been told by a doctor that you were suffering from a medical condition of the kidney called Chronic Kidney Disease (CKD)?

21. Have you ever been told by a doctor that you were suffering from a condition called enlargement of the prostate?

22. Have you ever been told by a doctor that you were suffering from a medical condition called Cancer?

23. Have you ever been told by a doctor that you were suffering from a medical condition called HIV and AIDS?

24. Have you ever been told by a doctor that you were suffering from a medical condition causing high blood sugar called diabetes mellitus?

25. Have you ever been told by a doctor or a nurse that you were suffering from a medical condition causing immunity to call down called HIV/AIDS infection?

26. Have you ever been told by a doctor or a nurse that you were suffering from a medical condition causing immunity to call down for any other reason?

27. Are you on dialysis for treatment of kidney disease by blood or abdominal cleaning?

28. Have you currently or in the recent past (90 days) undergone any invasive surgical procedure?

29. What did the doctor say the operation was done for?

30. Do you currently have a medical device called urinary catheter inserted into your body?

31. Do you currently have an intravenous cannula inserted into your body?

32. Are you currently or in recent past (90 days) taking any medications?

33. Did a doctor prescribe to you pills called steroids within the past 90 days?

34. Did a doctor prescribe to you pills referred to as "immunosuppressant agents" within the past 90 days?

35. Are you taking some medicine used to treat infections called antibiotics?

36. Have you recently (past 90 days) been prescribed by, a doctor medicines known as broad spectrum antibiotics?

37. Have you in a recent past decide to buy medications over the counter without medical prescription to treat symptoms of diseases in you or people you live with?

38. Is there among the people you live with or interact with someone who has fever?

39. Is there among the people you live with or interact with someone who currently or in a recent past had a cough?

40. Do you smoke cigarettes?

41. Do you sniff traditional tobacco leaves in Zambia known as nsunko?

42. Do you drink alcoholic beverages?

43. What types of alcoholic beverages to you drink?

44. Could you estimate quantities of alcohol you consume per day and per week?

45. Have you recently visited a medical institution such as a hospital, a clinic or a home for the sick?

46. Are you feeling any pain or itching when passing urine?

47. Have you ever been told by a doctor that you have a disease of the pancreas called pancreatitis?

48. Prior to this illness, have you ever been told by a doctor that you have a disease of the lungs called pneumonia?

49. How long has the patient been admitted to the hospital?

III. CLINICAL ASSESSMENT (PHYSICAL EXAMINATION)

1. How are the patient's Vital signs during examination?

a. What is the Temperature during examination in ^{o}C? _____

b. How did the patient temperatures fluctuate during Nursing reviews (Refer to the temperature chart and whenever possible keep a copy)?

c. What is the blood pressure in _____mmHg during reviews?

d. How did the blood pressure fluctuate during medical reviews (Refer to blood pressure chart whenever possible)?

e. What is the Respiratory Rate per minute during examination? _____/min

f. How did the patient respiration rate vary since observation began?

g. What is the Pulse Rate during examination?

h. How did the pulse rate vary from the time observation began?

i. What is the oxygen saturation at room air during examination?

j. How did the oxygen saturation at room air vary since observation began?

2. General Examination

a. General condition

i. How is the patient Glasgow Coma Scale?

ii. Is the patient oriented in Time, Place and Person?

iii. Does the patient appear in obvious respiratory distress?

iv. Is the patient having medical devices connected?

v. How are the nails on inspection? (Schamroth's angle open or closed?)

vi. If yes, kindly specify the type of medical device from the list below:

Intravenous Cannula_____ Urinary Catheter_____

Endotracheal Tube_____ Surgical Stiches_____

Nasogastric tube _____Ear implant_____

Others_____

vii. What is the duration of the medical device insertion?

viii. IS the patient bedridden for a chronic medical condition?

ix. How would that medical condition expose to infection

b. Body Mass Index

i. What is the patient's Weight?

ii. How has the patient Weight changed recently?

iii. What is the patient height?

iv. What is the patient abdominal circumference?

3. Systemic Examination

a. **Skin:**

i. Is the patient having skin lesions on inspection?

1. Pressure sores_____

2. Ulcers_____ 3. abscess_____ 4. Others_____ 5. Burns_____ 6. Vesicles_____

7. Pustules_____

ii. Inspect pressure point for bedridden patients: 1. Presence of changes prior to pressure sore formation _____ 2. Pressure sore present_____

iii. Examine for signs of infections: 1. Discharging pus_____ 2. Green discoloration _____3. Yellow discoloration_____

4. Other _____

b. Respiratory examination

i. Is the patient in respiratory distress? Use of accessory respiratory muscles? _____ Is the respiratory rate above 18 per minute? _____

ii. Is the vocal fremitus sound modified as follow? Reduced_____ Increased_____ Normal _____

iii. Are Percussion sounds modified? Normal _____Area of dullness or stony dullness_____ Resonant? _____

iv. Is the vesicular breath sound on auscultation modified as follows? 1. Normal_____ 2. Presence of crepitation's _____ 3. Presence of Bronchial Breath Sound (BBS) _____

c. Urinary System examination

i. How is the urine appearance on inspection? (From urinary catheter or urine samples) Normal amber _____ Cloudy_____

ii. Is there presence of tenderness of the following areas? Renal angle tenderness _____ flanks tenderness _____ lower abdominal tenderness _____

d. Gastro-intestinal tract

i. What is the appearance of the patient stool? Bloody? _____ Watery? _____

ii. Is the patient having areas of abdominal tenderness?

e. **IS the patient having signs of systemic inflammatory response syndrome (SIRS) as follows?**

i. Presence of Systolic Blood pressure value < 90 mmHg Mercury _____

ii. Presence of Diastolic Blood Pressure < 60 mmHg Mercury _____

iii. Presence of Heart beat < 90 bpm _____

iv. Presence of Temperature < 36 ^{0}C _____

v. Presence of Temperature > 37.5 ^{0}C _____

vi. Presence of respiratory rate > 20/minutes _____

vii. Presence of respiratory rate < 16/minutes _____

viii. Presence of White Blood Count < 4,000 X 10^9 _____

ix. Presence of White Blood Count > 10,000 X 10^9cells/mm^3 _____

4. HOSPITAL ENVIRONMENT ASSESSMENT.

Using agar fingertip impression plates to investigate for the hands or gloves of health professionals as sources of bacterial contamination in the following:

Screen for the presence of *Pseudomonas aeruginosa* on hands of wards doctors

- The participants will be asked to put all 4 fingertips, excluding the thumb, in a practically horizontal position for 2 seconds onto the agar plate (Pickering, 2011).
- This will be followed by placing the thumb onto the same side of the plate in the remaining space at the center of the plate.
- The fingertips of the other hand will be put onto the opposite half of the plate in the same way.
- Each agar plate will be transported from the collection point in a cooler maintaining a low temperature with gel ice packs. Once in the laboratory, the plates will be placed in the incubator at 37 degrees Celsius for 24 h.
- Then Pseudomonas will be identified from the colonies using biochemical and other phenotypic analysis.

ii. Screening for the presence of *Pseudomonas aeruginosa* on wards nurses' hands

- Participants (In a ratio of 1:3) will be asked to put all 4 fingertips, excluding the thumb, in a horizontal position for 2 seconds onto the agar plate (Pickering, 2011).
- The rest as above

iii. Screening for the presence of *Pseudomonas aeruginosa* on bedsider's hands

- Participants will be asked to put all 4 fingertips, excluding the thumb, in a horizontal position for 2 seconds onto the agar plate (Pickering, 2011).
- The rest as above

a. investigate surfaces biofilm of the hospital environment in the patient ward as possible deposits and source of resistant bacteria hospital acquired infections in the following (de Oliveira, 2010):

Selection of sampling sites will be made in consultation with the heads of departments and attending doctors targeting the most representative and most critical location in each ward and patient immediate environment as follows:

Random, undirected sampling will be collected. Sterile swabs will be moistened in sterile normal saline and rolled over the targeted inanimate surfaces/equipment separately for the following:

- Ward hand wash station surfaces
- the presence of bacteria on monitors,
- bed grids,
- patient gowns
- bed linen
- bedside furniture
- tables
- tap/faucet handles,
- telephones,
- keyboards and other objects

IV. LABORATORY INVESTIGATIONS

General investigations

a. Full blood count

h. Collect 5 mls of blood in a sterile purple top (EDTA) specimen container

i. Using an automated blood count analyzer determine the profile of blood count

b. Urea & Creatinine

j. Collect 5 mls of blood in a sterile green top specimen container

k. Using an automated blood chemistry analyzer determine the chemistry profile

c. Urinalysis

Using aseptic procedure, collect 5 mls. midstream urine in a sterile plain bottle
Using urinalysis strips determine the following biochemical & cellular changes in urine:

*Presence of glucose in urine
*Presence of protein in urine
* Presence of nitrate

d. Urine Microscopy

l. Collect 5 mls of midstream urine in a sterile plain specimen container

m. Ensure analysis done within 30 minutes otherwise use refrigeration

n. Look for the following changes:

* Presence of more than 4 white cells
*Presence of tubular debris (granular cast)

e. Random or Fasting blood sugar

o. Using aseptic procedure, collect a drop of blood
p. Using an automated glucometer, determine the fasting and/or the blood sugar levels

f. Serum Retroviral Test for HIV (RVT)

q. Using aseptic procedure, collect a drop of blood

r. Using an HIV confirmatory Rapid Diagnostic Test according to the Ministry of Health Zambia (MoH) as follows:

HIV + ve _____HIV –ve _____HIV Undetermined

_____ HIV Unknown Status _____

g. **Cystic Fibrosis Screening: Suggestive history:** Recurrent ancreatitis_____ nasal polyps_____ Chronic sinusitis_____ Chronic lung infections_____

Laboratory test: Immunoreactive trypsinogen (Serum IRT) _____ Chloride sweat test_____ No available test_____

Diagnostic investigations

i. Sample Types (Tick where appropriate)

a. **Midstream Urine** _____
b. **Sputum** _____
c. **Bronchial secretion** _____
d. **Skin Swabs** (Specify from the following): Wound Swab_____ Pressure sore Swab_____ Burns Swab _____ Abscess Swab _____
e. **Medical devices Swabs** (Kindly specify) Catheter tips_____ Endotracheal tube tips _____ Peritoneal drain tip
f. **Other samples** (Kindly specify) _____

ii. Transportation of samples

a. Targeted labs for sample analysis

- Unza Laboratory Veterinary School
- UTH Laboratory – Microbiology
- TDRC Laboratory - Ndola

b. Specimen collection, handling and transportation

- Use of universal precautions for collecting and handling all specimens.

  Done_____ Not done_____

- Has patient received any antibiotics prior to specimen collection? (If yes, Specify)

  _____

- Avoid contamination with indigenous flora by respecting strict aseptic technic. Done _____ Not done _____

- Are all specimens appropriately labeled with two patient identifiers? Yes_____ No _____

- Are the following identifiers included: patient name (Only for principal investigator) _____, birthdate _____, hospital number _____, Ward _____, hospital service and department _____, date and time of collection _____, specimen type _____ and tests requested _____?

- Is a requisition written to accompany each different specimen type? Yes _____ No _____

- Is the sample transported to the nearest lab (Vet Lab, UTH for Lusaka and TDRC for Ndola) within time as follow? Within 30 minutes to one hour? _____

- Are specimen delivered to

c. Specimens should be in tightly sealed, leak proof containers and transported in sealable, leak-proof plastic bags. Specimens for TB should be double bagged. Specimens should not be externally contaminated. Specimens grossly contaminated or compromised may be rejected.

d. If anaerobic culture is requested, make certain to use proper anaerobic collection containers.

e. Further questions may be referred to Professor Muma on 0966744355, Dr Sody M. Munsaka or Dr Patrice Mukomena Ntanda on 0979832265.

iii. Bacterial Cultures

Bacterial Cultures: Transport at room temperature (Within one hour) unless otherwise specified.

1. Skin and soft-tissue infections:

- **Abscess**

Technique– To remove surface exudate by wiping with sterile saline or 70% alcohol. Then aspirate content with needle and syringe. Cleanse rubber stopper with alcohol;

Allow to dry 1 min before inoculating; push needle through septum and inject all abscess material on top of agar.

Or if using a swab must be used, pass the swab deep into the base of the lesion to firmly sample the fresh border. Then tick from the following:

Specimen Transport Time≥ 2 hours _____

Specimen Transport time ≤ 2 hours. _____

Culture Results _____

- **Burn**

Technique- To clean and debride burn (as cultures of surface samples can be misleading) and place tissue in sterile screw cap container than transfer aspirates to a sterile container (For aerobic culture only).

Culture Results _____

- **Decubitus ulcer**

Technique– A swab is not the specimen of choice. To cleanse surface with sterile saline. Collect tissue or aspirate inflammatory material from the base of the ulcer in a sterile tube or anaerobic system. Transport time ≤2 hours.

Culture Results_____

2. Urine Culture

Technique – Cultures should not be performed as an add-on test to urinalysis but collect separate sample. To collect 4 mL of urine in a sterile specimen container. Use sterile technique to transfer urine to a gray top C&S urine container. Tubes must be filled to 3 mls to prevent inhibition of bacterial growth. Transport to the microbiology laboratory. Transport urine specimens to the Microbiology Laboratory or refrigerate within 30 minutes. Refrigerated specimens should be delivered to the lab as soon as possible, and may be discarded if not analyzed within 24 hours of collection.

Culture Results _____

3. Urinalysis

Technique. To collect separate sample for urinalysis (random urine yellow top, round bottom tubes no additive) and culture (as above).

Midstream clean catch method: Patients should be instructed to wash hands prior to collection and offered exam gloves.

- **Technique for female patients** (Tick if this technique used). _____ Instruct female patient to wash hand, sit on toilet with legs apart and cautiously opening sterile container without touching the insides of the jar or lid. Spread labia with one hand.

Void first in the toilet and then, while continuing voiding, to hold specimen container in "midstream" to collect sample. While touching only the outside of the lid, put the lid on the cup. Carefully replace the lid. Handle the specimen as sterile.

Results _____

Technique for male patients. (Tick if this technique used). _____ Male patients should be instructed to wash hands, carefully open the sterile container without touching the inside of the jar or lid, and retract foreskin if uncircumcised. At first patient voids in toilet and then, while continuing to void, hold specimen container in "midstream" to collect sample. Patient should carefully replace the lid and handle the specimen as sterile.

Results _____

Technique for collecting directly from the catheter (Tick if this technique used). _____: After washing hands, sterilely insert catheter into bladder. Allow urine to drain then place sterile specimen container under catheter to catch 4 mL "midstream" sample. Collect in a container midstream of urine in a gray top C&S urine container. Do not collect urine from the collection bag.

Results _____ _____

Technique for collecting directly from the indwelling catheter (Tick if this technique used). _____: Clamp catheter below port and allow urine to collect in tubing. Disinfect the catheter collection port with 70% alcohol. Use needle and syringe to aseptically collect 20 mL freshly voided urine though catheter port.Transfer to gray top urine container. Do not collect urine from collection bag.

Results _____

Technique for collecting directly from the catheter tips (Tick if this technique used)

_____

5.1.1.1.Results

_____

_____

4. Ventilator-Associated Pneumonia & Collection of samples from the lower respiratory track. (Transport for analysis within ≤ 2 hours). (Tick if this sample collected) _____

- **Technique for Broncho alveolar lavage or brush, endotracheal aspirate** – To collect fluid in a sputum trap; transfer to leak-proof container for transport to microbiology laboratory; place brush in sterile container with 1 mL sterile saline.
- **Technique for collection of expectorated sputum** (Tick if technique used). _____ Patient should rinse mouth and gargle with sterile water prior to sputum collection; instruct patient to cough deeply and expectorate. Collect sputum specimen in sterile transport containers.

Results _____

- **Technique for collection of induced sputum** (If technique used indicate)

_____

To have the patient brush gums and teeth, then rinse mouth thoroughly with sterile water. Using a nebulizer, have the patient inhale 20-30 mL of 3 to 10% sterile saline. Instruct patient to cough deeply and expectorate. Collect sputum in sterile container.

Results _____

5. **Collection of samples from the upper respiratory track.** (To transport sample to be analyzed within ≤ 2 hours).

Collection of oral samples (If technique used indicate here) _____ To remove oral secretions and debris from surface of lesion with a swab and use a second swab to vigorously sample lesion, avoiding normal tissue. Tissue or needle aspirates are preferred when indicated.

Results _____

6. **Blood Stream Infections** (Indicate if sample collected) _____

Blood Sample collection – (To transport for analysis whining ≤ hours).

To cleanse skin with antiseptic as per routine protocol. Use a side-to-side motion to scrub the site with the friction pad for a full 30 seconds; allow site to dry completely (at least 30 sec) before venipuncture. Do not touch site after preparation. Remove over caps from bottles and cleanse each rubber septum with separate 70% alcohol swabs. Allow septum to dry for 1 min before inoculating.

Draw 10 mL of blood and inoculate bottle with 10 mL of blood. Do not vent or overfill bottles. Adding low (<8 mL) or high (>10 mL) volumes may adversely affect the recovery of organisms. In case of suspected bloodstream infections (BSI), collect three initial sets of blood cultures sequentially from separate phlebotomy procedures. Ideally, three venipunctures should be performed immediately but a third set of bottles can be drawn at a 4-6-hour delay. Three sets of blood cultures to be collected within a 24-hour period (J Clin Microbiol 2007; 45:3546).

Results _____

Phenotypic analysis

will primarily be done by identification of the following:

colony morphology (specify)_____

_____

_____,

Gram stain (specify) _____,

standard biochemical tests.

Glucose fermentation test _____

Oxidase reaction test _____

Antimicrobial susceptibility testing _____

_____

_____

_____

List of antibiotics tested by disk diffusion

_____

_____

_____

_____

_____

The Etest metallo-β-lactamase (bioMérieux Etest MBL) (Epsilometer test) used on aerobe Mueller-Hinton agar (MH) (Indicate if done)

_____

Results

Genotypic Analysis

Primers Used

PCR analysis using specific primers (Indicate from the following, if done)

Detection of metallo-beta lactamase (MBL) genes (bla*IMP*, bla*VIM*, bla*SPM* and bla*NDM*):

PCR analysis will be performed to confirm the presence of the metallo-lactamase with a standard cycle program (95°C for 1 min, 52°C for 1 min, and 72°C for 1 min) for 30 cycles. Then amplification of the metallo-lactamase genes will be done using specific primers.

Results_____
_____
_____

Carbapenem-resistant isolates will be tested by real-time PCR for the expression levels of the mexB, mexY, ampC, and oprD genes.

Results

_____
_____
_____
_____

PCR to detect overproduction of the MexABeOprM

Results _____
_____
_____
_____

Pulsed-Field Gel Electrophoresis (PFGE) (Indicate if done)

_____
___
_____

Results _____

Multilocus Sequence Typing (MLST) (Curran et al., 2004)
(Indicate if done) _____
Results _____
_____
Presence of new allele sequences? _____
_____

Information from already-known alleles _____
_____
_____

16S rRNA Sequence Data Submitted & Compared to International Website

_____

_____

_____

Phylogenic Analysis

The population structure of the species will be determined by a nucleotide-based analysis of the entire *PA* MLST database and further localized on the phylogenetic tree

The sequence types (STs)

_____

_____

Comparison to the STs from other continents

_____

_____

_____

Compare with ST with ESBL- and MBL-producing STs from the MLST database
_____
_____

_____**Genetic distance** (Indicate if calculated)

_____

Genetic tree _____

_____

_____

_____

V. Clinical outcomes

a. Site of Infections:

- Respiratory Tract Infection
- Urinary Tract Infection
- Skin infection
- Blood infection

b. Types of Infections:

- Carbapenem-susceptible_____

  _____

  _____

  _____

- Carbapenem –non susceptible _____

_____

_____

c. What is the severity of patient illness (estimated using **the acute physiology and chronic health status scoring system II [APACHE II] and Pitt score**)?

d. Outcome:

- Disease Free Survival? (Specify) _____
- Progression Free Survival? _____
- Response duration? _____
- Overall survival? _____

Appendix 4

Ethical Approval Letter

UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
Federal Assurance No. FWA00000338

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
E-mail: unzarec@unza.zm
IRB00001131 of IORG0000774

22nd April 2020.

Your REF. No. 671-2019.

Dr. Patrice Ntanda Mukomena,
University of Zambia,
School of Veterinary Medicine,
Department of Disease Control,
P.O. Box 32397,
Lusaka.

Dear Dr. Mukomena,

RE: "CLINICAL AND MOLECULAR CHARACTERIZATION OF PSEUDOMONAS AERUGINOSA AMONG ADULT PATIENTS IN SELECTED HOSPITALS IN LUSAKA AND COPPER BELT PROVINCES OF ZAMBIA" (REF. NO. 671-2019)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 25th March, 2020. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) Study proposal
b) Questionnaires
c) Participant Consent Form

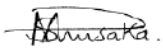
APPROVAL NUMBER : REF. 671-2020

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 21st April 2020**
- **TYPE OF APPROVAL : Expedited**
- **EXPIRATION DATE OF APPROVAL : 20th April 2021**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

- **TERMINATION OF STUDY**: On termination of a study, a report must be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.
- **NHRA:** You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.
- **QUESTIONS**: Please contact the UNZABREC on Telephone No.256067 or by e-mail on unzarec@unza.zm.
- **OTHER**: Please be reminded to send in copies of your research findings/results for our records. You're also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study. Use the online portal: unza.rhinno.net for further submissions.

Yours sincerely,



Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON
Tel: +260977925304
E-mail: s.munsaka@unza.zm

Appendix 5

NHRA Approval Letter

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@gmail.com | www.nhra.org.zm

Ref No:…… **Date: 5th May, 2020**

The Principal Investigator
Dr. Patrice Ntanda Mukomena,
University of Zambia,
School of Veterinary Medicine,
Department of Disease Control,
P.O. Box 32397,
Lusaka.

Dear Dr. Mukomena,

Re: Request for Authority to Conduct Research

The National Health Research Ethics Board (NHREB) is in receipt of your request for authority to conduct research titled **"Clinical and Molecular Characterization of Pseudomonas aeruginosa among adult patients in Selected Hospitals in Lusaka and Copper Belt Provinces of Zambia."**
I wish to inform you that following submission of your request to the Board, its review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. **A Material Transfer Agreement is obtained and cleared by the National Health Research Ethics Board should there be any need for samples to be sent outside the country for analysis.**
2. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
3. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
4. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
5. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, and all key respondents.

Yours sincerely,

Prof. Patrick Musonda
Chairperson
National Health Research Ethics Board

Appendix 6

16S rRNA GENES SEQUENCING AND *PSEUDOMONAS AERUGINOSA* STRAINS

>MZ618953.1:201-1052 Pseudomonas aeruginosa strain MDM1 16S ribosomal RNA gene, partial sequence
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-
TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGGT-
GGTTCAGCAAGTTGG-ATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAAGGGTGGGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CTTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
TGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCASCTCGTGTCSTGAGAT

>MG966347.1:219-1070 Pseudomonas aeruginosa strain P2 16S ribosomal RNA gene, partial sequence
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-
TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGGT-
GGTTCAGCAAGTTGG-ATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAGGGT--GGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CYTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
TGCCTTCGGGAAMTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT

>KF031123.1:182-1032 Pseudomonas aeruginosa strain SN3 16S ribosomal RNA gene, partial sequence
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-

TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGT--
GGTTCAGCAAGTTGG-ATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAGGGT--GGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CTTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
TGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT

>FM997148.1:203-1054 Uncultured bacterium partial 16S rRNA gene,
clone 16sps24-1d07.p1k
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-
TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGGT-
GGTTCAGCAAGTTGG-ATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAGGGT--GGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCAGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CTTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
TGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT >

>CP097557.1:713091-713942 Pseudomonas aeruginosa strain C1.3
chromosome, complete genome
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-
TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGGT-
GGTTCAGCAAGTTGG-ATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAGGGT--GGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC

ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CTTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
TGCCTTCGGGAGCTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT

>CP036492.1:221946-222797 Pseudomonas aeruginosa strain Paer4
chromosome, complete genome
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-
TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGGT-
GGTTCAGCAAGTTGG-ATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAGGGT--GGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCATAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CTTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
TGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT

>JQ659969.1:211-1065 Pseudomonas aeruginosa strain R8-590-1 16S
ribosomal RNA gene, partial sequence
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-
TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGGT-
GGTTCAGCAAGTTGGAATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAGGGGTGGGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CTTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
NGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT

>JQ659968.1:211-1065 Pseudomonas aeruginosa strain R8-590 16S
ribosomal RNA gene, partial sequence
CTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCC
AGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGC
GTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTT---AAG-

TTGGGAGGAAGGGCAGTAAGTTAATAC-
CTTGCTGTTTTGACGTTACCAACAGAATAAGCACCGGCTAACTTC--GTG-CCAGC-AGCCGC-
GGTA-ATACG-AAGGGTGCAA-GCGTTAATCGGAA-TTACTGGGCGTAAAGCGC-GCGTAGGT-
GGTTCAGCAAGTTGGAATGTGAAA--TCCCCGGGCTCAA-CCTGGGAA-CTGCAT--CCAAAA-
CTACTG-AGCTAGAGTACGGTAGAGGGGTGGGTGGAA-TTT--
CCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCG-
AAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGTCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGC-
TAAC--
GCGATAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGC
ACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCA--ACGCGAAGAACCTTACCT-GGC-
CTTGACATGCTGA-GAACTTTCCAGAG-ATGGATTGG-
NGCCTTCGGGAACTCAGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT

>KT759016.1:188-1041 Bacterium F7-16 16S ribosomal RNA gene, partial sequence CTACCAAGGCGACGATCCGTAACTGGTCT

Appendix 7

PA Genetic Analysis

Table 9. *Pseudomonas aeruginosas*' DNA with results of PA-SS, PA-GS, AMPC, OXA (23&51), and IMP Genes

CODE	DNA #	PA-SS	PA-GS	AMP-C	OXA-23	OXA-51	IMP
1070-S	1	POS	NEG	NEG	NEG	NEG	NEG
661-S	2	POS	NEG	POS	NEG	NEG	NEG
1086-U	3	POS	NEG	NEG	NEG	NEG	NEG
1044-U	4	POS	POS	POS	NEG	NEG	NEG
903-U	5	POS	NEG	POS	NEG	NEG	NEG
500-S	6	POS	NEG	POS	NEG	NEG	NEG
590-S	7	POS	NEG	NEG	NEG	NEG	NEG
662-S	8	POS	POS	POS	NEG	NEG	NEG
489-U	9	POS	POS	POS	NEG	NEG	NEG
1088-U	10	POS	NEG	NEG	NEG	NEG	NEG
629-U	11	POS	NEG	NEG	NEG	NEG	NEG
477-U	12	POS	NEG	POS	NEG	NEG	NEG
537-U	13	POS	POS	NEG	NEG	NEG	NEG
503-U	14	POS	POS	NEG	NEG	NEG	NEG
629-U	15	POS	POS	POS	NEG	NEG	NEG
708-U	16	POS	POS	POS	NEG	NEG	NEG
489-S	17	POS	POS	POS	NEG	NEG	NEG
1069-Sa	18	POS	POS	POS	NEG	POS	NEG
560-S	19	POS	NEG	POS	NEG	POS	NEG
1069-Sb	20	POS	POS	POS	NEG	POS	NEG
1053-U	21	POS	NEG	NEG	NEG	NEG	NEG
666-U	22	POS	NEG	POS	NEG	NEG	NEG
566-U	23	POS	POS	POS	NEG	POS	NEG
591-S	24	NEG	NEG	NEG	NEG	NEG	NEG
2086-S	25	POS	POS	POS	NEG	NEG	NEG
3009-S	26	POS	POS	POS	NEG	POS	NEG
3002-S	27	NEG	POS	POS	NEG	POS	NEG
3015-S	28	POS	POS	POS	NEG	NEG	NEG
3005-S	29	POS	POS	POS	NEG	POS	NEG
3087-U	30	POS	POS	POS	NEG	NEG	NEG
429-S	31	POS	POS	POS	POS	POS	NEG
466-S	32	POS	POS	POS	POS	NEG	NEG
428-U	33	POS	POS	POS	POS	POS	NEG
467-S	34	POS	POS	POS	NEG	POS	NEG
449-U	35	POS	POS	POS	NEG	POS	NEG
469-S	36	POS	NEG	POS	NEG	NEG	NEG
3003-S	37	POS	POS	POS	NEG	POS	NEG

457-U	38	NEG	POS	POS	NEG	POS	NEG
478-U	39	POS	POS	POS	NEG	NEG	NEG
279-U	40	POS	POS	POS	NEG	POS	NEG
517-S	41	POS	POS	POS	NEG	NEG	NEG
594-SB	42	POS	POS	POS	POS	NEG	NEG
593-SA	43	POS	POS	POS	POS	POS	NEG
592-S	44	POS	POS	POS	NEG	POS	NEG
585-UB	45	POS	POS	POS	NEG	POS	NEG
576-U	46	POS	POS	POS	NEG	POS	NEG
589-S	47	POS	NEG	POS	NEG	POS	NEG
587-S	48	POS	POS	POS	NEG	POS	NEG
3004-S	49	POS	POS	POS	NEG	POS	NEG
3076-U	50	POS	POS	POS	NEG	NEG	NEG
2069-U	51	POS	POS	POS	NEG	POS	NEG
2067-U	52	POS	POS	POS	NEG	POS	NEG
3000-U	53	POS	POS	POS	NEG	POS	NEG
2088-U	54	POS	POS	POS	NEG	POS	NEG
2084-U	55	POS	NEG	POS	NEG	POS	NEG
2094-U	56	POS	POS	POS	NEG	NEG	NEG
2090-U	57	POS	POS	POS	NEG	POS	NEG
2065-U	58	POS	POS	POS	NEG	POS	NEG
2048-U	59	POS	NEG	POS	NEG	POS	POS
2087-U	60	POS	NEG	POS	NEG	POS	NEG
2089-U	61	POS	NEG	POS	NEG	POS	NEG
3011-U	62	POS	POS	POS	NEG	POS	NEG
2070-U	63	POS	POS	POS	NEG	POS	NEG
2064-U	64	NEG	POS	POS	NEG	POS	NEG
2062-U	65	POS	POS	NEG	NEG	POS	NEG
637-S	66	POS	NEG	POS	NEG	POS	NEG
649-S	67	POS	POS	POS	NEG	POS	NEG
639-S	68	POS	NEG	POS	NEG	POS	NEG
3057-U	69	POS	POS	POS	NEG	POS	POS
357-U	70	POS	POS	POS	NEG	POS	POS
3055-U	71	POS	POS	POS	NEG	POS	POS
3007-U	72	POS	POS	POS	POS	POS	POS
2004-U	73	POS	POS	POS	NEG	POS	NEG
1085-U	74	POS	POS	NEG	NEG	POS	POS
2008-S	75	POS	POS	POS	NEG	NEG	NEG
2005-U	76	POS	POS	POS	NEG	POS	NEG
1054-U	77	POS	POS	POS	NEG	NEG	NEG
2007-S	78	POS	NEG	POS	NEG	NEG	NEG
1056-U	79	POS	POS	POS	NEG	POS	NEG
1043-U	80	POS	NEG	POS	NEG	POS	NEG
1084-U	81	POS	NEG	POS	NEG	POS	NEG
2044-U	82	POS	POS	POS	NEG	POS	NEG

2010-U	83	POS	NEG	POS	NEG	POS	NEG
2011-U	84	POS	NEG	POS	NEG	POS	NEG
2025-U	85	POS	NEG	POS	NEG	POS	NEG
207-U	86	POS	POS	NEG	NEG	POS	NEG
33-S(Ndola)	87	POS	POS	POS	NEG	POS	NEG
198-Spt	88	POS	NEG	POS	NEG	POS	NEG
16	89	POS	POS	NEG	NEG	NEG	NEG
1011-U	90	POS	NEG	POS	NEG	POS	NEG
426-U	91	POS	POS	NEG	POS	POS	NEG
2059-U	92	POS	NEG	POS	NEG	POS	NEG
427-U	93	POS	POS	POS	NEG	POS	NEG
906-S	94	POS	POS	POS	NEG	POS	NEG
908-S	95	POS	POS	POS	NEG	POS	NEG
904-S	96	POS	POS	POS	NEG	POS	NEG
903-S	97	POS	POS	NEG	NEG	POS	NEG
436-S	98	POS	POS	POS	NEG	POS	NEG
1014-U(Rep)	99	POS	POS	POS	NEG	POS	NEG
101-S	100	POS	POS	NEG	NEG	POS	NEG
2076-U	101	POS	POS	POS	NEG	POS	NEG
1000-S	102	POS	POS	POS	NEG	POS	NEG
907-S	103	POS	POS	POS	NEG	POS	NEG
905-S	104	POS	POS	POS	NEG	POS	NEG
52-S(Ndola)	105	POS	POS	POS	POS	NEG	NEG
33-s	106	POS	POS	NEG	NEG	NEG	NEG
84-C/S	107	POS	POS	POS	NEG	NEG	NEG
52-S	108	POS	POS	POS	NEG	NEG	NEG
34-S	109	POS	POS	POS	NEG	POS	NEG
7	110	POS	POS	POS	NEG	NEG	NEG
36	111	POS	POS	POS	NEG	NEG	NEG
64 C/S	112	POS	POS	POS	NEG	POS	NEG
37-S	113	POS	POS	POS	NEG	NEG	NEG
14	114	POS	POS	POS	NEG	NEG	NEG
NTH	115	POS	POS	POS	NEG	NEG	NEG
ATCC 27853 PA (Control)	116	POS	POS	POS	NEG	NEG	NEG

Positive (POS), Negative (NEG)

APPENDIX 8

Published article 1

'Nosocomial infections and associated risk factors at two tertiary healthcare facilities in Lusaka and Copperbelt Provinces, Zambia'

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ABSTRACT

Background: Nosocomial infections are a serious public health problem affecting both developed and developing countries. They are caused mainly by multi-drug-resistant pathogens that limit treatment options, leading to high morbidity and mortality, longer hospital stays and increased costs of health care. This study aimed to evaluate nosocomial infections, risk factors and causative pathogens at two large teaching hospitals in Zambia.

Material and Methods: A year-long hospital-based cross-sectional study was conducted from April 2020 to April 2021 at two large tertiary-level hospitals in Zambia. Hospitalised and out-patients with previous hospital contact were screened for nosocomial infections, followed by the collection of specimens (skin swabs, urine or sputum) for bacteriological culture and Polymerase Chain Reaction (PCR) amplification of 16S rRNA gene fragments. Nosocomial infections were defined according to the World Health Organization case definitions. Frequencies were estimated, and the association between the outcome variable (positive culture) and categorical predictor variables were analysed using the Chi-square test.

Results: Eight hundred and forty-one clinical specimens (skin swabs, urine or sputum) were collected and analysed, 640 from the University Teaching Hospital in Lusaka and 201 from the Ndola Teaching Hospital in Ndola. Of these, 71.2% were from male, with only 28.8 % from female patients. The median age was 50 years old. Catheter-associated urinary tract infections (57%) were the most common, followed by those from pressure sores (38.7%). The most

frequently observed pathogens included *Escherichia coli* (17.8%), *Pseudomonas aeruginosa* (13.7%), *Klebsiella pneumonia* (5.6%) and *Proteus vulgaris* (5.5%).

Conclusions: The hospital infection rate at the two urban tertiary hospitals was very high. Age over 65 years, male gender, presence of medical devices, presence of a wound, longer hospital stays, previous hospital contacts and low systolic blood pressure were associated with the risk of developing nosocomial infections. Despite improved infection control following the COVID-19 waves, nosocomial infections have remained a significant public health threat.

Keywords: Nosocomial infections, hospital-acquired infections, risk factors, Zambia

Background

Nosocomial Infections (NI), also called Healthcare-Associated Infections (HAI), are infections acquired while receiving health care, initially absent at admission. It can occur within 48 hours of hospital admission, three days after discharge or 30 days after medical or surgical procedure for conditions unique to the infection [1][2]. Surgical site infections (SSI), urinary tract infections (UTI), bloodstream infections (BSI), and respiratory tract infections (RTI) have been reported as the most common types of NI [3][4]. Nosocomial Infections (NI) affect millions of people globally, and many of these infections are entirely preventable. According to the World Health Organization (WHO), seven in 100 patients in high-income countries and 15 in low/middle-income countries (LMIC) will acquire at least one NI in an acute care setting, with one in 10 affected dying from these infections [5], [6]. It has been estimated that this burden of NI is twice that of other 32 infectious diseases and that 75% of disability-adjusted life years (DALY) are attributed to antimicrobial (AMR) in Europe [6][7].

Often caused by multidrug-resistant pathogens, NIs have limited treatment options resulting in high morbidity and mortality, longer hospital stays and high cost of care [6], [7]. According to the Centers for Disease Control and Prevention (CDC), Gram-negative bacteria, such as

Pseudomonas aeruginosa, play an important role, especially in critically ill and immunocompromised patients [8].

In sub-Saharan Africa (SSA), there is a scarcity of data on NI, but the burdens appear to be high, and this has been attributed to a lack of surveillance data on these infections [9]. Two previous studies in Zambia reported high levels of microbial contamination from white coats worn by healthcare workers and the hospital environment [10], [11]. However, these studies did not report clinical data on bacterial infections; instead focused on the hospital environment. Therefore, this study aimed to evaluate nosocomial infections, risk factors and associated bacterial pathogens at two major teaching hospitals in Zambia.

Materials and Methods

Study Site and Design

A hospital-based cross-sectional study was conducted between April 2020 and April 2021 at the University Teaching Hospital (UTH) in Lusaka and the Ndola Teaching Hospital (NTH) in Ndola, Zambia. UTH is a 2000-bed capacity tertiary level hospital and the largest referral centre in Zambia, whilst NTH has a bed capacity of 948 and is the second largest hospital in the country after UTH.

Patient recruitment and data collection

Patients were recruited from the Out-Patient Department (OPD) (patients with a history of hospital contact within 30 days) [2] and in-patients in medical and surgical wards. Participants were enrolled from consecutive patients aged 15 years and older after obtaining their consent or that of their proxy and screened for nosocomial infections. Baseline information on demographic and clinical details was collected. Nosocomial infections were defined using the World Health Organization case definitions [5], [12]. These included an infection acquired in the hospital by a patient admitted for a reason other than that infection and infections acquired

in the hospital but appearing after discharge. A physical examination was performed, and appropriate clinical specimens were collected. Clinical specimens were collected and processed for pathogen identification and molecular analysis. Medical records were reviewed for all enrolled patients to collect relevant clinical and therapeutic data.

Specimen Collection

After the labelling of sterile containers, clinical specimens were collected using the Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Hospital and wards of origin, date and time of collection, specimen type and tests performed were carefully noted. All specimens were placed in tightly sealed, leak-proof containers, transported in sealable, leak-proof plastic bags, and delivered to the laboratory within 30min to 2 hours of collection for processing. Skin swabs were collected from patients with septic burns, pressure sores or other wounds, and infected intravenous and catheter sites. We collected swabs at infected Seldinger's wire insertion sites for those in the dialysis unit. For decubitus ulcers, the surface was first cleansed with sterile normal saline, and then inflammatory material was collected from the base of the ulcer into a sterile tube for bacterial culture.

Further, four millilitres of urine were collected in a sterile specimen container from patients with suspected urinary tract infections and those with a history of in-dwelling urinary catheter use.

Patients were instructed to wash their hands, correctly open the sterile container without touching the inside of the jar or lid to collect "midstream" urine and correctly replace the lid while they observed the specimen as sterile to avoid contamination. For catheterized patients, the catheter was clamped below the port to allow for urine to collect in the tubing. The catheter was disinfected with 70% alcohol, and 20 mls of freshly voided urine was aseptically collected through the port using a syringe. While changing catheters, the medical device tips were also taken for cultures. For patients with respiratory tract infections, sputum or tracheal secretions

were collected in sterile containers. Patients were instructed to rinse their mouths, gargle with sterile water before collecting, and then cough deeply. For those unable to produce sputum, a nebulizer with 3 to 10% sterile saline was used to induce tracheal sputum production. The collected specimens were immediately transported to the Microbiology Laboratory at the University of Zambia, School of Veterinary Medicine in Lusaka or at the Tropical Diseases Research Centre Laboratory in Ndola for immediate Processing.

Bacteriological analysis

Samples were inoculated onto blood agar (Oxoid Ltd, Basingstoke, UK) and MacConkey (Oxoid Ltd, Basingstoke, UK) and incubated aerobically at 37°C for 24 hours. Non-lactose fermenting colonies from MacConkey plates were sub-cultured onto nutrient agar (Oxoid Ltd, Basingstoke, UK) and subjected to conventional biochemical tests: Oxidase, Simon's citrate, Urease, Sulphur Indole Motility testing (SIM), Lysine Iron Agar (LIA), Triple Sugar Iron (TSI) and Gram staining. The large flat dark greenish colonies from blood agar (after sub-culturing on nutrient agar) were also Gram-stained and tested for catalase and coagulase production. The isolates were further sub-cultured onto two nutrient agar (Oxoid) plates and incubated separately (at 37°C for pigment production and growth at 42°C). ATCC strains were used as reference strains.

Molecular detection of bacterial pathogens

In addition to the bacteriological analysis, molecular analysis was also conducted as another means of pathogen's identification. DNA was extracted from purified presumptive positive bacterial cultures using commercial DNA extraction kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Polymerase Chain Reaction (PCR) amplification was used to amplify 16S rRNA gene fragments from purified genomic DNA of culture isolates. The PCR master mix contained 1 x PCR buffer [50 mM KCl, 1.5mM $MgCl_2$, 10mM Tris/HCl (pH9.0), 10µg gelatin ml^{-1}], 200µM dNTPs, 0.3µM each of the 16S rRNA primers, 1.5 U *Taq*

polymerase (Gibco BRL) and approximately 10-100ng DNA in a final reaction volume of 100µl. Polymerase chain reaction based on Extaq protocol according to the manufacturer's instructions (Takara Biotechnology (Dalian) Co., Ltd.) was used for species identification using specific primers targeting the 16sRNA gene as previously prescribed [14]. The following thermal cycling conditions were used: 98˚C for 10s, 35 cycles of 98˚C for 30s, 54˚C for 30s, and 72˚C for 1min and final extension at 72˚C for 5min. The positive amplicons were purified using Wizard® SV Gel and Clean-Up System (Promega, Madison, WI, USA) according to the manufacturer's protocol. The purified DNA was later sequenced directly using a Big Dye terminator cycle sequencing ready reaction kit v3.1 and analysed on a 3500 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

Data analysis

The collected data was validated and entered into Excel® Spreadsheets before exporting to STATA® version 14.0 (College Station, TX: StataCorp LP, USA) for analysis. The frequency of nosocomial infection was determined at a 95% confidence interval. The proportions (%) of the different variables were estimated as the number of positive outcomes divided by the number examined. Frequency distributions of the variables were produced and checked for inconsistencies and input errors. A Chi-square test was performed to identify factors associated with nosocomial infection. Quantitative variables were summarised using mean and standard deviation. Variables with binary outcomes were analysed using odds ratios. The level of significance was set at a p-value of less than 0.05. The nucleotide sequences obtained from the 16S rDNA analysis were compared for similarity with other previously published sequences using the BLAST search on PubMed https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch.

Ethical consideration

Ethical clearance was sought from the University of Zambia Biomedical Research Ethic Committee (UNZA REC) [REF. No. 671-2019]. Participants provided informed written consent to collect anonymised clinical and demographic data. The consent form was verbally explained to all participants in their local language. For illiterate participants, a witnessed fingerprint was obtained. All obtained information was kept confidential and only used for research or academic purpose. Electronic devices used to collect and store the data were password protected.

Results

Participant's enrolment per study sites

A total of 640 participants were enrolled from UTH and 201 from NTH. At UTH, 360 patients were enrolled from the out-patient department (OPD), while 280 were from the in-patient department (IPD). Of these, 48 were from medical OPD, 158 from medical IPD, 312 from surgical OPD and 122 from surgical IPD. Among 201 participants enrolled at NTH, 82 were from OPD, while 119 were from in-patient wards. Of these, 53 came from medical OPD, 75 from medical IPD, 44 from surgical IPD and 29 from surgical OPD. Further details are described in Table 1.

Socio-demographic characteristics of participants

Of the 841 participants enrolled in the study (from UTH and NTH), 683 participants were males, and 158 females; the median age for males and females was 50 years. The majority of the participants originated from Lusaka province (761), with only 93 from the Copperbelt province. Only 75 participants (9%) completed tertiary and 150 (18%) secondary education, while 563 achieved at least primary education. Around 6% (53) of participants reported no formal education. Only 10% (85) of participants reported being in formal employment, while

the majority (756) were either in the informal sector or unemployed. The majority of participants (71%) were married.

Specimen collected

The most frequently collected specimen types were skin swabs (38.7%) and midstream urine (37.3%). Of the 326 swabs, 103 were collected from UTH medical department, 127 in UTH surgical wards, 51 from the NTH medical department and 45 from the NTH surgical department.

A total of 314 midstream urine (MSU) specimens were collected. Of these, 67 were obtained from UTH medical department, 201 from UTH surgical department, 35 from the NTH medical department and 11 from the NTH surgical department. Other details are shown in Table 1.

Culture specimen and bacteriological profile

The majority (69.9%) of clinical specimens had positive bacterial cultures (Table 2). The distribution of the commonly isolated pathogens per ward are highlighted in Table 2. More pathogens were isolated from surgical (55.6%) compared to medical wards; returning OPD patients had more pathogens isolated (56.2%) compared to admitted patients. Of the 588 pathogens isolated, 199 (17.8%) were Escherichia coli, 116 (13.7%) Pseudomonas aeruginosa, 87 (5.6%) Proteus vulgaris, 79 (5.5%) Klebsiella pneumonia and 107 (11.4%) other bacteria (Table 2).

16S rDNA sequencing

Approximately 500 bp nucleotide sequences of the 16S rDNA gene from study and reference strains were generated. Upon alignment and comparison, all the 16S rDNA gene sequences were determined. Comparison with previously published sequences was done using a Basic Local Alignment Search Tool (BLAST) search on NCBI (National Center for Biotechnology Information) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). The sequences were deposited in National Centre for Biotechnology Information (NCBI).

Clinical features and Risk factors of nosocomial infections

Medical devices-related nosocomial infection was a common presentation. Among surgical patients, a urinary catheter was frequently inserted in patients being managed for BPH. Catheter-associated UTI was the most common nosocomial infection (57%). The other common diagnosis was infected pressure sores (38.7%). Table 3 highlights the factors associated with nosocomial infection in univariate analysis. These were medical device insertion ($p=0.0000$), age ≥ 65 years ($p=0.0003$), male gender ($p=0.000001$), prior admission and prolonged hospital admission ($p=0.0000$). Other associated risk factors are summarized in Table 3. Co-morbidities included hypertension (8%) and diabetes mellitus (4%). Nine (N=9) participants were psychiatric patients presenting with infected wounds. More than half (62%) of participants reported a history of previous hospital exposure within the past 30 days, which were prior medical visits or being ex-bed-siders taking care of patients.

Discussion

The hospital environment is known to select for and promote the spread of nosocomial pathogens. Therefore, clinical characteristics, causes and risk factors of nosocomial infections at two urban tertiary-level hospitals in Zambia were evaluated.

Most participants were enrolled from UTH compared to NTH. At NTH, the data was collected during the pick of the COVID-19 third wave, hence the low enrolment (23.9 %) of participants at this site due to a decrease in the number of patients attending hospital for non-COVID 19 related illnesses during the study period. These observations agree with Quadros et al. [15], who linked the decrease in the number of patients with non-COVID-19-related diseases to public anxiety about acquiring the viral infection in the hospital and the subsequent risk of mortality and lockdown. Nosocomial infection pathogens were isolated in most (71.1%) patients with inserted medical devices. The prior hospital visit was significantly associated with the risk of nosocomial infection, as previously observed by Baggs et al. [16] in a retrospectively

identified cohort of hospitalized patients from the Truven Health MarketScan Hospital Drug Database in the United States of America (USA).

Catheter-associated UTI (57%) was the most common nosocomial infection. A disparity was observed with the finding by Pezhman et al. [17], who observed that the most common sites of NI were the respiratory tract (39.4%). The low frequency of respiratory NIs could be due to infection prevention measures scaled up during the COVID-19 waves. The other reason could be that cases of respiratory infections were considered suspected Covid-19. People with suspected cases were told to go into self-quarantine and would not go to the hospital as the case was prior to the pandemic.

The most common causes of NIs in Tehran's [17] non-teaching hospitals were Klebsiella pneumonia (31.4%), Escherichia coli (30.9%), Pseudomonas aeruginosa (26.7%), and Staphylococcus (23.6%). This current study also showed that Escherichia coli and Pseudomonas aeruginosa were the most common microorganisms isolated from our patients.

We did observe a high frequency of NI at the two tertiary hospitals in Zambia. This finding was contrary to the observation by Su et al. [18], who observed that infection prevention and control measures for the COVID-19 pandemic reduced nosocomial infection in almost all departments in a Chinese tertiary hospital during the waves. However, they found that catheter-related infections did not differ, as observed in our study.

These findings agree with Mekonnen et al. [19], who found a high frequency of nosocomial infection, such as that caused by Pseudomonas aeruginosa (13%), in an Ethiopian study. The observed difference with other studies could be explained by factors such as the presence of co-morbidities in participants, such as benign prostate enlargement, presence or frequency of medical device insertion, hospital settings, standards of infection prevention practice, and length of hospitalization. We observed that the nosocomial infection rate was significantly higher among patients with BPH due to an inserted medical device such as a urinary catheter.

We further observed that out-patient attendance was associated with a high risk of nosocomial infection. Most of these were out-patients who frequented the hospital for complications related to urinary catheters. These patients were catheterized and then discharged with the urinary catheter in-situ with options for self-catheterization while awaiting prostate surgery. This potentially puts them at risk of being infected with either community or hospital-acquired bacteria, leading to nosocomial infection, and these infections were commonly associated with the urology Department. This finding was consistent with a French study [20], which observed that aged males were more likely to be catheterized for reasons such as BPH.

In addition, Mekonnen et al. [19] reported that factors contributing to the high prevalence of NIs could be due to the commonly observed overcrowding of patients, the hospital environment, and poor implementation of infection control measures, particularly hand hygiene practices and decontamination of the hospital environment. Surprisingly, the Acquired Immune Deficiency Syndrome secondary to Human Immune Deficiency Virus (HIV/AIDS) was not significantly associated with an increased risk of nosocomial infection. This is because most HIV patients in Zambia are on anti-retroviral treatment. According to data from UN/AIDS, the Zambian Ministry of Health (MoH) and the Centre for Infectious Diseases Research in Zambia (CIRDZ) indicated that out of 1,300,000 Zambians estimated to be living with HIV/AIDS, 1,176,000 (90.5%) were on treatment [21].

Conclusions

The hospital infection rate at the two urban tertiary hospitals was very high with E. coli and Pseudomonas causing the majority of infections. Being aged ≥65 years, male gender, presence of medical devices, presence of a wound, prolonged hospitalisation, hospital exposure in the last 30 days and low systolic blood pressure was associated with the likelihood of acquiring NI. We also found no statistical association between developing nosocomial infection and HIV+ status, probably due to improved HIV care in Zambia. These data, generated from two

major teaching hospitals during and after the third wave of COVID-19, are important to predict the infections and the antimicrobial resistance profile that will develop in the future.

5.1.2. Limitations

The study was conducted at two large teaching hospitals, though including data from the regional hospital could give an idea of the burden of NI countrywide.

Recommendations

The recommendation is to expand this study to regional hospitals to obtain the burden and AMR profile to put evidence-based infection prevention measures in place.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix 9

Published article 2

Antimicrobial resistance profiles of and associated risk factors for *Pseudomonas aeruginosa* nosocomial infection among patients at two tertiary healthcare facilities in Lusaka and Copperbelt Provinces, Zambia

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Abstract

Background

Antimicrobial resistance (AMR) of pathogens such as *Pseudomonas aeruginosa* is among the top 10 threats to global health. However, clinical and molecular data are scarce in Zambia. We, therefore, evaluated the AMR profiles of *P. aeruginosa* nosocomial infections (NIs).

Methods

A year-long hospital-based cross-sectional study was conducted at two large tertiary-level hospitals in Zambia. Patients with current or previous hospital contact were screened for NIs. The current study focused on patients diagnosed with *P. aeruginosa* NIs. Clinical specimens were collected for bacteriological culture, and PCR amplification of 16S rRNA gene fragments was performed on pure isolates. Hospital or NIs were defined as infections that arise during hospitalization, occurring at least 48 h after admission. The Kirby–Bauer's disk diffusion method was used to evaluate antibiotic resistance patterns. The association between AMR and risk factors was analysed using the χ^2 test.

Results

Eight hundred and forty-one patients were screened, and clinical specimens were collected and analysed. Of them, 116 (13.7%) were diagnosed with *P. aeruginosa* NIs. The participants' ages ranged from 15 to 98 years, with a mean of 51 (SD±18). Catheter-associated urinary tract infections (57%) were the most common, followed by pressure sores (38.7%). *P. aeruginosa* isolates were primarily susceptible to amikacin, which had the highest resistance to FEP. We observed a high prevalence of multidrug resistance (73.6%). The AMR was associated with carbapenem-hydrolysing β-lactamase gene blaOXA-51 and surgical care.

Conclusions: This study has demonstrated that multidrug-resistant *P. aeruginosa* is prevalent in hospitals in Zambia's Lusaka and Ndola districts and possibly countrywide.

Introduction

The discovery of PEN at St Mary's Hospital in London in 1928 by Alexander Fleming was a breakthrough in medical science and practice. This discovery led to the introduction of antibiotics, significantly reducing the number of infection-related deaths. Therefore, predictions were made that infectious diseases would finally be eliminated as more antimicrobial compounds were discovered.[1] Unfortunately, developing and rising antimicrobial resistance (AMR) to these antibacterial agents quickly diminished this optimism.[2] Since then, the WHO has declared AMR as one of the top 10 global public health threats facing humanity.[3] It is estimated that AMR will lead to 10 million annual human deaths globally by 2050 if no interventions are implemented to combat it at global, regional, and national levels.[4] Despite scanty information in Africa, the available data show evidence of increasing trends in AMR, suggesting that the region shares the worldwide trend of this problem.[5]

Gram-negative bacteria, especially non-fermenting pathogens, have been implicated in nosocomial infections (NIs). *Pseudomonas aeruginosa*, one of the most common hospital pathogens, is a causative agent of many severe opportunistic infections, particularly in immunocompromised patients.[6] High morbidity and mortality occur due to weakened host defences, bacterial resistance to antibiotics and the production of extracellular enzymes and toxins.[7] β-Lactamase production has significantly contributed to the rise in resistance to beta-lactam antibiotics, becoming a public health threat.[8] The emergence and the spread of metallo-β-lactamases, especially in non-fermenters like *P. aeruginosa*, have become therapeutic challenges. Class C β-lactamases (Amp C) confer resistance to the same antibiotics as ESBLs with additional resistance to cephamycin and β-lactamase inhibitors.[9] Infections caused by AMR bacteria pose serious challenges that include prolonged hospital stays, higher hospital costs and poorer clinical outcomes in that they cause severe morbidities and mortality.[9,10]

Due to the need for country-specific data on the burden of AMR and the factors driving its spread, the Zambia National Public Health Institute recently developed an integrated AMR surveillance framework to guide the fight against AMR.[11] A recent study by Kaluba *et al.*[12] found low carbapenem resistance in *P. aeruginosa*. It recommended improved AMR surveillance, antimicrobial stewardship and infection prevention and control at the University Teaching Hospital (UTH) in Zambia. Nevertheless, specific clinical and molecular AMR data on resistant *P. aeruginosa*, such as data on associated genes, are scarce.[11,12] Therefore, this

study evaluated antimicrobial and molecular profiles of *P. aeruginosa* from patients with NIs at two sizeable tertiary healthcare facilities in Zambia.

Materials and methods

Study site and design

A hospital-based cross-sectional study was conducted between April 2020 and April 2021 at the UTH in Lusaka district and the Ndola Teaching Hospital (NTH) in Ndola district, Zambia. The UTH is a 2000-bed capacity tertiary-level hospital and the largest referral centre in Zambia. At the same time, the NTH has a capacity of 948 beds and is the second largest hospital in the country after the UTH. The UTH is located in the Lusaka district, the capital city of Zambia, and serves as a national referral hospital. On the other hand, the NTH is a referral hospital mainly serving the northern part of the country.

These two teaching hospitals' size and pyramid-type referral systems make them ideal places to study NIs in Zambia. The two hospitals, located more than 320 km apart, were included as study sites to ascertain the diversity of AMR patterns in these two hospitals.

Sample size estimation

According to Gosling *et al*.,[13–15] data on the prevalence of hospital-acquired infections in Zambian hospitals were scarce. In this study, we assumed an estimated prevalence of 10% positivity to *P. aeruginosa* among patients screened for NI,[16] a 95% confidence level of obtaining a true estimate and an allowable error of 2%. Based on these assumptions, we used the AusvetEpitool (https://epitools.ausvet.com.au/oneproportion) to estimate the sample size of 841 patients. Considering the size of the two hospitals, sample estimates were distributed proportionally according to the bed capacity. Therefore, we set to screen three quotas (640) and one quota (201) of patients at the UTH and NTH, respectively.

Sampling

In this study, we applied consecutive sampling. We included everyone who met the inclusion criteria as they attended the hospitals (UTH and NTH) either as returning outpatients or inpatients. All consecutive (until meeting sample size) *P. aeruginosa* suspected infected patients meeting the eligibility criteria were identified and enrolled after informed consent.

Patient selection

Participants were enrolled among adult medical and surgical patients after obtaining consent from patients or their next of kin. Hospital or NIs were defined as infections that arose during hospitalization, occurring at least 48 h after admission.[17] The history of prior hospital contact within a month was also documented. Patients who developed clinical evidence of nosocomial wounds (surgical or pressure sore) and respiratory or urinary tract infections with a positive *P. aeruginosa* culture were included in the study.

Patients who presented with localized swelling, pain, purulent discharge, redness or heat in the skin, subcutaneous tissue, deep soft tissue, organ, or spaces and at least one positive culture for *P. aeruginosa* after 48 h post-surgical intervention were considered as nosocomial surgical site infection. Patients with fever (> 38°C), dysuria, frequency, urgency or suprapubic tenderness with no other recognized cause after 48 h of admission or recent history of catheterization were also considered. Non-catheterized patients were considered to have nosocomial urinary tract infections after obtaining a positive culture from their midstream urine.

Furthermore, patients with fever (> 38°C), chills, cough or hypotension and at least one positive culture for *P. aeruginosa* in sputum or respiratory secretions after 48 h of admission were considered to have a nosocomial respiratory infection and included as study participants. However, those patients who could not give either consent, clinical details or samples due to different conditions were excluded from the study.

Clinical data and laboratory specimen collection

Questionnaire data were administered to enrolled patients using the Epi collect five electronic tools (https://five.epicollect.net/).[18] Additional data were obtained from attending internists and surgeons for their decision if we could not select based on the above criteria. Face-to-face interviews were used to collect information on each patient's socio-demographic variables and potential risk factors for NI. For those unable to provide information, the caregiver was interviewed. Clinical data on chronic diseases, hospitalization, previous admission, ward type and antimicrobial taking history were collected by reviewing the patient's medical record and consulting the attending physician and surgeon. Clinical specimens such as urine, respiratory secretions and wound swabs were collected as soon as NI was suspected.

Wound, urine and respiratory sample collection and processing

Wound sample collection

Amies media sterile swabs were used to collect discharge from patients' septic burns, pressure sores or other wounds and at intravenous injection sites for those in a dialysis unit. Using Levine's technique, wound/pus specimens were collected aseptically by sterile cotton swabs dipped in normal saline.[19] This technique has been reported to detect more organisms in acute and chronic wounds than other techniques.[19,20]

Urine sample collection

Patients suspected of non-catheterized urinary tract infection were instructed to collect 10 mL midstream clean-catch urine samples using a wide-mouth sterile container. For catheterized patients, the catheter was clamped below the port to allow for urine to collect in the tubing. The catheter was disinfected with 70% alcohol, and 10 mL of freshly voided urine was aseptically collected through the port using a syringe. The urine sample was transferred to a sterile container. While changing catheters, the medical device tips were put in normal saline and taken for cultures.

Respiratory sample collection

In patients with chest symptoms, we collected the 'deep cough' sample of the early morning before eating or drinking anything to avoid bias in interpreting the results. At first, the patients needed to rinse out the mouth with clear water for 10–15 s to eliminate any contaminants in the oral cavity. After expelling saliva, the patients then breathed in deeply three times to cough at 2-min intervals until bringing up some sputum. At least 5 mL of sputum was then released in a sterile, well-closed container. ICU, the tip of the endotracheal tube was aseptically submitted for culture.

The hospital and wards of origin, date and time of collection, specimen type and tests performed were carefully noted. All specimens were placed in tightly sealed, leak-proof containers and transported in sealable, leak-proof plastic bags.

After labelling sterile containers, clinical specimens were transported to microbiology laboratories at the University of Zambia (UNZA) for UTH specimens and the Tropical Diseases

Research Centre for the NTH. All samples were transported for processing within 30min to 2 h of collection.

Processing, isolation and identification

All the specimens (swabs, urine and respiratory) were inoculated onto blood agar and MacConkey (Oxoid Ltd, Basingstoke, UK) and incubated aerobically at 37°C for 24 h. Urine was inoculated using the calibrated loop that measures about 1 µL, and after 24 h of incubation, plates were examined and inspected for bacterial growth. Colonies were counted using a colony counter and checked for significant bacteriuria. Cultures from catheterized and non-catheterized patients that grew $\geq 10^2$ and 10^5 cfu/mL, respectively, were taken as considerable bacteriuria and processed further.[21]

Pure bacterial growths from wounds, urine and respiratory samples were examined for colony morphology. All flat, large and greenish non-lactose fermenting colonies on MacConkey were sub-cultured onto nutrient agar (Oxoid Ltd) and subjected to Gram staining and conventional biochemical tests for presumptive identification. Isolates that were Gram-negative rods, oxidase-positive, catalase-positive, Simon's citrate-positive and urease-negative were considered presumptive *P. aeruginosa.*[22]

Molecular conformation of isolates

In addition to the phenotypic analysis, molecular analysis was performed to confirm the isolates. According to the manufacturer's instructions, DNA was extracted from purified presumptive positive cultures of *P. aeruginosa* using commercial DNA extraction kits (Qiagen, Hilden, Germany). PCR amplification was used to amplify 16S rRNA gene fragments from purified genomic DNA of culture isolates. The PCR master mix contained 1×PCR buffer [50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris/HCl (pH 9.0), 10 µg gelatine mL^{-1}], 200 µM deoxynucleotide triphosphates, 0.3 µM each of the 16S rRNA primers, 1.5 U *Taq* polymerase (Gibco BRL) and ~ 10 – 100 ng DNA in a final reaction volume of 100 µL. According to the manufacturer's instructions, PCR based on Extaq protocol [Takara Biotechnology (Dalian) Co, Ltd] was used for species identification using specific primers targeting the 16S rRNA gene as previously described.[23,24] PCR was also performed to identify resistant genes using specific primers for *Bla OXA 23*, *Bla OXA 51*, and *Bla IMP* genes.[25] The following thermal cycling conditions were used: 98°C for 10 s, 35 cycles of 98°C for 30 s, 54°C for 30 s and 72°C for 1 min, and final extension at 72°C for 5 min. The positive amplicons were purified using Wizard

SV Gel and Clean-Up System (Promega, Madison, WI, USA) according to the manufacturer's protocol. The purified DNA was later sequenced directly using a Big Dye terminator cycle sequencing ready reaction kit v3.1 and analysed on a 3500 Genetic Analyser (Applied Biosystems, Foster City, CA, USA).

Antimicrobial susceptibility testing

The susceptibility of *P. aeruginosa* isolates to different antibiotics was determined by Kirby–Bauer's disk diffusion agar method on cation-adjusted Mueller–Hinton agar (Merck, Darmstadt, Germany) according to the Clinical and Laboratory Standards Institute recommendations.[26] Antibiotic disks (MAST Diagnostics, Merseyside, UK) tested were as follows: CAZ (30 µg), TZP (100 µg/10 µg), CIP (5 µg), GEN (10 µg), AMK (30 µg), TOB (10 µg), IPM (10 µg), ATM (30 µg) and FEP (30 µg). The turbidity standard of a 0.5 McFarland (1.5×108 cfu/mL) of *P. aeruginosa* was prepared and spread on Mueller–Hinton agar as a lawn culture. The control strain of *P. aeruginosa (ATCC 27853)* was used to quality control antibiotic disks. *P. aeruginosa* is intrinsically resistant to CRO and CTX; therefore, *P. aeruginosa*'s sensitivity to third-generation cephalosporin was assessed based on susceptibility to CAZ.[27]

Data analysis

The collected data were validated and entered into Excel Spreadsheets before exporting to STATA version 14.0 (College Station, TX: StataCorp LP, USA) for analysis. Descriptive statistics were computed and presented using words and tables. The OR and 95% CI were calculated to measure the strength of the association.

The frequency of NI was determined at a 95% CI. The proportions (%) of the different variables were estimated as the number of positive outcomes divided by the number examined. Quantitative variables were summarized using mean and SD. Frequency distributions of the variables were produced and checked for inconsistencies and input errors. A χ^2 test was performed to screen potential factors associated with AMR in univariable analysis. Predictors of AMR were determined using step-wise logistic regression. The logistic regression model was tested for goodness of fit using the Hosmer–Lemeshow test. The level of significance was set at a P value of < 0.05. The nucleotide sequences obtained from the 16S rDNA analysis were compared for similarity with other previously published sequences using the BLAST search on NCBI/PubMed https://blast.ncbi.nlm.nih.gov/Blast.cgi? PAGE_TYPE=BlastSearch.

Ethical consideration

The study protocol was ethically approved by the University of Zambia Biomedical Research Ethic Committee (UNZA REC) (ref. no. 671-2019). Participants provided informed written consent to collect anonymized clinical and demographic data. For illiterate participants, a witnessed fingerprint was obtained. All obtained information was confidential and only used for research or academic purposes. Electronic devices used to collect and store the data were password protected.

Furthermore, authorization for data collection was obtained from all legally recognized district and hospital representatives. After clarifying the study's objective, written informed consent was obtained from adult study participants. The results were maintained anonymously and used only for the study. Positive findings were communicated to the attending clinician for appropriate treatment.

Results

Socio-demographic characteristics

Eight hundred and forty-one patients were screened for NIs. The majority were male (81.2%). The participants ranged from 15 to 98 years, with a mean age of 51, and most (90.4%) lived in Lusaka. Only 9.6% of participants completed tertiary and 18% (150) secondary education, while 563 achieved at least primary education. Around 6% (53) of participants reported no formal education. Only 10% (85) of participants reported being in formal employment, while the majority (756) were either in the informal sector or unemployed. Most participants (71%) were married. Of 841 screened, 588 patients (69.9%) had positive bacterial cultures (Figure1).

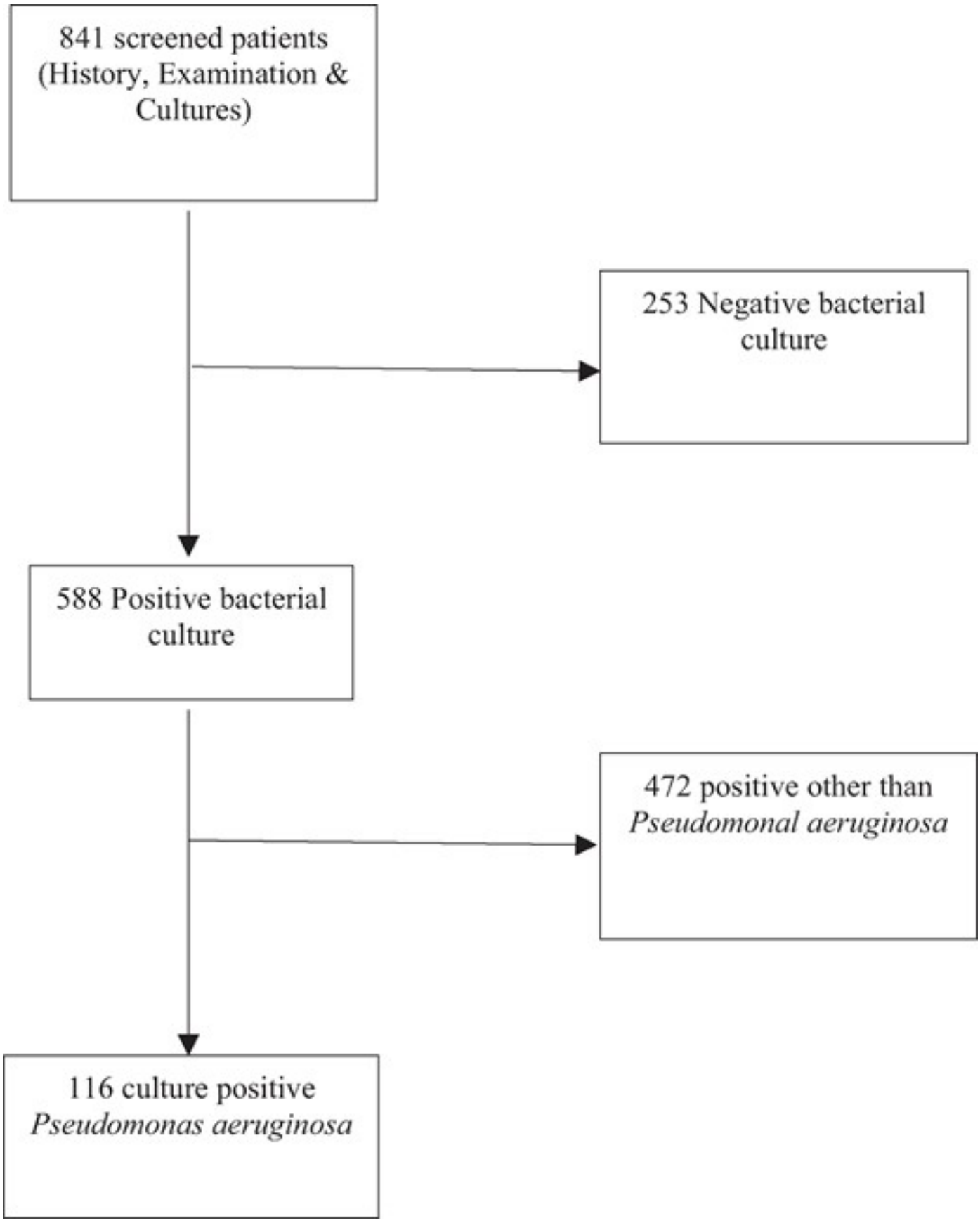

Figure 1.

Schematic diagram of the sample processing and *P. aeruginosa* isolation.

The full details of these were described in a previous publication.[26] The current study focussed on AMR of the 116 (13.7%) patients diagnosed with *P. aeruginosa* infections. Of them, 96 were male and 20 were female.
Table1 highlights the factors associated with AMR in univariable analysis.

Table 1.
Association of clinical and molecular characteristics with AMR to *P. aeruginosa* among patients treated for NI at two tertiary hospitals, Lusaka and Copperbelt, Zambia

Independent variables		**AMR**				
		Yes	**No**	**OR**	**CI**	***P* value**
Gender	Male	50	46	1.49	0.55–4.04	0.441
	Female	8	11			
Age (years)	15 – 65	49	50	0.84	0.35–2.00	0.826
	> 65	14	12			
Education status	Illiterate	27	16	2.28	1.05–5.07	0.033*
	Literate	30	41			
Residence	High density	36	41	0.68	0.31–1.47	0.432
	Low density	22	17			
Occupation	Employed	14	13	0.98	0.41–2.32	1.000
	Unemployed	48	44			
Previous antimicrobial use	Yes	13	17	0.73	0.31–1.69	0.522
	No	40	38			
Hospital	UTH	53	48	2.57	0.63–10.52	0.202
	NTH	3	7			
Department	Inpatient	31	27	1.37	0.65–2.88	0.453
	Outpatient	25	30			
Wards	Surgical	48	17	4.16	1.30–13.29	0.014*

Independent variables		AMR		OR	CI	*P* value
		Yes	**No**			
	Medical	47	4			
History of previous admission (30 days)	Yes	25	33	0.86	0.39–1.86	0.843
Prior medication	Yes	42	46	0.66	0.24–1.80	0.458
	No	11	8			
Invasive medical device	Yes	3	92	0.19	0.03–1.24	0.081
	No	3	18			
Comorbidity	HIV+ve	10	3	0.70	0.18–3.47	0.615
	HIV−ve	85	18			
	BPH+ve	9	10	2.25	0.78–6.45	0.165
	BPH−ve	18	45			
Presence of resistance genes	Amp C+ve	43	20	0.80	0.27–2.36	0.792
	Amp C−ve	16	6			
	BLA OXA 23+ ve	7	1	3.36	0.39–28.86	0.425
	BLA OXA 23−ve	52	25			
	BLA OXA 51+ ve	65	6	5.33	1.91–16.3	0.001*
	BLA OXA 51−ve	30	15			
	BLA IMP+ve	5	1	1.16	0.13–10.52	0.481
	BLA IMP−ve	90	21			

IPD, inpatient department; MSU, midstream urine; NTH, Ndola Teaching Hospital; OPD, outpatient Department; RTI, respiratory tract infection; UTH, University Teaching Hospital; UTI, urinary tract infection.

Participant's enrolment per study sites and wards

One hundred and sixteen patients were diagnosed with *P. aeruginosa* NI out of 841 screened participants from the UTH (640) and NTH (201). NI types and samples collected from patients at the UTH and NTH from different departments are shown in Table 2.

Table 2.
NI types and samples collected from the UTH and NTH

Hospital	Ward	Department	UTI (MSU)	Wound (swab)	RTI (sputum)	Total
UTH	Medical	OPD	9	13	1	24
		IPD	14	12	2	28
	Surgical	OPD	17	12	3	32
		IPD	12	8	1	21
	Medical	OPD	0	0	0	0
NTH		IPD	1	3	2	6
	Surgical	IPD	1	3	0	4
		OPD	0	1	0	1
Total			51	56	9	116

IPD, inpatient department; MSU, midstream urine; NTH, Ndola Teaching Hospital; OPD, outpatient department; RTI, respiratory tract infection; UTH, University Teaching Hospital; UTI, urinary tract infection.

Factors associated with *P. aeruginosa* NI

Invasive medical device-related NI was a common presentation (78.5%) among participants. However, the presence of the medical device was not associated with AMR (*P* value 0.4581). A urinary catheter was frequently inserted among surgical patients being managed for benign prostate enlargement (BPH). Catheter-associated urinary tract infection was the most common NI (57%). The other common diagnosis was infected pressure sores

(38.7%). AMR was associated with carbapenem-hydrolysing β-lactamase gene *BlaOXA-51* (*P* value 0.001), literacy level (*P* value 0.033) and surgical ward attendance (*0.014).

Factors that were not associated with AMR include having an age of ≥ 65 years, male gender, prior admission and prolonged hospital admission. More than half (62%) of participants reported a history of previous hospital exposure within the past 30 days, which were prior medical visits or being ex-bed-siders taking care of patients.

AMR profiles

The antimicrobial susceptibility and resistance profiles of all 116 clinical isolates of *P. aeruginosa* are shown in Table 3. The antibiotic sensitivity of *P. aeruginosa* was best with aminoglycosides (AMK 87%, TOB 86% and gentamycin 79%), quinolones (CIP 70%) and carbapenem (IPM 58.2%). The susceptibility to monobactam (ATM 34%), cephalosporins third-generation (ceftazidime 55.4%) and fourth-generation (FEP 3.8%) antibiotics was moderate to poor.

Table 3.
P. aeruginosa antimicrobial susceptibility and resistance patterns

Antibiotic name	**R (%)**	**I (%)**	**S (%)**	**R 95% CI**	**S 95% CI**
Piperacillin/tazobactam	30.09	13.59	56.31	21.7–40.0	46.2–65.9
Ceftazidime	34.54	10	55.45	25.9–44.3	45.7–64.8
FEP	69.23	26.92	3.84	59.3–77.7	1.2–10.1
Aztreonam	39.80	25.24	34.95	30.4–49.9	26.0–45.0
Imipenem	6.79	34.95	58.25	3.0–14.0	48.1–67.8
Amikacin	2.73	10	87.27	0.7–8.4	79.2–92.6
Gentamicin	19.42	0.97	79.61	12.5–28.6	70.3–86.7

Antibiotic name	R (%)	I (%)	S (%)	R 95% CI	S 95% CI
Tobramycin	8.74	4.85	86.40	4.3–16.4	77.9–92.1
Ciprofloxacin	24.27	4.85	70.87	16.6–33.9	61.0–79.2

I, intermediate; R, resistant; S, sensitive.

A high prevalence of multidrug resistance (MDR) (73.6%) and extensive drug resistance (XDR) (37.2%) was observed in the current study at all the two tertiary hospitals included in the present study (see Table1).

Table 4.
Distribution of MDR and XDR *P. aeruginosa* at the UTH and NTH

	Frequency			
AMR pattern	**UTH (*n*=106)**	**NTH (*n*=11)**	**Total**	**Associated resistance genes**
MDR	80 (75.5%)	5 (45.4%)	85 (73.3%)	*amp* C, *Bla OXA 23*, *bla*OXA 51
XDR	40 (39.6%)	1 (0.9%)	41 (37.2%)	*Amp* C, *bla*OXA 23, *bl*OXA 51, *bla*IMP

MDR, multidrug resistance: resistance to at least one agent in three or more antibiotic classes; XDR, extensive drug resistance: resistance to at least one agent in all but two or fewer antimicrobial categories.

The multivariate logistic regression model results showed that *P. aeruginosa* MDR was related to province, admission to the surgical department, current antibiotic therapy, self-medication and *bla*OXA 51 genes. Details of the logistic regression are shown in Table 5 (MDR) and Table 6 (XDR).

Table 5.
Logistic regression analysis of the factors associated with *P. aeruginosa* NI and MDR at the UTH and NTH

Provinces	*0.11*	*−2.14*	*0.032*	*0.0141587*	*0.83*
Department	*0.15*	*−2.73*	*0.006*	*0.038*	*0.583*
Current antibiotics treatment	*4.36*	*2.07*	*0.039*	*1.08*	*17.59*
blaOXA-51gene1	*8.80*	*3.54*	*0.000*	*2.63*	*29.39*
Self-medication	*2.05*	*1.13*	*0.260*	*0.586*	*7.20*
Cons	*180.97*	*2.84*	*0.005*	*4.99*	*6561.30*

_cons estimates baseline odds.

Table 6.
Logistic regression analysis of the factors associated with *P. aeruginosa* NI and XDR at the UTH and NTH

Variables	**OR**	***Z***	***P*>[*z*]**	**95% CI**	
Age group	1.92	2.14	0.033	1.06	3.51
blaOXA23 gene	12.52	2.72	0.007	2.02	77.63
blaOXA51 gene1	4.67	3.00	0.003	1.71	12.78294
Cons	0.02	−3.71	0.000	0.003	0.17

_cons estimates baseline odds.

Genomic analysis

The results of amplified genes by PCR showed that six (5.1%) *P. aeruginosa* clinical isolates contained *bla*IMP. These six clinical isolates all belonged to the UTH in Lusaka and were cultured from urinary tract infection (n=4) and skin swabs from patients with infected decubitus ulcers (n=2). All these six patients were from the surgical outpatient department (50%) and the medical outpatient department (50%) with a history of prior medical

contact within 90 days and self-medication. These clinical isolates were MDR and harboured *amp* C and *bla*OXA51 genes. Table 7 highlights the frequency of targeted resistance genes.

Table 7.

P. aeruginosa analysis of targeted resistance genes

Genomic analysis				
Genes and primers used		**+ve**	**−ve**	**%**
Primers	*PA-SS*	87	25	112 (96%)
	GGGGGATCTTCGGACCTCA (F)			
	TCCTTAGAGTGCCCACCCG (R)			
	PA-GS	70	16	86 (74%)
	GACGGGTGAGTAATGCCTA (F)			
	CACTGGTGTTCCTTCCTATA (R)			
	AMP C	79	20	99 (85.3%)
	CGGCTCGGTGAGCAAGACCTTC (F)			
	AGTCGCGGATCTGTGCCTGGTC (R)			
	Bla Oxa23	7	1	8 (6%)
	GATCGGATTGGAGAACCAGA (F)			
	ATTTCTGACCGCATTTCCAT (R)			
	Bla Oxa51	65	7	72 (62%)
	TAATGCTTTGATCGGCCTTG (F)			
	TGGATTGCACTTCATCTTGG (R)			
	Bla IMP	6	0	6 (5%)

Genomic analysis				
Genes and primers used		**+ve**	**−ve**	**%**
	GTTTGAAGAAGTTAACGGGTGG (F)			
	ATAATTTGGCGGACTTTGGC (R)			

The results of a PCR assay for 116 clinical isolates showed that the majority 99/116 (85.3%) of *P. aeruginosa* clinical isolates harboured *the amp* C *gene.* These 99 clinical isolates were recovered from the UTH (90) and NTH (9) in patients presenting with urinary tract infection (49) (49.5%), infected wounds (37) (37.4%) and respiratory tract infection (5%).

Figures 2-4 show agarose gel electrophoresis of the PCR product to determine the presence of PA-SS, *amp* C and *bla*OXA51 genes in *P. aeruginosa* clinical isolates.

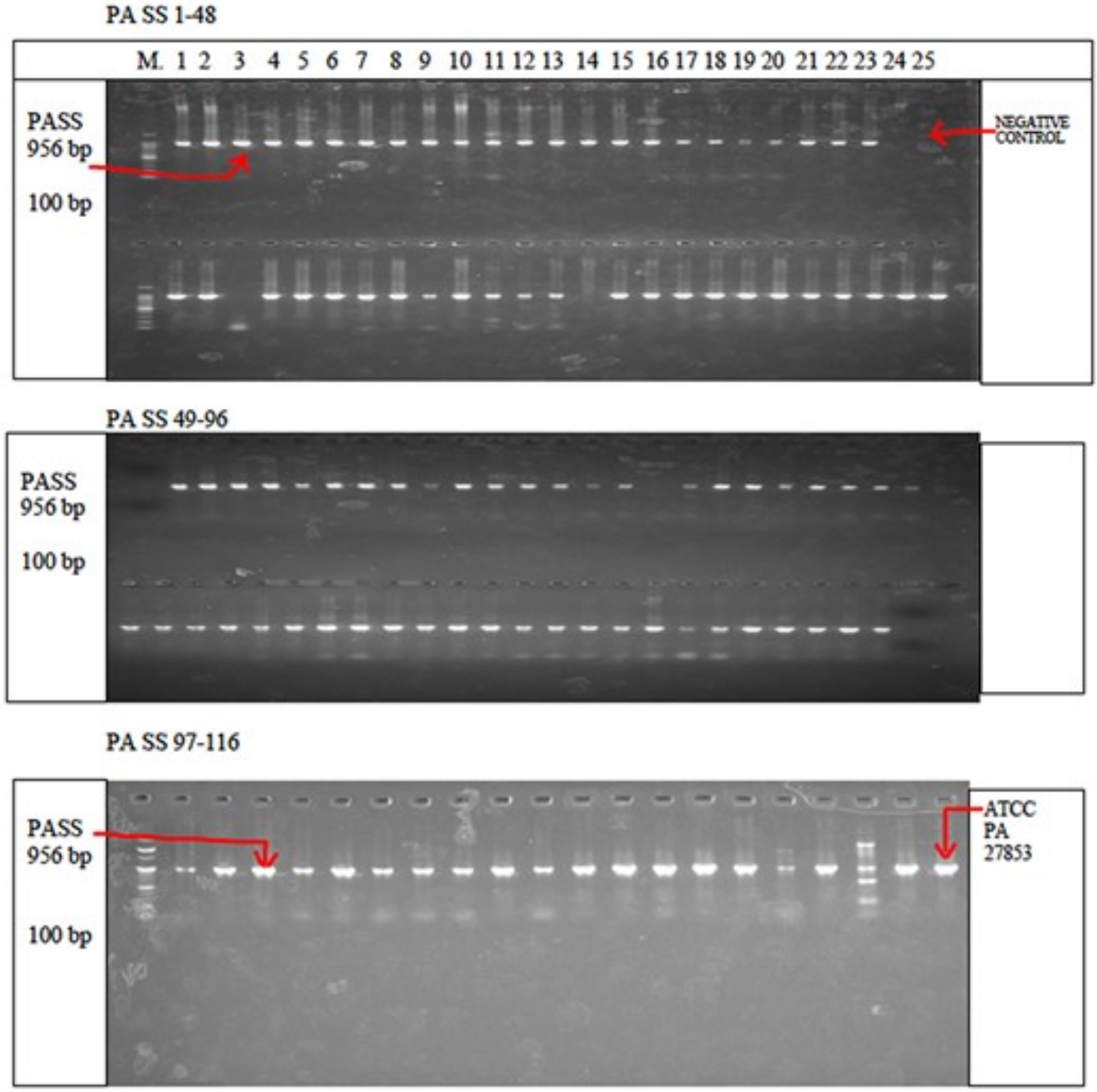

Figure 2.

An electrophoretogram showed amplicons of 116 clinical isolates of *P. aeruginosa* using specific primers (*PA-SS*). This figure contains clinical isolates from 1 to 48, 49 to 96 and 97 to 116. PA-SS-positive amplicons are shown in lanes 1–23, 26, 28–37, 39–48, 49–63, 65–72, 73–96 and 97–115. PA-SS-negative amplicons are shown in lanes 24, 27, 38 and 64. Ladder (lane M): 100 bp molecular maker, P: positive control, N: negative control, *25*. Positive control *P. aeruginosa* ATCC 27853 (116) (New England Biolabs Inc.).

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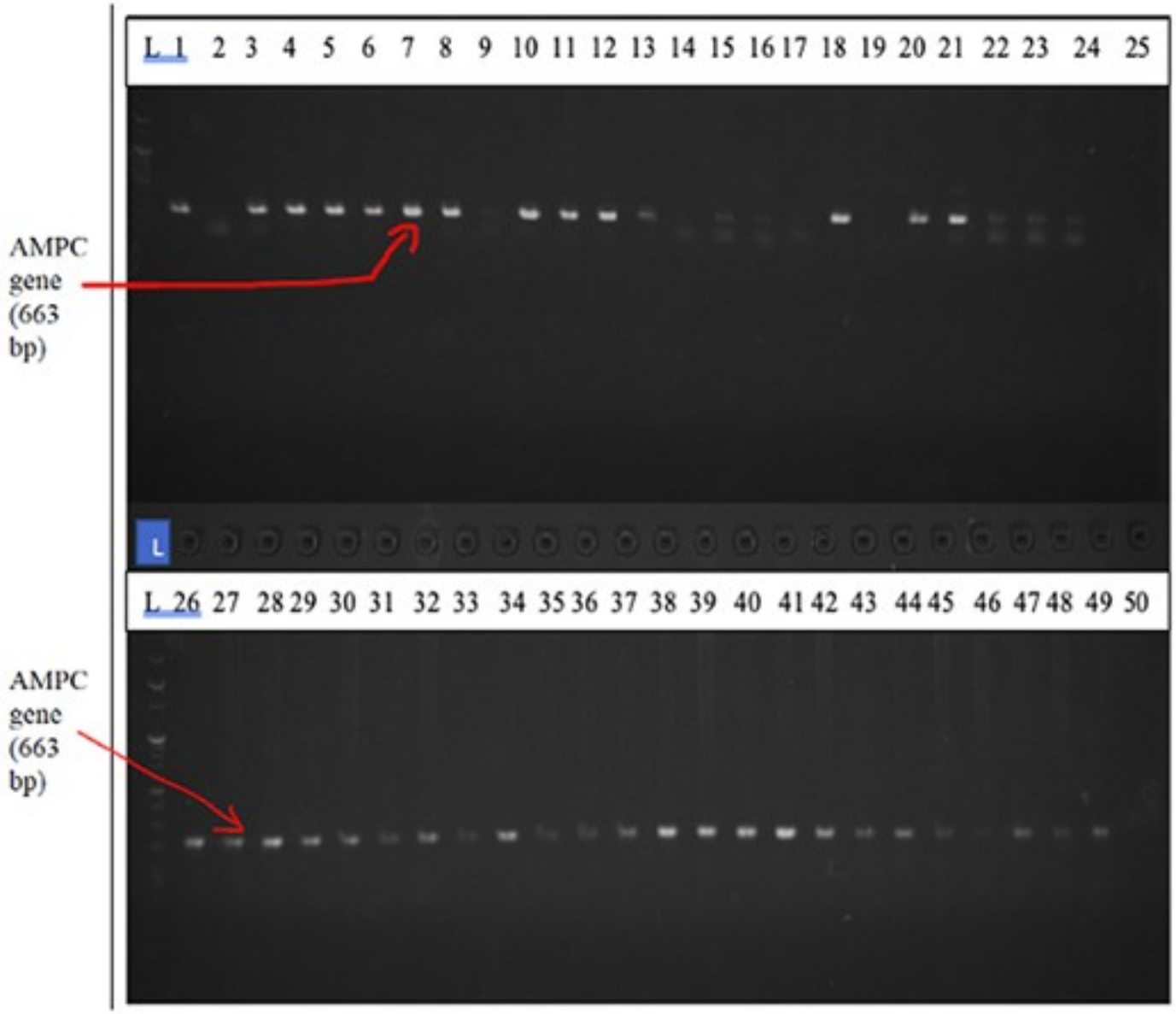

Figure 3.

Agarose gel electrophoresis of PCR product for the presence (1, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, 18, 20–49) or absence (2, 9, 14–17, 19) of *amp* C gene. A negative control is shown in well 50. Lane L represents the 100 pb DNA ladder.

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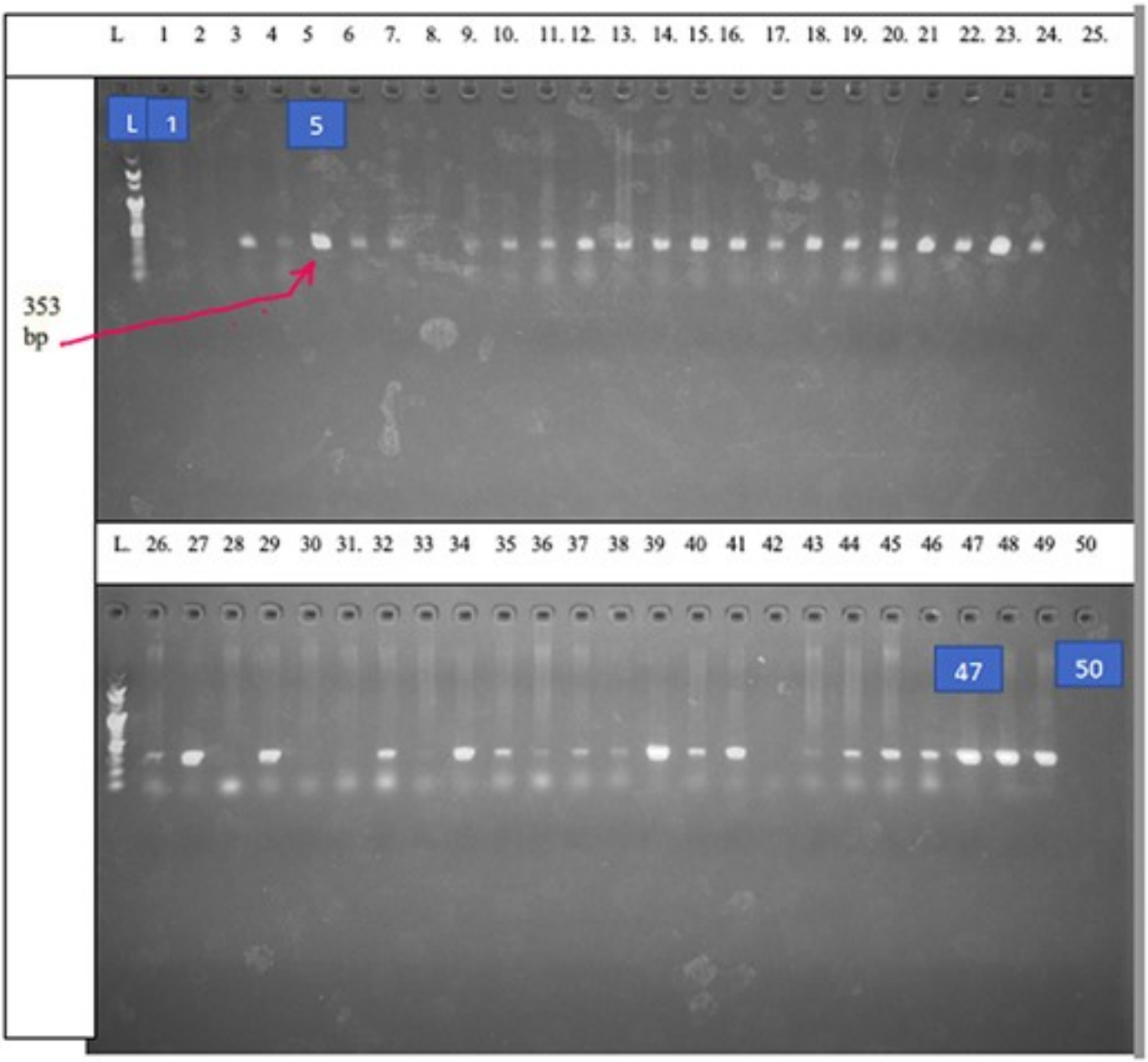

Figure 4.

Agarose gel electrophoresis of the PCR product for the presence (3, 4, 5, 6, 9–24, 26, 29, 32, 34, 35, 37, 38, 39, 40, 41, 44–49) or absence (1, 2, 4, 8, 9, 25, 28, 30, 31, 33, 42 and 43) of the *blaOXA51* gene. Wells 25 and 50 show a negative control. Lane L represents the 100 pb DNA ladder.

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The results of amplified genes by PCR showed that only eight (6%) clinical isolates contained *bla*OXA23. At the NTH, *bla*OXA23 was found in 10% of isolates from infected wounds and 6% of isolates from the UTH from patients

with infected wounds (n=2), urinary infection (n=3) and respiratory tract infection (n=2).

The results of amplified genes by PCR also showed that 72 (62%) clinical isolates contained *bla*OXA51. At the NTH, 40% of *P. aeruginosa* recovered from infected wounds (n=4) harboured *bla*OXA51. At the UTH in Lusaka, 64.1% of *P. aeruginosa* recovered from infected wounds (n=21), urinary tract infection (n=40) and respiratory tract infection (n=5) harboured *the bla*OXA51 gene.

Discussion

NIs caused by antibiotic-resistant *P. aeruginosa* have emerged as a primary concern in clinical care settings due to the increasing development of MDR strains.[6] This study evaluated AMR in patients in two tertiary hospitals in Zambia. The majority of participants were male patients with indwelling urinary catheters. In a study conducted in Ethiopia, Taye *et al*.[28] reported a 4.62 higher risk of developing NIs in patients with urinary catheters than non-catheterized chronic patients. This observation is consistent with other studies done in Poland,[29] China,[30] India,[31] Morocco[32] and Egypt.[33]

In the current study, most participants were enrolled from the UTH compared with the NTH. At the NTH, the data were collected during the pick of the COVID-19 third wave, hence the low enrolment (23.9%) of participants at this site due to a decrease in the number of patients attending hospital for non-COVID 19-related illnesses during the study period. Similarly, Quadros *et al*.[34] linked the reduction of patients attending hospitals with non-COVID-19-related diseases during an outbreak to public anxiety about acquiring the viral infection in the hospital and the subsequent risk of mortality and lockdown.

The current study found self-medication to be associated with AMR. Similarly, Miliani *et al*.[35] reported that AMR was associated with the common usage of antibiotics. Likewise, Leopold *et al*.[36] and Tadesse *et al*.[37] reported a high resistance level to commonly used antibiotics compared with less prescribed antibiotics in sub-Saharan Africa. Despite the development by the WHO of the Access, Watch and Reserve classification system to guide the use of antibiotics

and prevent AMR,[38] there are numerous challenges in the implementation in various countries due to a lack of awareness, limited resources and capacity to implement the framework in many countries, especially in lower- and middle-income countries, including Zambia. This could be due to the low availability of affordable and good-quality antibiotics, leading to the inappropriate use of antibiotics and AMR.[39] The association between self-medication and AMR observed in our study could also result from repeated exposure to antimicrobial agents through over-the-counter access to these drugs.[40,41]

The current study found high resistance levels to CAZ and ATM despite the report that *P. aeruginosa* is naturally susceptible to carboxypenicillins, ceftazidime and aztreonam.[6] This could be explained by the high prevalence of Amp C β-lactamase in the current study.

The present study also observed a high resistance level to piperacillin despite being combined with a β-lactamase inhibitor (tazobactam). This could also result from the increased expression of the Amp C gene observed in our clinical isolates of *P. aeruginosa*. Amp C cephalosporinase activity is not inhibited by β-lactamase inhibitors used in clinical practice, such as CLA, SUL and TZB.

Resistance of *P. aeruginosa* to CIP is a rising problem in many parts of the world. In the current study, the resistance rate to CIP was low but higher than that reported in some countries in the Middle East.[42] This was similar to the findings by Yang *et al*.[42] who reported low AK and CIP resistance among *P. aeruginosa* in South China. Due to the relatively low resistance of *P. aeruginosa* to fluoroquinolones among our clinical isolates, the mutation of *gyrA*/*gyrB* genes within the quinolone-resistance-determining region linked to fluoroquinolones resistance of *P. aeruginosa* was not investigated.

The multivariate logistic regression model in the present study showed that *P. aeruginosa* AMR was associated with self-medication, surgical OPD attendance or ward admission and the carbapenem-hydrolysing β-lactamases gene *blaOXA-51*. Generally, self-medication has been reported to be associated with emergency of AMR in bacteria,[43,44] and thus, this practice needs public

sensitization. In a related study, Yamba *et al.*45 observed high morbidity and mortality rates associated with AMR pathogens in the same hospital (UTH) surgical ward, indicating the need to improve infection prevention and control in the surgical wards.

Conclusion

This study has demonstrated that multidrug-resistant *P. aeruginosa* is highly prevalent in hospitals in Zambia's Lusaka and Ndola districts and possibly countrywide. *P. aeruginosa* AMR was associated with self-medication, surgical care and carbapenem-hydrolysing β-lactamase gene *blaOXA-51*. This calls for the establishment and implementation of antimicrobial stewardship programmes and the strengthening of the surveillance system.

Limitations

The study was conducted at two large teaching hospitals, though including data from the regional hospital could give an idea of the actual burden of *P. aeruginosa* AMR countrywide. This study should expand to regional hospitals to obtain the real-burden AMR profiles.

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Transparency declarations

None to declare.

Author contributions

P.N.M., J.B.M., G.K., J.-M.K. and S.M. conceived, designed and supervised the study and revised the manuscript. P.N.M. and J.K. performed the clinical and molecular experiments. P.N.M., F.B. and K.Y. drafted the manuscript. J.B.M. and P.N.M. analysed and interpreted the data and contributed to statistical analysis. All authors read and approved the final manuscript.

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