CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA* RECOVERED FROM SURGICAL SITES AT KAKAMEGA COUNTY GENERAL HOSPITAL

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I dedicate this research work to God and my family who supported and encouraged me during this academic journey.

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ABSTRACT

Pseudomonas aeruginosa is a significant pathogen in surgical site infections (SSIs) due to its diverse virulence factors and high antibiotic resistance. It is important to understand its prevalence, resistance profiles, genotypic diversity, and physiological attributes in order to formulate effective treatment and infection control strategies. The objective of the investigation was to ascertain the prevalence of *P. aeruginosa* among bacterial isolates from surgical sites, evaluate the antibiotic resistance profiles of these isolates, assess their genotypic diversity, and determine major physiological characteristics that contribute to their pathogenicity. Standard microbiological techniques were employed to confirm the presence of *P. aeruginosa* isolates among the 128 bacterial isolates collected and identified from surgical sites. The disc diffusion method was employed to conduct antibiotic susceptibility testing. Sequence typing and phylogenetic analysis were employed to investigate genotypic diversity. Protease secretion, motility, exoprotein production, pigment production, and quorum sensing (QS) activity were evaluated to ascertain their contribution to virulence. In order to identify resistance patterns and ascertain the susceptibility of isolates to a variety of antibiotics, a statistical analysis of antibiotic resistance data, including MIC values, was performed. The genetic relationships among the isolates were revealed through the construction of phylogenetic trees using sequence typing data. Categorical variables were investigated using chi-square analysis, with a significance level of $P<0.05$. Principal component analysis (PCA) was employed to identify clustering patterns associated with biofilm formation and QS activity, and physiological data were analysed to compare the virulence characteristics of various isolates. *P. aeruginosa* was identified as the most prevalent bacterium, accounting for 38.9% of the isolates. The antibiotic susceptibility testing demonstrated significant multidrug resistance, with resistance rates of 60.7% for Ceftazidime, 60.7% for Ciprofloxacin, and 71.4% for Amikacin. Meropenem demonstrated the maximum sensitivity, with a value of 53.6%. Genotypic analysis revealed substantial diversity, including multiple sequence types (STs) and distinct phylogenetic clusters, which suggest both genetic variability and clonal dissemination. Physiological evaluations demonstrated that protease secretion, motility, exoprotein production, and QS activity were subject to variation, with certain isolates exhibiting heightened pathogenic potential. The study emphasises the high prevalence and multidrug resistance of *P. aeruginosa* in surgical site infections, as well as the significant genotypic diversity and complex physiological traits that contribute to its pathogenesis. These results underscore the necessity of enhanced infection control, targeted antibiotic stewardship, and ongoing research into innovative therapeutic strategies to effectively manage *P. aeruginosa* infections.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ACRONYMS AND ABBREVIATIONS

ABHR	Alcohol-Based Handrub
AMR	Antimicrobial Resistance
CDC	Centre for Disease Control and Prevention
CP	Carbapenemase-Producing
CPE	Carbapenemase-Producing Enterobacteriaceae
EQAS	External Quality Assurance System
HAI	Health Care-Associated Infection
KCTRH	Kakamega County Teaching and Referral Hospital
KNBS	Kenya National Bureau of Statistics
MTC	Medical Therapeutic Committee
NACOSTI	National Commission for Science, Technology, and Innovation
PSSIs	Post-surgical site infections
SSIs	Surgical site infections
SOP	Standard Operating Protocols
WHO	World Health Organization

OPERATIONAL DEFINITION OF TERMS

Antimicrobial prophylaxis: The process of administering antimicrobials to prevent the development of infection.

Antimicrobial resistance: The ability of a microorganism to stop or prevent the activity of an antimicrobial that was once effective against it.

Antimicrobial stewardship: A coherent set of actions which promote the responsible use of antimicrobials. This definition can be applied to actions at the individual level, as well as the national and global level, and across human health, animal health and the environment.

Antimicrobial: products that kill or slow the spread of a microorganism.

Debridement - This is the wide removal of unhealthy tissues from a surgical wound to promote healing of the remaining healthy tissues.

Emplaced - This is when something is put in place for example an implant. Extrinsic - Features that are external to the patient.

Healthcare associated infection - This is an infection that a patient can get while receiving treatment in a hospital and the infection was not present during the admission period.

Surgical site infections (SSI): Infections that occur at the site of incision within 30 days of any surgery.

Standard operating procedure: Set of step-by-step instructions compiled by an organization to help workers carry out routine operations in the most effective manner.

CHAPTER ONE

INTRODUCTION

The study background, gaps, statement of the problem, objectives, research questions, justification, significance, scope, and limitations are presented in this chapter. Additionally, the conceptual model has been explained.

1.1 Background information

Post surgery, a surgical site infection (SSI) develops in the region of the body where the operation was performed. Surgical site infections may be superficial, affecting only the epidermis. Other surgical site infections are more severe and may affect tissues beneath the epidermis, organs, or implanted material (Berríos-Torres *et al*., 2017). SSIs are a significant global issue in the field of surgery, resulting in numerous complications, increased morbidity, and mortality. The majority of SSIs are hospital-acquired, with the prevalence of occurrence varying from one hospital to another (Sharma *et al*., 2023). In the United States, these infections are responsible for 38% of all nosocomial infections and affect 2-5% of patients who undertake surgical procedures (Anderson *et al*., 2008). In Kenya, the rates of surgical site infections at Aga Khan University Hospital and Kenyatta National Hospital are 7%, 30.8%, and 37.7%, respectively (Dinda *et al*., 2013; Miima *et al*., 2016). Thus, SSIs are a significant issue, accounting for approximately 20% of all health care-associated infections that lead to postoperative complications (Morad Asaad & Ahmad Badr, 2016).

SSIs are those caused by *P. aeruginosa*, a Gram-negative bacterium that is versatile and has a well-known but geographically limited ability to cause severe infections in healthcare contexts. *P. aeruginosa* is a significant pathogen because of its ability to induce severe sirgical site infections and its adaptability. In addition to the capacity to acquire resistance genes through mobile genetic elements, the pathogen has intrinsically developed drug resistance mechanisms, which further exacerbates these infections. Additionally, reports have indicated the emergence of multi-drug-resistant strains of *P. aeruginosa*, which further impedes the available treatment options and requires effective surgical patient care (Berríos-Torres *et al*., 2017; Sharma *et al*., 2023). Therefore, it is essential to possess an understanding of the antimicrobial resistance profile and virulence potential of the pathogen isolated from surgical site infections in order to develop strategies for infection control and treatment (Morris & Cerceo, 2020). Furthermore, understanding the transmission dynamics of the pathogen within the designated and other hospital settings can be facilitated by an understanding of the genetic intricacies of the pathogen recovered from the SSIs in Western Kenya (Behzadi *et al*., 2021). Other research has further elucidated on the necessity of ongoing identification of virulence determinants to facilitate the comprehension of the adaptability and pathogenicity of this microbe, which is associated with surgical wound infections (Elmouaden *et al*., 2019).

Despite the global efforts to combat antimicrobial resistance by *P. aeruginosa*, recent studies in Kenya continue to report concerning trends in surgical site infections. Additionally, resistance to commonly used antibiotics, such as fluoroquinolones like Ciprofloxacin, beta lactams like Piperacillin, aminoglycosides like amikacin, and cephalosporins like Ceftazidime, is developing (Dinda *et al*., 2013; Miima *et al*., 2016). This persistent resistance has resulted in an increase in the prevalence of certain genotypes that are classified as multi-drug resistant, which has further exacerbated the necessity for urgent, customised treatment and management strategies. Furthermore, the pathogenesis of *P. aeruginosa* is associated with the production of numerous virulence factors, which are categorised as either cell-associated or secreted. In previous research on virulence genes and antibiotic resistance in *P. aeruginosa* isolated from in-patients, it was found that lasB (98.7%) and exoS (98.7%) were the most frequently detected virulence genes in *P. aeruginosa* strains, followed by plcH (96.1%) and algD (87.7%). The least frequently detected virulence factor gene was exoA (74.2%) (Elmouaden *et al*., 2019). The presence of certain virulence genes caused by mutation and codes for specific antibiotic resistance patterns is not necessarily associated with the presence of similar virulence factor genes in *P. aeruginosa* strains, as indicated by a variety of studies (Schroeder *et al*., 2017). Consequently, it is imperative to comprehend the interplay between the physiological traits of the isolated isolates, genotypic diversity, and antimicrobial resistance in order to mitigate the global health consequences of *P. aeruginosa*.

Consequently, the current investigation investigated the distribution, antibiotic resistance, genotypic diversity, and specified physiological attributes of the recovered *P. aeruginosa* in Kakamega County General Hospital (KCGH), Kenya. The data generated in this study has the potential to inform healthcare practices at the local, national, and global levels in accordance with the guiding principles of antimicrobial stewardship.

1.2 Statement of the problem

Surgical site infections (SSIs) are a significant cause of postoperative complications, resulting in increased healthcare costs, lengthy hospital stays, and significant morbidity. Due to its high level of intrinsic antibiotic resistance, its capacity to rapidly acquire multidrug resistance (MDR), and its diverse array of virulence factors, *P. aeruginosa* is particularly challenging among the pathogens implicated in SSIs (Pachori *et al*., 2019; Wood *et al*., 2023). *P. aeruginosa* is a critical target for therapeutic intervention and infection control due to these characteristics. Nevertheless, the current state of knowledge regarding the prevalence, antibiotic resistance profiles, genotypic diversity, and physiological traits of this pathogen in the context of SSIs is insufficiently detailed, despite its significance.

Previous research has primarily concentrated on the prevalence of *P. aeruginosa* in clinical settings or its resistance to specific antibiotics, frequently employing isolated or small-scale data sets (Elfadadny *et al*., 2024; Pang *et al*., 2019; Wood *et al*., 2023). Traditional microbiological techniques, including disc diffusion for antibiotic susceptibility testing and basic genotyping methods, were frequently employed in these investigations. Although these methods have yielded valuable insights, they are subject to a number of constraints. The complete spectrum of resistance mechanisms is not always captured by conventional susceptibility testing methodologies, particularly for complex organisms such as *P. aeruginosa* (Munita & Arias, 2016). Moreover, the genetic diversity and evolutionary dynamics of *P. aeruginosa* populations were frequently not completely investigated in previous genotyping studies (Botelho *et al*., 2023). Furthermore, the physiological characteristics of *P. aeruginosa*, including motility, protease secretion, biofilm formation, and quorum sensing (QS), have been inadequately investigated in the context of SSIs, resulting in an incomplete comprehension of the pathogen's virulence and persistence (Qin *et al*., 2022; Tuon *et al*., 2022).

The necessity of a more comprehensive and integrated approach to the study of *P. aeruginosa* in SSIs is underscored by the limitations of these previous methodologies. This study offers a more comprehensive and precise evaluation of *P. aeruginosa* in the context of SSIs by incorporating these methodologies. The exhaustive analysis of physiological traits provides new insights into the mechanisms underlying *P. aeruginosa*'s virulence and resistance, while the use of advanced genotyping techniques enables a more in-depth exploration of the genetic diversity and evolutionary relationships among the isolates. This method not only mitigates the deficiencies of prior investigations, but it also provides a comprehensive framework for the enhancement of patient outcomes, the optimisation of antibiotic utilisation, and the development of more effective infection control strategies in the management of SSIs caused by *P. aeruginosa*.

1.3 Objectives

1.3.1 General Objective

To characterise *P. aeruginosa* recovered from surgical sites at Kakamega County General Hospital (KCGH).

1.3.2 Specific Objectives

1. To determine the prevalence of *P. aeruginosa* among bacterial isolates from surgical sites among patients attending KCGH
2. To evaluate the antibiotic resistance profiles of *P. aeruginosa* isolates from surgical sites among patients attending KCGH
3. To assess genotypic diversity in *P. aeruginosa* isolates obtained from surgical sites among patients attending KCGH

iv. To determine physiological traits of *P. aeruginosa* isolates from surgical sites among patients attending KCGH

1.4 Research questions

i. What is the prevalence of *P. aeruginosa* isolates from surgical sites vary across different patient demographics at KCGH?
ii. What are the antibiotic resistance patterns of *P. aeruginosa* isolates to key classes of antibiotics at KCGH?
iii. How diverse are the genotypes of *P. aeruginosa* isolates from surgical sites infections at KCGH?
iv. What physiological traits characterise *P. aeruginosa* isolates from surgical sites KCGH?

1.5 Justification

P. aeruginosa is a significant pathogen that causes surgical site infections (SSIs), which have a significant impact on patient recovery, increase healthcare costs, and contribute to protracted hospital stays. *P. aeruginosa* poses a significant challenge in clinical contexts due to its diverse virulence factors and inherent capacity to resist multiple antibiotics (Qin *et al*., 2022). Despite its acknowledged significance, there is an urgent need for a more comprehensive comprehension of the pathogen's behaviour, particularly in the context of SSIs, across a variety of geographical settings and hospital environments.

The recognition that the prevalence, resistance patterns, and genetic diversity of P. aeruginosa can vary significantly depending on regional factors, healthcare practices, and environmental conditions is one of the primary reasons for studying this pathogen in various geographical locations and healthcare institutions (Al-Orphaly *et al.*, 2021; Sid Ahmed *et al.*, 2022). The evolution of *P. aeruginosa* strains and the emergence of multidrug-resistant (MDR) variants may be influenced by the significant differences in antibiotic usage policies, infection control protocols, and the availability of healthcare resources across hospitals and regions (Cristina *et al.*, 2021; Sathe *et al.*, 2023). It is essential to comprehend these distinctions in order to create strategies that are specifically designed to address the unique obstacles encountered in various environments. Furthermore, the examination of *P. aeruginosa* in a variety of environments is instrumental in the identification and monitoring of isolates that are particularly virulent or resistant, which is beneficial for global surveillance efforts and informs regional public health interventions.

Many studies have been restricted by their narrow focus on either prevalence, basic antibiotic resistance profiles, or rudimentary genotyping methods, despite the fact that previous research has provided valuable insights into the general behaviour of *P. aeruginosa* (Elfadadny *et al.*, 2024; Pachori *et al.*, 2019). Although informative, these methods have not completely captured the complexity of *P. aeruginosa* infections in surgical settings. Additionally, the pathogen's virulence is not adequately examined in the context of SSIs due to the physiological characteristics that contribute to it, including motility, protease secretion, biofilm formation, and quorum sensing (QS). The development of effective treatment protocols and targeted infection control strategies is impeded by this knowledge gap.

The investigation substantiates the implementation of sophisticated methodologies to surmount these constraints and offer a more thorough comprehension of *P. aeruginosa* in SSIs. The study provides a comprehensive evaluation of antibiotic resistance profiles by utilising a combination of disc diffusion and minimum inhibitory concentration (MIC) methodologies, which is crucial for identifying even the most minute variations in susceptibility among isolates. Sequence typing and phylogenetic analysis are employed to investigate genotypic diversity, thereby facilitating a more comprehensive comprehension of the evolutionary dynamics and genetic relationships of *P. aeruginosa* strains. These techniques are essential for the identification of clonal lineages, the comprehension of the dissemination of resistance genes, and the guidance of the development of molecular diagnostics.

In addition, the investigation's emphasis on physiological characteristics, including motility, exoprotein production, quorum sensing activity, and protease secretion, offers crucial insights into the virulence mechanisms of *P. aeruginosa*. The bacterium's capacity to cause infection, persist in the host, and resist treatment is significantly influenced by these characteristics. The study guarantees a comprehensive and robust characterisation of the isolates' pathogenic potential through the utilisation of principal component analysis (PCA) and advanced phenotypic assays to investigate these characteristics.

1.6 Significance of the study

The genetic characterization of *P. aeruginosa* isolates is one of the first steps to understand the diversity of the pathogen at local and global level, and helps in the comprehension of transmission dynamics, establishment of surveillance strategies, reduce risk of outbreaks and enhance knowledge of virulence and resistance roles in infection process. The study findings provide novel information to Medical Therapeutic Committee (MTC) who are part of the health policy makers in the county, for the effective management of *Pseudomonas aeruginosa* infections in KCGH. Data collected serves as a baseline for antibiotic resistance surveillance to detect invasive *P. aeruginosa* and intrusive resistance genes. This data supports and guide the policy makers on treatment regimens at the hospital and appraise prevention measures and prevent AMR transmission. Through the findings and implementation of recommendations, the overall study's significance lies in its potential to improve patient care, reduce the impact of antimicrobial resistance, and contribute to the global efforts in combating infectious diseases caused by multidrug-resistant pathogens like *P. aeruginosa*. The impact of this research in Kakamega County extends to better healthcare practices, enhanced infection control measures, and ultimately, improved outcomes for patients undergoing surgical procedures in the region.

1.7 Scope of the study

This study identified the patterns of antibiotic resistance and genetic characteristics of *Pseudomonas aeruginosa* isolates from surgical site infections among patients attending KCGH. It spanned into the geentic diversity of the pathogen, while identifying the virulence factors responsible for its resistance. By exploring selected physiological characteristics, the study underscored various factors for its pathogenicity and resistance mechanisms whic can help explain about the infections observed at the hospital.

1.7.1 Limitations

Following the review of the study, a limitation could stem from restrictions observed while picking the most populous microbe from the plates. Wounds can have polymicrobial infections. However, this study has given a baseline assessment on which other studies assessing surgical wound infections can compare.

1.8 Conceptual Model

The conceptual model is as presented in **figure 1.1** The One Health Conceptual Framework emphasizes the interconnectedness of human and environmental health, recognizing that the health of individuals and populations is influenced by complex interactions between microbial pathogens, antimicrobial resistance, virulence factors, and environmental factors. *Pseudomonas aeruginosa* infections is dependent on the multifaceted relationships between factors such as the presence of multi-drug and extensive drug resistant strains of Pseudomonas, antimicrobial resistance gene mutations, virulence patterns, host factors, and environmental conditions, which greatly influences the burden of surgical site infections.

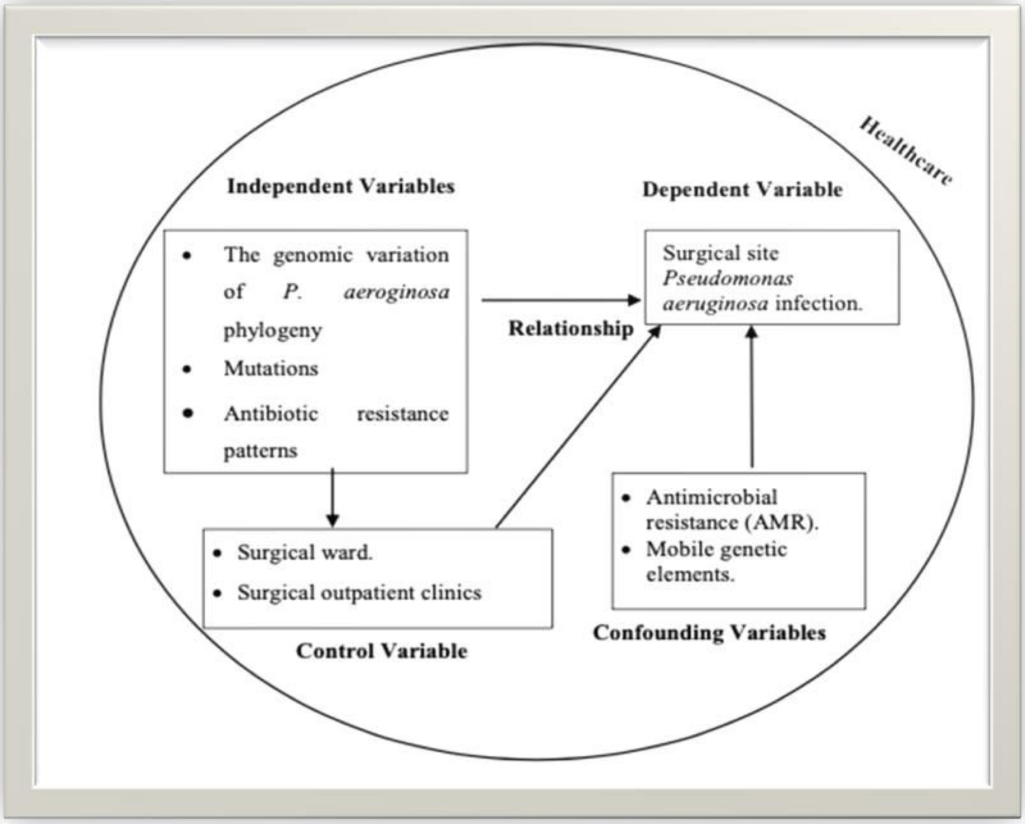


Figure 1 1: Conceptual model showing the interplay between *P. aeruginosa* antimicrobial resistance gene mutation and virulence patterns and other relevant variables.

CHAPTER TWO

LITERTAURE REVIEW

2.0 Introduction

This chapter sheds light into the available literature on the study objectives at large. While progressively identifying the knowledge gap and emphasizing on the need for the study, the chapter reviews the phenotypic antimicrobial resistance patterns of *P. aeruginosa,* the genes responsible for these resistances, the genetic variations among strains and their evolutionary relationships and the virulence factors of the pathogen.

2.1 Overview of *Pseudomonas aeruginosa*

P. aeruginosa is an aerobic, motile gram-negative bacillus. Due to its diverse metabolic pathway and repertoire of pathogenic mechanisms, *P. aeruginosa* is adapted to various ecosystems such as soil, water, and plants (Sainz-Mejías *et al.,* 2020) *Pseudomonas aeruginosa* produces a characteristic grape like odor (fruity odor) on culture media. The compound responsible for the odor has been identified as 2-aminoacetophenone by mass spectroscopy. *P. aeruginosa* also produces a blue – green pigment which is a combination of two metabolites of pyocyanin (blue) and pyoverdine (green), which impart the blue-green characteristic color of cultures (Cox & Parker, 1979). The colonies produced by *P. aeruginosa* on culture media are usually of two types: 1) Large and smooth colonies with flat edges and elevated centers resulting in fried-egg appearance and 2) Small, rough, and convex type (Cheesbrough, 2006). On MacConkey agar *P. aeruginosa* presents as non-lactose fermenting colonies which are flat with rough edges. Colonies on Blood agar are usually mucoid with a typical metallic sheen. Beta-hemolysis is observed on blood agar with a clear zone of hemolysis (Reyes *et al.,* 1981). On Nutrient agar (NA) *P.*

aeruginosa forms large opaque and flat colonies with irregular margins and fruity or earthy odor (LaBauve & Wargo, 2012). Usually, on NA, green-colored colonies can be seen due to the production of Pyoverdine pigment. On Cetrimide Agar colonies are medium sized with irregular margins. The visual examination of the plates is performed by using ultraviolet light to detect the presence of fluorescein. Colonies have blue-green pigment. On triple sugar iron (TSI) agar *P. aeruginosa* shows pink, red slope and butt, no gas and no H_2S produced Alkaline/Alkaline, no gas and no H_2S produced. Ability to grow at temperatures of up to 42^0C is a feature that helps to distinguish *P. aeruginosa* from other Pseudomonas species (Cheesbrough, 2006).

In clinical settings, *P. aeruginosa* is the most common Gram-negative pathogen causing nosocomial infections such as pneumonia (Restrepo *et al.,* 2018), urinary tract and bloodstream infections (Nathwani *et al.,* 2014). This occurs when medical equipment such as inhalers, respirators, and vaporizers are contaminated with *P. aeruginosa* resulting in more than a third of the hospital acquired infections. It is a ubiquitous bacterium known for its ability to cause a wide range of infections, particularly in individuals with compromised immune systems or underlying health conditions. It is often associated with healthcare-associated infections (HAIs), including pneumonia, urinary tract infections, surgical site infections, and bloodstream infections. The most frequent types of infections include the central line associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia (Khan *et al.,* 2017). Central line associated bloodstream infections (CLABSI) are deadly nosocomial infections with the death incidence rate of 12%–25%. Catheters are placed in central line to provide fluid and medicines, but prolonged use can cause serious bloodstream infections resulting in compromised health and increase in care cost. Although there is a decrease of 46% in CLABSI from 2008 to 2013 in US hospitals yet an estimated 30,100 CLABSI still occur in ICU and acute facilities wards in US each year (Khan *et al.,* 2017). Catheter associated urinary tract infections (CAUTI) is the most common nosocomial infection globally. According to acute care hospital statistics conducted in 2011, UTIs accounted for more than 12% of reported infections (ACH, 2011). Catheters serve as a conduit for entry of bacteria whereas the imperfect drainage from catheter retains some volume of urine in the bladder providing stability to bacterial residence. CAUTI can develop to complications such as, orchitis, epididymitis and prostatitis in males, and pyelonephritis, cystitis, and meningitis in all patients. Surgical site infections (SSIs) are nosocomial infections affecting 2%–5% of patients who've undergone surgery. These are the second most common type of nosocomial infections resulting in prolonged hospitalization and risk of death globally (Restrepo *et al.,* 2018). The incidence may be as high as 20% dependent on the procedure and surveillance criteria used. Ventilator associated pneumonia (VAP) occurs in 9–27% of patients on mechanically assisted ventilators. It usually occurs within 48 hours after tracheal intubation. Approximately 86% of nosocomial pneumonia is associated with ventilation.

Pseudomonas aeruginosa is an opportunistic pathogen belonging to the multi-drug resistant (MDR) pathogens comprising of *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Enterobacter* ("ESKAPE") (Sainz-Mejías *et al.,* 2020). *P. aeruginosa* is responsible for opportunistic infections including respiratory tract, blood, urinary tract, and skin infections in immunocompromised individuals and patients with malignant diseases or HIV infection (Hosu *et al.,* 2021). *Pseudomonas aeruginosa* infections are prevalent worldwide, with varying incidence rates depending on the region, healthcare settings, and patient populations. It is a leading cause of nosocomial (hospital-acquired) infections. One of the significant challenges associated with *Pseudomonas aeruginosa* infections is its high level of antibiotic resistance. The emergence of multidrug-resistant strains, including carbapenem-resistant *P. aeruginosa* (CRPA), has limited treatment options. Infections caused by *P. aeruginosa* can be life-threatening, especially in critically ill patients. High mortality rates are often associated with bloodstream infections and ventilator-associated pneumonia caused by this bacterium.

2.2 Epidemiology of *Pseudomonas aeruginosa* infections

Global studies to evaluate *P. aeruginosa* isolates amongst inpatients shows antibiotic resistance of 4.2% out of which 2.0% of *P. aeruginosa* isolates were antibiotic resistant. A broad spectrum of drugs is available for treating *P. aeruginosa* infections. However, *P. aeruginosa* rapidly develops tolerance to these agents due to its intrinsic resistome (Horcajada et al., 2019). According to the studies performed in Iran, *Pseudomonas aeruginosa* is considered as the major cause of infectious diseases like burn and wound infection that makes it one of the most threatening opportunistic pathogens (Shahbazzadeh *et al.,* 2020). In Africa, as in many other regions, *Pseudomonas aeruginosa* infections are a concern, particularly in healthcare facilities. Limited access to healthcare resources and infrastructure challenges may contribute to the spread of these infections. In hospital settings *Pseudomonas aeruginosa* is frequently identified in African hospitals, and healthcare-associated infections are a significant issue. Inadequate infection control practices and the use of broad-spectrum antibiotics can contribute to the prevalence of *Pseudomonas* infections in healthcare settings. While *Pseudomonas aeruginosa* infections are often associated with healthcare facilities, cases of community-acquired infections have also been reported in Africa, especially in individuals with chronic illnesses or compromised immune systems. Moreover, antibiotic resistance is a growing concern in Africa, and this extends to PA. The emergence of multidrug-resistant strains in the region poses a serious threat to patient outcomes.

Kenyan healthcare facilities, particularly those with limited resources, may face challenges in preventing and controlling *Pseudomonas aeruginosa* infections. Inadequate hygiene practices and overcrowding can contribute to the spread of nosocomial infections. Recently, *Pseudomonas aeruginosa* has been found to be a leading cause of nosocomial infections in Kenya and it can produce a range of debilitating infections, have a propensity for developing antimicrobial resistance, and present with a variety of potent virulence factors (Musila *et al.,* 2022). Recent studies in Kenya have found out that *P. aeruginosa* isolates from SSIs are developing resistant to the most routinely used antibiotics including piperacillin, clavulanic acid meropenem and cefepime (Sharma *et al.,* 2022, Musila *et al.,* 2022). Antibiotic resistance is a global concern, and Kenya is no exception. Inappropriate antibiotic use and limited access to effective antibiotics can lead to the emergence of drug-resistant Pseudomonas strains and as many studies suggest, continued research and surveillance efforts are crucial to understand the epidemiology of PA infections in Kenya better. Local data can inform healthcare practices and policies to reduce infection rates.

Therefore, *Pseudomonas aeruginosa* infections are a global health concern, with varying incidence rates in different regions. In Africa, including Kenya, limited healthcare resources and antibiotic resistance can exacerbate the problem. Ongoing research and surveillance are thus essential to combat these infections effectively. However, for the most up-to-date information, it is advisable to consult more recent literature and reports specific to the region.

2.2.1 Transmission and control of nosocomial *P. aeruginosa*

P. aeruginosa is spread through improper hygiene, such as from the unclean hands of healthcare workers, or via contaminated medical equipment that wasn't fully sterilized. Common hospital-associated *P. aeruginosa* infections include bloodstream infections, pneumonia, urinary tract infections, and surgical wound infections. It is necessary that healthcare professionals take active roles in infection control. Personal hygiene, hand decontamination with proper disinfectants before and after handling patients, safe injection practices and use of personnel protective equipment (PPE) is essential for healthcare delivery (Khan *et al.,* 2017). Clinical wastes are a potential reservoir for pathogens. Infectious healthcare waste should be stored in restricted areas and cleaners informed of waste management.

Health care policies are critical to ensure the cleaning and use of cleaning agents on walls, floor, windows, beds, baths, toilets, and other medical devices. Proper ventilated and fresh filtered air can eliminate airborne bacterial contamination. Regular check of filters and ventilation systems of general wards, operating theatres and ICUs must be maintained and documented (WHO, 2019). Infections associated with contaminated water are due to the lack of testing procedures in healthcare institutions. Microbiological monitoring methods should be used for water analysis and infected patients given separate bath sections, a practice commonly known as cohorting (WHO, 2019).

2.3 Antimicrobial resistance in *P. aeruginosa*

Antimicrobial resistance (AMR) in *Pseudomonas aeruginosa* is a pressing global health issue that has garnered significant attention in scientific literature. *P. aeruginosa* is notorious for its ability to develop resistance to multiple antibiotics, which poses substantial challenges in clinical management and infection control. Recent reports on the antibiotic sensitivity patterns of *P. aeruginosa* in the UK have highlighted the problem of antibiotic resistance in cystic fibrosis (CF) strains in comparison with other hospital isolates (Hosu *et al.,* 2021). Its general resistance is due to a combination of factors: It is intrinsically resistant to antimicrobial agents due to low permeability of its cell wall. It has the genetic capacity to express a wide repertoire of resistance mechanisms. It can become resistant through mutation in chromosomal genes which regulate resistance genes. Moreover, it can acquire additional resistance genes from other organisms via plasmids, transposons, and bacteriophages.

Antimicrobial resistance in *Pseudomonas aeruginosa* is increasingly becoming a public health concern due to its low cell wall permeability and its ability to quickly express multiple resistance mechanisms such as porin deletions and excessive expression of efflux pumps (Nathwani *et al.,* 2014). Resistance is transferred between *Pseudomonas aeruginosa* strains by means of interchangeable genetic material such as plasmids, integrins and transposons, commonly referred to as mobile genetic elements (MGEs). MGEs are implicated in transmitting resistance to beta-lactams, aminoglycosides, and fluoroquinolones. Plasmids can encode enzymes that confer resistance to different antimicrobial substances (e.g., antibiotics, disinfectants, heavy metals, and UV radiation) (Subedi, Vijay, & Willcox, 2018). A critical analysis of thirty-seven studies conducted in different sites globally reported *Pseudomonas aeruginosa* resistance rate to commonly used antibiotics as follows: gentamicin (22%), ceftazidime (13%) and ciprofloxacin (9%) (Subedi, Vijay, & Willcox, 2018). Recently, the emergence and the global spread of MDR and XDR *P. aeruginosa* clones ST111, ST175, ST235, ST253 and ST274 and the Liverpool epidemic strain (LES-1) are responsible for the high mortality rates associated with nosocomial infections (Aguilar-Rodea *et al.,* 2017).

P. aeruginosa employs both intrinsic and acquired resistance mechanisms. The acquired resistance mechanism is facilitated by mobile genetic elements through horizontal gene transfer (HGT) posing a greater risk to human health due to the ease of expression and dissemination (Hosu *et al.,* 2021). *P. aeruginosa* resistance to antibiotics is classified as: 1). Multidrug resistant (MDR) which is resistance to at least one agent in three or more antimicrobial categories; 2). Extensively drug resistant (XDR) which is resistance to at least one agent in all but two or fewer antimicrobial categories and, 3). Pan drug resistant (PDR)-resistance to all antimicrobial agents in all categories (Aguilar-Rodea *et al.,* 2017). In 2017, multidrug-resistant *Pseudomonas aeruginosa* caused an estimated 32,600 infections among hospitalized patients and 2,700 estimated deaths in America while the government of UK in April 2017 extended the surveillance of SSI caused by gram-negative organisms to include *P. aeruginosa* and *Klebsiella spp* by 50%. In a study in Kenya, it found out that total SSI incidence rate was 6.8%. Pathogens isolated from SSI included *S. aureus* (30%), Coagulase negative Staphylococcus (16%), Klyuvera spp. (13%), *E. coli* (13%), *P. aeruginosa* (13%), *Klebsiella spp.* (9%) (Dinda et al., 2013).

2.3.1 Intrinsic antimicrobial resistance mechanisms of *P. aeruginosa.*

The intrinsic antibiotic resistance of a bacterial species refers to its innate ability to diminish the efficacy of a specific antimicrobial through inherent structural or functional characteristics (Botelho *et al.,* 2019). In *P. aeruginosa,* the intrinsic antimicrobial resistance mechanisms include low outer membrane permeability, expression of efflux pumps that expel antimicrobials from the cell and the production of antibiotic inactivating enzymes (Subedi, Vijay, & Willcox, 2018). *Pseudomonas aeruginosa* exhibits a high level of intrinsic resistance to most antibiotics through restricted outer membrane permeability, efflux systems that pump antibiotics out of the cell, and production of antibiotic-inactivating enzymes such as β-lactamases (Pang et al., 2019a).

2.3.1.1. Outer membrane permeability

Antibiotics used to treat *P. aeruginosa* infections must penetrate the cell membrane and reach intracellular targets (Lord *et al.,* 2021). The aminoglycoside family of antibiotics such as tobramycin, gentamicin, and amikacin inhibit bacterial protein synthesis by binding to ribosomal 30S subunits (Krause et al., 2016a). Quinolones such as ciprofloxacin and levofloxacin interfere with DNA replication by inhibiting DNA gyrase and topoisomerase IV (Bush *et al.,* 2020). The β-lactam antibiotics such as penicillin, cephalosporin, carbapenem and monobactam contain a β-lactam ring in their molecular structures. *β*-lactams block bacterial cell wall biosynthesis by targeting enzymes involved in peptidoglycan synthesis (Bush *et al.,* 2020). Polymyxins are a group of polypeptide antibiotics that bind to the lipopolysaccharides (LPS) on the outer membrane of Gram- negative bacteria, leading to increased cell membrane permeability and enhanced antibiotic uptake (Khondker & Rheinstädter, 2020). Polymyxin B and polymyxin E, also known as colistin, are the two polymyxins used in clinical practice, and they kill bacteria by induction of a hydroxyl radical-mediated cell death pathway (Lenhard *et al.,* 2019). To enter the bacterial cell, β-lactams and quinolones penetrate cell membranes through porin channels, whereas aminoglycosides and polymyxins promote their own uptake by interacting with bacterial LPS on the outer membrane of Gram-negative bacteria (Bush et al., 2020; Krause et al., 2016a; Lima et al., 2020).

The outer membrane of *P. aeruginosa* acts as a selective barrier preventing antibiotic penetration. It is an asymmetric bilayer of phospholipid and LPS, embedded with porins to form *β*-barrel protein channels (Pang et al., 2019a) that are divided into four classes namely: 1). the non-specific porins, which allow for slow diffusion of most of the small hydrophilic molecules; 2). specific porins, which possess specific sites to bind a particular set of molecules; 3). gated porins, which are ion-regulated outer membrane proteins responsible for uptake of ion complexes; and 4). efflux porins, which are important components of efflux pumps (Chevalier *et al.,* 2017). The OprF protein is the major non-specific porin; OprB, OprD, OprE, OprO and OprP are specific porins; and OprC and OprH belong to the class of gated porins. The class of efflux porins include OprM, OprN and OprJ (Chevalier *et al.,* 2017). The carbohydrate-specific porin (OprB), the basic amino acid-specific porin (OprD), the phosphate-specific porin (OprP), and the pyrophosphate-specific porin (OprO) are some of the specific porins in *Pseudomonas aeruginosa*.

The OprD porin is involved in antibiotic uptake and contains the binding sites for carbapenems, a class of β-lactam antibiotics (Chevalier *et al.,* 2017). The absence of OprD increases the resistance to β-lactam antibiotics. OprH is the smallest *P. aeruginosa* porin and its overexpression in the absence of Mg^{2+} is associated with increased resistance to polymyxin B and gentamicin through stabilization of the outer membrane by inducing LPS modification (Pang et al., 2019a). The OprF porin, a homolog of the *E. coli* outer membrane protein A (OmpA), is the predominant porin in *P. aeruginosa* and is responsible for the non-specific uptake of ions and saccharides (tri- and tetra-saccharides), but has a low efficiency for antibiotic permeation (Ude *et al.,* 2021). OprF can fold into two conformations: 1). the two-domain closed conformer consisting of an N-terminal transmembrane β-barrel and a C- terminal periplasmic globular domain, and 2). the one-domain open- channel conformer containing a single transmembrane domain. The closed conformer is dominant while the OprF open channels make up less than 5% of the protein population (Pang et al., 2019a). The high frequency of closed OprF channels is likely to be associated with low outer membrane permeability in *P. aeruginosa* compared with other bacteria. Additionally, the absence of *P. aeruginosa* OprF leads to increased biofilm formation through upregulation of bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP), which is an important messenger for controlling biofilm formation (Bouffartigues *et al.,* 2015).

2.3.1.2. Efflux systems

Pseudomonas aeruginosa has multiple efflux pumps that can expel antibiotics from within the bacterial cell, reducing intracellular drug concentrations. These pumps re classified into five families namely: 1). Resistance-nodulation-division (RND) family, 2). Major facilitator super family (MFS), 3). ATP-binding cassette (ABC) superfamily, 4). Small multidrug resistance (SMR) family, and 5) Multidrug and toxic compound extrusion (MATE) family (Du *et al.,* 2018). Proteins belonging to the RND family of efflux pumps play a key role in antibiotic resistance in *P. aeruginosa* (Soto, 2013).These consist of cytoplasmic membrane transporters, periplasmic linker proteins and outer membrane porin channel proteins (Daury *et al.,* 2016).

The cytoplasmic and periplasmic components of *P. aeruginosa* RND pumps are named multidrug efflux (Mex) along with a letter, and the outer membrane porin is named Opr along with a letter. *Pseudomonas aeruginosa* expresses twelve RND family efflux pumps, four of which (MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM) contribute to antibiotic resistance (Soto, 2013). MexAB-OprM is responsible for efflux of *β*-lactams and quinolones (Daury *et al.,* 2016). MexCD-OprJ can pump out β-lactams while MexEF-OprN and MexXY-OprM are capable of extruding quinolones and aminoglycosides, respectively (Pang et al., 2019a).

Overexpression of multiple efflux pumps has been documented in clinical strains of *P. aeruginosa*, broadening bacterial antibiotic resistance and contributing to the development of multidrug resistance (Bassi *et al.,* 2020; Shigemura *et al.,* 2015). Efflux pump inhibitors have emerged as potential therapeutic strategies for treating *P. aeruginosa* infections (Askoura *et al.,* 2011). Phenylalanine arginyl βnaphthylamide (PAβN) is an efflux pump inhibitor that impairs antibiotic efflux through competitive inhibition of efflux pumps and also increases the permeability of bacterial outer membranes (Pang et al., 2019a), thus reducing virulence, diminishing quorum sensing and increasing antibiotic susceptibility of *P. aeruginosa.*

2.3.1.3 Antibiotic-inactivating enzymes

Antibiotic-inactivating enzymes either break down or modify antibiotics thus inactivating them. This is one of the major mechanisms of intrinsic resistance in bacteria. Antibiotics have chemical bonds such as amides and esters that are susceptible to hydrolysis by enzymes produced by *P. aeruginosa* such as β-lactamases and aminoglycoside-modifying enzymes (Lord *et al.,* 2021; Yoon & Jeong, 2021). *β*-lactams are the most widespread class of human antibacterial (Singh *et al.,* 2019). The development of resistance to *β*-lactam antibiotics in gram-negative pathogens is a result of the production of *β*-lactamase enzymes, which endues the microbes with the ability to hydrolyse the *β*-lactam ring (Singh *et al.,* 2019). Beta (β)-lactamases are the most important agents that confer drug resistance among gram-negative bacteria due to the continuous mutations that make them diverse (Singh *et al.,* 2019). There are 3 classes of beta-lactamases namely the extended-spectrum β-lactamases (esBL), Metallo β-lactamases (MBL), and AmpC β-lactamases (ABL).

P. aeruginosa possesses an inducible ampC gene, encoding the hydrolytic enzyme *β*-lactamase capable of breaking the amide bond of the *β*-lactam ring, leading to inactivation of β-lactam antibiotics (Subedi, Vijay, & Willcox, 2018). B-lactamases can be divided into four classes, A, B, C and D, based on their amino acid sequences.

The enzyme classes A, C, and D hydrolyze β-lactams through an active site serine. By contrast, the class B β-lac- tamases are metallo enzymes that require divalent zinc ions for β-lactam hydrolysis (Hong *et al.,* 2015) . The class C *β*-lactamase inhibit anti pseudomonal cephalosporins (Lima *et al.,* 2020). Extended-Spectrum β-lactamases (ESBLs) and plasmid-borne AmpC *β*-lactamases (ABLs) hydrolyse penicillin, cephalosporins, and bactams while ABLs degrade cephamycins and are resistant to *β*-lactamase inhibitors. Clavulanic acid (CA) inhibits ESBL but not ABLs. The resulting *β*-lactam-resistant phenotype in *P.aeruginosa* is due to the acquisition of plasmid-mediated *β*-lactamases such as class A ESBL (*TEM*, *SHV*, *CTX-M* and *OXA*) (Yoon & Jeong, 2021), class C plasmid-mediated AmpC (*ACT*, CMY and *DHA*) or by the hyper production of the chromosomal AmpC enzyme, and class A, B or D carbapenemases (Hamdy Mohammed *et al.,* 2016). Metallo β-lactamases (MBLs): *VIM*, *IMP*, and *NDM*, reported from Enterobacteriaceae further limits the treatment options. The plasmid mediated ABLs are less common compared to ESBLs. Carbapenemases are β-lactamases composed of MBLs and serine-β-lactamases (*KPC*, *OXA*, *GES*) (Musila *et al.,* 2021; Singh *et al.,* 2019). The MBLs require zinc ion for their action.

2.4 Virulence factors of *Pseudomonas aeruginosa*

The principal virulence factors of *P. aeruginosa* are elastase, phospholipase C, protease A, exotoxins and cytotoxins, flagella and pili, pigment production, and Quorum Sensing (QS) regulatory system proteins, which regulate both virulence factor transcription and biofilm formation (Elmaraghy *et al.,* 2019). The pathogenesis of *P. aeruginosa* is linked to the production of several virulence factors classified into cell-associated and secreted. One of these virulence factors that play a main role in tissue lysis and bacterial invasion is exotoxin A (*exoA).* The gene encoding the major DNA exonuclease of *Streptococcus pneumonia,* exoA, was cloned in a streptococcal host vector system. Its location was determined by sub cloning and by insertion mutations. Transfer of a DNA segment containing the gene to an *Escherichia coli* expression vector showed that exoA was the structural gene for the enzyme and that it was adjacent to its promoter.

The hemolysin phospholipase H (*plcH*) act to destroy lipids and lecithin contributing to tissue invasion. *Pseudomonas aeruginosa* haemolytic phospholipase C, (PlcH), can degrade phosphatidylcholine (PC) and sphingomyelin in eukaryotic cell membranes and extracellular PC in lung surfactant. Numerous studies implicate PlcH in *P. aeruginosa* virulence (Restrepo *et al.,* 2018). The phosphorylcholine released by PlcH activity on phospholipids is hydrolyzed by a periplasmic phosphorylcholine phosphatase, PchP. *P. aeruginosa* also produces exoenzyme S (*exoS*), it is cytotoxin responsible for damage to many types of host cells and elastase B (*lasB*) that play an important role during the acute infection(Elmouaden et al., 2019b). Some strains produce alginate that forms the matrix of the biofilm which protect bacteria from the host defence during the chronic infection. The GDP-mannose 6-dehydrogenase (algD) is one of three proteins that are implicated in the production of alginate (Elmouaden et al., 2019). A previous study on virulence genes and antibiotic resistance of *Pseudomonas aeruginosa* isolated from patients in a hospital highlighted that *lasB* (98.7%) and *exoS* (98.7%) were the most frequent virulence genes in *P. aeruginosa* strains, followed by *plcH* (96.1%), and *algD* (87.7%), while the least commonly detected virulence factor gene was *exoA* (74.2%). Recent findings have shown that *P. aeruginosa* strains do not necessarily have similar virulence factor genes. Moreover, various studies suggest that there is an association between the presence of some virulence genes and certain antibiotic resistance patterns (Hosu *et al.,* 2021). Recent studies show that 31% of 56 clinical isolates of *Pseudomonas aeruginosa*, collected from different medical centres in Kenya between 2015 and 2020, harbour both virulence genes and a multidrug resistance phenotype (Darmancier *et al.,* 2022). The biofilm formation which reflects a community of cells attached to either a biotic or an abiotic surface and enclosed in a complex exopolymeric substance is nowadays a major problem as it increases the potential of the pathogen to be resistant to antibiotics and disinfectants, is difficult to be eradicated and contributes to localized or systemic inflammation, which prolongs wound healing (Solano *et al.,* 2014). To overcome this phenomenon, different strategies have been proposed in order to (i) avoid microbial attachment to a surface, (ii) disrupt biofilm development to increase the penetration of antimicrobials; and (iii) affect biofilm maturation (Rasamiravaka *et al.,* 2015).

2.5 Mutational factors of *Pseudomonas aeruginosa*

There have been numerous studies identifying mutational genes in *Pseudomonas aeruginosa* responsible for antimicrobial resistance. Mutations in genes encoding various components of antibiotic resistance mechanisms contribute to the development of resistance in this bacterium. Mutations in these genes can result in overexpression of the MexAB-OprM efflux pump, which extrudes a wide range of antibiotics, including beta-lactams and fluoroquinolones. Overexpression due to mutations can lead to increased resistance. Other mutations in AmpC Beta-Lactamase genes including ampC have been attributed to increased expression of this chromosomal beta-lactamase, which hydrolyzes beta-lactam antibiotics like cephalosporins. Moreover, Porin gene known as oprD have shown mutations which can result in loss or alteration of the outer membrane porin protein, reducing the entry of antibiotics, particularly carbapenems, into the bacterial cell. Other mutations leading to changes in DNA gyrase and topoisomerase enzymes, which are targets of fluoroquinolone antibiotics have been documented (Abdelkreem et al., 2020). Gyrase and Topoisomerase including gyrA, gyrB, parC, parE form a large number in this category. These mutations can reduce drug binding and increase resistance.

It's important to note that *P. aeruginosa* can develop resistance through mutations in various combinations of these and many other genes, often resulting in multidrug-resistant (MDR) or extensively drug-resistant (XDR) phenotypes. Additionally, the prevalence and distribution of specific mutations may vary over time and by geographical location, emphasizing the importance of ongoing surveillance and research in the field of antimicrobial resistance.

2.6 Novel therapeutic strategies for *P. aeruginosa* treatment

Studies have reported several novel non-antibiotic therapeutic approaches that are highly effective against antibiotic-resistant *P. aeruginosa* strains. These approaches include inhibition of quorum sensing and bacterial lectins, use of iron chelation, phage therapy, vaccine strategy, nanoparticles, antimicrobial peptides, and electrochemical scaffolds. These therapeutic approaches can be used as either an alternative or in combination with conventional antibiotic treatments (Pang et al., 2019a).

2.6.1 Quorum sensing inhibition

Quorum sensing allows bacteria to control gene expression in a cell density-dependent manner (Pena *et al.,* 2019). *Pseudomonas aeruginosa* utilizes quorum sensing to regulate virulence and biofilm formation (Möker *et al.,* 2010). Las and Rhl are two major quorum-sensing systems *of P. aeruginosa* responsible for the synthesis of the N-acyl homoserine lactone (AHL) signal molecules, N-(3-oxododecanoyl)-L-homoserine lactone (3O-C12- HSL) and N-butanoyl-L-homoserine lactone (C4-HSL), respectively (Pang et al., 2019a). The 3O-C12-HSL and C4-HSL bind and activate LasR and RhlR respectively, inducing biofilm formation and expression of various virulence factors including elastase, proteases, pyocyanin, lectins, rhamnolipids and toxins. Inhibition of quorum sensing prevents and reduces biofilm formation, decrease bacterial virulence with a low risk of bacterial resistance. For example, the carotenoid zeaxanthin, commonly found in plants, algae, and lichens, reduced the biofilm formation in *P. aeruginosa* by binding to quorum sensing signal receptors, LasR and RhlR, and blocking expression of virulence genes, lasB and rhlA (Thi *et al.,* 2020). Flavonoids are plant metabolites that act as antagonists of LasR and RhlR, significantly reducing their binding ability to the promoters of quorum sensing- regulated genes in *P. aeruginosa* (Paczkowski *et al.,* 2017)

2.6.2. Lectin inhibition

Lectins are bacterial outer membrane proteins that recognize host glycoconjugates and allow bacteria to adhere to the host tissues (Jurado-Martín *et al.,* 2021). The adhesion of *P. aeruginosa* to lung epithelial cell surface is mediated by two specific lectins, LecA and LecB, that bind to the galactose and fructose surface receptors of the lung epithelial cells, respectively (Chemani *et al.,* 2009) . Furthermore, *P.*

aeruginosa lectins have been found to be involved in biofilm formation by interacting with host cell glycoconjugates (Pang et al., 2019a). Inhibition of lectin binding is useful for prevention and treatment of *P. aeruginosa* infections due to its high stability and low risk of bacterial resistance. Lectin binding to host cell surfaces may be blocked by lectin inhibitors, such as glycoclusters, glycopolymers and glycodendrimers (Chemani *et al.,* 2009).

2.6.3. Iron chelation

Iron is essential for bacterial growth and is involved in a variety of cellular processes, such as energy production, DNA replication and electron transport (Cheesbrough, 2006). *Pseudomonas aeruginosa* utilizes the siderophores pyoverdine and pyochelin to acquire iron from the extracellular environment (Cornelis & Dingemans, 2013) Thus, limiting the concentration of extracellular iron or disrupting iron uptake by *P. aeruginosa* is a strategy to counter infections. Iron analogues and chelators are potential therapeutic agents against *P. aeruginosa*. For instance, the iron chelators, 2, 2′- dipyridyl (2DP), diethylenetriaminepentaacetic acid (DTPA) and EDTA, impaired *P. aeruginosa* growth and biofilm formation (O'May et al., 2006). Galliumis a non-redox iron III analogue disrupts bacterial iron metabolism by acting as an iron substitute in biologic processes (Goss *et al.,* 2018).

2.6.4 Phage therapy

Bacteriophages (phages) are viruses that infect and kill bacteria by causing lysis (Principi *et al.,* 2019). There are several advantages of phage therapy, including replication at the infection site, high specificity to target bacteria without effects on commensal flora, fewer side-effects than other treatments, bactericidal activity against antibiotic- resistant bacteria and easy administration (Chatain-Ly, 2014). Use of phages for the treatment of *P. aeruginosa* infections has been extensively studied as an alternative to antibiotics. To date, there are 137 different phages targeting the Pseudomonas genus that have been characterized (Chegini *et al.,* 2020; Secor *et al.,* 2020). Pre-treatment of hydrogel-coated catheters with phage M4 significantly reduces *P. aeruginosa* biofilm formation (Chegini *et al.,* 2020) . Thus, pre-treatment of the surface of indwelling medical devices with phages may be a potential benefit for patients, preventing *P. aeruginosa* infections during medical treatment.

2.6.5 Vaccine strategy

Presently, no licensed vaccine is as available against *Pseudomonas aeruginosa. P. aeruginosa* antigens elicit potent immune responses, and are responsible for pathogenesis (Gonzaga *et al.,* 2021) .The potential candidates for *P. aeruginosa* vaccines are the LPS O-antigen, polysaccharide- protein conjugates, outer membrane proteins OprF and OprI, the type III secretion system component PcrV, flagella, pili, DNA, live-attenuated *P. aeruginosa* and whole killed cells (Pang et al., 2019a).

2.6.6 Nanoparticles

Nanoparticles are tiny materials (~100nm) with a large surface area to mass ratio with high penetrability into bacterial membranes making them suitable vectors of antibiotics (Wang et al., 2017). Silver nanoparticles produce silver ions that inhibit bacterial enzymatic systems including DNA synthesis making them suitable antimicrobials (Wang et al., 2017). These silver nanoparticles have shown significant antimicrobial effects on *P. aeruginosa* clinical strains, effectively killing *P. aeruginosa* and inhibiting its growth *in vitro*. Moreover, silver nanoparticles show low cytotoxicity to mammalian cells (Salomoni et al., 2017). As mentioned earlier, nanoparticles are able to deliver antimicrobial.

2.6.7 Electrochemical scaffolds

Electrochemical scaffolds generate low and constant concentrations of H_2O_2 that destroy bacterial biofilms and facilitate antibiotic penetration (Sultana et al., 2015). A study by Sultana *et al.,* (2016) demonstrated that an electrochemical scaffold enhanced tobramycin susceptibility of *P. aeruginosa* PAO1 and effectively eradicated persister cells in biofilms (Sultana et al., 2016). To date, electrochemical scaffolds have not been implanted into patients, thus the clinical efficacy of this approach remains to be demonstrated.

2.6.8 Phylogenetic relatedness

Phylogenetic relatedness has been used to characterize and identify the risk of transmission and spread of *P. aeruginosa* outbreaks in hospital (Ramanathan *et al.,* 2017; Saati-Santamaría *et al.,* 2021; Tran *et al.,* 2021). Through genomic comparisons, phylogenetic analysis and sequencing have revealed new antibiotic resistant variants of *P aeruginosa* strains worldwide (Subedi, *et al.,* 2018) . Currently, the use of next-generation sequencing is expanding because it offers information on not only genetic relatedness at a greater resolution but also relatedness between resistance-associated genes, providing deeper understanding of bacteria that are resistant to antibiotics (Tshibangu-Kabamba *et al., 2020).* The phylogenetic tree places the *P. aeruginosa* isolates in genotype groups each having different characteristics of antibiotic resistance genes and Sequence types (Tran *et al.,* 2021).

2.7 Physiological Traits

Physiological traits such as motility, biofilm formation, and metabolic capabilities are critical to the pathogenicity of *P. aeruginosa*. Studies have shown that its motility, facilitated by flagella and pili, enhances colonization and infection spread (Tuon et al., 2022). Biofilm formation, initiated by environmental cues, contributes to the persistent infection observed in surgical sites (Sharma et al., 2023). Additionally, *P. aeruginosa* demonstrates remarkable metabolic versatility, allowing it to utilize various carbon and nitrogen sources, augments its survival in nutrient-limited environments, such as those found in surgical wounds (Qin et al., 2022). This adaptability also poses challenges for treatment, as biofilm-associated bacteria are significantly more resistant to antibiotics.

CHAPTER THREE

METHODS

3.0 Overview

This chapter gives a detailed description of the study area, study design and the study population. The methods used for sample size determination, inclusion/exclusion criteria, specimen collection, laboratory analyses, quality assurance, data analysis, and logistical and ethical considerations are described in depth.

3.1 Study Area

This study was conducted at the Kakamega County General Hospital in Western Kenya **(Appendix I).** This hospital is a level 5 government health facility in Kakamega Town**.** Kakamega town is the administrative headquarters for Kakamega County. Kakamega County lies on (Latitude: 0° 16' 60.00" N Longitude: 34° 45' 0.00" E). Temperatures range from a minimum of 10.3°C to a maximum of 30.8°C with an average of 20.5°C. Kakamega County has a population of 1.8 million people and an area of 3,033.8km2 (KNBS, 2019). Human activities conducted in the county and around the hospital include small scale agriculture, livestock rearing, poultry farming, sugarcane farming, and bathing and sewage disposal. Kakamega County General Hospital has a wide catchment of patients as the main public hospital serving residents of the Western region including Kakamega County as well as other neighboring counties such as Vihiga, Bungoma and Busia. Kakamega County General Hospital receives approximately 15,000 patients monthly (both in and out - patients) and has a bed capacity of 400 at the time of study, therefore, one of the largest referral hospitals in Western Kenya.

3.2 Study Design

This study adopted a cross sectional design with a purposive sampling. Samples for study were selectively taken during the period of hospitalization.

3.3 Study population

The study population comprised of female and male patients of any age group who underwent surgery at the facility. A total of 209 patients presented with surgical wounds during the period of the study.

3.3.1 Inclusion criteria

Post-operative patients at the facility were included in the study, provided they gave consent. Children were included with guardian consent, while those able to consent for themselves were also considered. Guardians consented for adult patients who were too ill to provide consent. Patients who had undergone any surgical procedure within the previous month were recruited if a prosthetic was implanted. Additionally, all post-surgical wounds exhibiting pain, redness, warmth, swelling, and purulent drainage at the incision site were included in the study.

3.3.2 Exclusion criteria

Patients admitted in other hospital wards other than surgical wards or had not undergone any surgical procedure at the hospital were excluded. Patients who declined to sign the informed consent form were excluded from the study. Patients who had been referred for surgery out of disease complications that is being managed and those on multiple antimicrobial exposures due to chronic care prior to the surgical procedure were excluded.

3.4 Study variables

3.4.1 Dependent variables

The dependent variable was *Pseudomonas aeruginosa* infection.

3.4.2 Independent variables

These were surgical site sites, prevalence, antibiotic resistance, diversity and general biology of the PA infecting, enzyme production, biofilm production.

3.5 Sampling design

Purposive sampling was employed to select patients who were most likely to provide relevant data about *P. aeruginosa* infections. Only patients exhibiting surgical wounds were considered, ensuring that the sample was relevant to the study's focus on wound infections. Patients who were likely to exhibit *P. aeruginosa* infections based on clinical assessments or previous medical history were prioritized.

3.6 Sample size determination

The sample size for this study was estimated based on the Cochran's formula for qualitative variable (Cochran, 1977) to determine the proportion of participants with surgical site *Pseudomonas aeruginosa* infection.

$$n_0 = \frac{Z^2 \cdot p \cdot (1 - p)}{e^2}$$

Where: n_o = the number of subjects required.

Z=1.96 (for 95% confidence level)

P =0.31 Thus, the expected proportion of antibiotic resistant *P. aeruginosa* infections from previous studies conducted in Kenya is 31% (0.31) (Kiyaga et al., 2022).

$1-P$ =0.69

e^2= margin of error 0.05 for 5%)

$$n_0 = \frac{(1.96)^2 \cdot 0.31 \cdot (1-0.31)}{(0.05)^2}$$

$$n_o = 329$$

Adjusting for Small Proportions:

For small proportions (where P is much smaller than 0.5). The patients presenting with surgical wounds in the entire period of study were 209.

$$n = \frac{n_0}{1+\frac{n_0-1}{N}}$$

Finite population in the wards is 209 patients

Therefore, adjusting for this sample size

$$n = \frac{329}{1+1.5619} \approx \frac{329}{2.5619} \approx 128.37$$

Thus, the adjusted sample size n is approximately 128**.**

3.7 Specimen collection

3.7.1 Specimen collection processes

Non-invasive specimen collection techniques were employed. Swabs were collected from infected surgical sites suspected of SSI by passing the swab deep into the base of the lesion to firmly sample the fresh border. The swabs were inserted into the Stuarts transport media. All specimens were appropriately labeled with two patient identifiers used at the various hospital set-ups and clinics, including the patient's name, birthdate and/or hospital number. The swabs were then packed in a Biosafety Transport Box and delivered to KCGH microbiology laboratory at room temperature, within 2 hours of being collected for culture process. The requisition accompanying each individual specimen included the patient's name, hospital number, date and time of collection, specimen type, tests requested and patient's history. Where transport delayed the specimens were refrigerated as they awaited transportation. Specimens were hand delivered to the laboratory. On arrival to the KCGH microbiology laboratory the specimens were accorded unique codes to ease downstream processes and for confidentiality purposes.

3.7.2 Determination of the prevalence of *Pseudomonas aeruginosa* isolates from surgical sites among patients attending KCGH

3.7.2.1 Isolation and identification procedures for *Pseudomonas aeruginosa*

From the transport media, samples were picked and inoculated simultaneously into enrichment broth, tryptic soy broth, and selective media: MacConkey agar (MAC) and blood agar (BA) (Oxoid, UK). The cultures were incubated at 37°C for 18 hours. The enrichment broth promotes the growth of *Pseudomonas aeruginosa* (PA) while inhibiting other bacteria. After incubation, sub-cultures were streaked onto selective media: Cetrimide Agar, which contains cetrimide to inhibit most bacteria, and Pseudomonas Agar F, which is selective for Pseudomonas species and allows for differentiation of colony types. The plates were incubated at 37°C for 24 hours in an aerobic environment and examined for visible growth. Typical colonies appeared greenish or blue-green from pyocyanin production, were flat and spreading, and had a distinctive fruity or sweet odor (Cheesbrough, 2006). Biochemical tests, including the oxidase test, catalase test, and sugar fermentation tests, confirmed the identity of

PA. For storage, a single colony was purified from the primary culture plate into nutrient agar by streak method. When different colony morphologies were observed, a single colony from each type was purified.

3.7.3 Evaluation of the antibiotic resistance profiles of *Pseudomonas aeruginosa* isolates from surgical sites among patients attending KCGH

P. aeruginosa isolates were tested against disk (ciprofloxacin (CIP, 5 µg), Tobramycin (10 µg), amikacin (AK, 30 µg), Piperacillin/Tazobactam (100/10) µg, piperacillin (PRL, 100 µg), meropenem (MRP, 10µg), Ceftazidime (30 µg); using the Kirby - Bauer disk diffusion technique (Hudzicki, 2009). The test was done on Mueller Hinton agar (MHA) (Oxoid, UK).

The method involved evenly spreading the bacterial inoculum onto the surface of a Mueller-Hinton agar plate. Paper discs impregnated with specific concentrations of antibiotics were then placed on the agar surface. The disks were placed 15mm away from the central disc using sterile forceps. The plates were then incubated at 37℃ for 24 hours. During incubation, the antibiotics diffused radially, inhibiting bacterial growth and forming clear zones of inhibition around the discs. The diameters of these zones were measured and compared to standardized interpretive criteria as per the standard zone sizes of Clinical and Laboratory Standards Institute that categorized the bacteria as susceptible, intermediate, or resistant to the antibiotics tested.

Briefly, categories and agents tested were 1) Aminoglycosides (Tobramycin, amikacin), 2) Antipseudomonal carbapenems: meropenem, 3) Antipseudomonal fluoroquinolones (Ciprofloxacin), 4) Cephalosporins (Ceftazidime) and 5) the Penicillins (piperacillin and Piperacillin/Tazobactam combination). Quality control was performed by inclusion of tests with the American Type Culture Collection (ATCC) standard reference strains (*P. aeruginosa* ATCC27853 and *Escherichia. coli* ATCC 25922), and MDR *P. aeruginosa* isolates.

3.7.4 Assessment of genotypic diversity in *Pseudomonas aeruginosa* isolates obtained from surgical sites among patients attending KCGH

Among the PA isolates recovered, only 14 with high resistance levels to the common antibiotics used at the facility were selected for sequencing. Genomic DNA was extracted from the selected 14 *Pseudomonas aeruginosa* isolates using the MagNA Pure Compact (MPC) nucleic acid isolation kit following the manufacturer's protocol. DNA was isolated using a Zymogen genomic DNA extraction kit (17062 Murphy Ave., Irvine, CA 92614) according to the manufacturer's instructions. For optimal performance, beta-mercaptoethanol, was added to the genomic lysis buffer to a final dilution of 0.5 %(v/v) *i.e.*, 500 µl per 100 ml. Next, 100 mg wet weight *P. aeruginosa* cells which had been resuspended in up to 200 µl of PBS was added to a ZR BashingBeadTM lysis tube and 750 µl BashingBead^{TM} buffer added. This was then secured in a bead beater fitted with a 2 ml tube holder assembly and centrifuged at maximum speed for 5 minutes. The ZR BashingBeadTM Lysis Tube (0.1 & 0.5 mm) was processed in a microcentrifuge at 10,000 x *g* for 1 minute and 400 µl supernatant transferred to a Zymo-Spin^{TM} III-F Filter in a collection tube and later centrifuged at 8,000 x *g* for 1 minute. Next, 1,200 µl of Genomic lysis buffer was added to the filtrate in the collection tube from the step above. Next, 800 µl of this mixture was transferred to a Zymo-Spin^{TM} IICR column in a collection tube and centrifuged at 10,000 x *g* for 1 minute. The flow was discarded, and the step repeated. Next, 200 µl DNA Pre-Wash buffer was added to the Zymo-Spin^{TM} IICR column in a new collection tube and centrifuged at 10,000 x *g* for 1 minute. 500 µl g-DNA wash buffer was then added to the Zymo-Spin^{TM} IICR Column and centrifuged at 10,000 x *g* for 1 minute. Finally, the Zymo-SpinTM IICR column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl (35 µl minimum) DNA elution buffer added directly to the column matrix and centrifuged at 10,000 x *g* for 30 seconds to elute the DNA.

Quality control of the Miseq Illumina sequencing reads was done using next generation sequencing (NGS) Tool kit v2.3. Filter parameters were set at cut-off quality (Phred) score of 20 with a cut-off read length for high quality set at 97% to exclude flawed-sequences due to sequencing error. The reads were trimmed using Trimmomatic and the quality assessed using in-house scripts combined with the Samtools 1.1.3, BedTools and bwa-mem software. Reads that were too short after quality truncation were discarded. Bowtie2 algorithm (Unipro UGENE v44.0) was used in aligning reads against the reference genome PA01. Next, PROKKA v1.14.5 (Kbase tool) was used to annotate the generated consensus sequences (Arkin *et al.,* 2018), and Comprehensive Antibiotic Resistance Database resistance gene identifier (CARD-RGI) (Alcock *et al.,* 2020) used to search for antimicrobial resistant genes.

Moreover, virulence determinants were searched using the reference virulence factor database (VFDB) (Chen *et al.,* 2011). For PubMLST, chromosomal sequences of the isolates were blasted to search for the internal fragments of the 7 house-keeping genes in the isolate sequences. Neighbor joining algorithm was used in the generation of the phylogenetic tree, after sequence alignment using MUSCLE (Edgar, 2004). *P. flurescence* [NC 00412] was used as the outgroup strain. For horizontally acquired genomic islands (GIs), their locations were identified by SeqWord Gene island Sniffer in line with Bezuidt and colleagues, (2009). All the molecular analysis tools in this study are summarized in **Appendix II.**

3.7.5 Determination of physiological traits of *Pseudomonas aeruginosa* isolates from surgical sites among patients attending KCGH

Streaking of recovered isolates and 2 reference strains was done on 1.5% *Pseudomonas* Isolation Agar (PIA) (Acumedia, US), a selective and differential medium. 27g of the PIA medium was suspended in 588mls of purified water containing 12 mL of glycerol. The flask was heated with frequent agitation for one minute to completely dissolve the medium. Autoclaving was done at 121ºC for 15 minutes. The media was cooled to 45-50ºC before dispensing into petri dishes. From the PA frozen stock cultures (-80^0C in 25%v/v glycerol solution), a scrape of a small number of bacteria was made, taking care not to allow thawing to avoid reducing viability of the organisms. The bacteria were streaked onto the plates using a sterile loop along with two wild type reference strains; PA14 and PA01(iglewski). Plates were incubated at 37^0C for 18hrs.

3.7.5.1 Preparation of Luria-Bertani broth (LB)

This media was used as a general-purpose bacterial culture medium for *Pseudomonas aeruginosa* isolates. It was primarily used in the preparation of *Pseudomonas aeruginosa* overnight cultures. Here, 10g of agar was suspended into 500mls of distilled water in a flask. The flask was heated with frequent agitation for one minute to completely dissolve the medium. The medium was autoclaved at 121^{0}C for 15 minutes. The overnight culture was used directly, reinoculated into a larger volume of LB broth (EO labs, Scotland) or plated to acquire either a lawn of bacterial growth or single isolated colonies. The culture was grown for 12 to 18 hours because beyond this period, the cells enter stationary phase and should not be used for critical procedures. Measured 10 mls of LB growth medium was added into 25ml universal tubes labelled with the sample IDs. A single colony was picked from the *Pseudomonas* Isolation Agar (PIA) plates and inoculated into the LB medium using a wire loop. This was done to all the tubes according to the sample labels. The tubes were placed in a roller drum housed at 37^{0}C for 16 hrs.

3.7.5.2 Auxotrophy

Agar plates containing 1.5% M9 minimal medium were prepared and supplemented with glucose only as a carbon source and as a control agar. Another set of agar plates were also prepared and supplemented with various amino acids (Arginine, Methionine, Leucine, isoleucine and valine) supplied by Sigma-Aldrich (Merck). Isolates were spot inoculated on each of these plates and specific requirement for amino acids was evident if the isolate failed to grow on the agar not containing the compounds. The wild type strains PA14 and PA01 were also spot inoculated in both plates to compare the auxotrophic characteristics. M9 minimal medium (1.5%) (Sigma-Aldrich, US) supplemented with glucose and amino acids was prepared and the overnight culture was centrifuged, and cells washed x3 using 100% PBS. A suspension of the cells was made in 10mls of sterile 100% PBS. The plates were labeled with the sample identification and 5µl of the suspension was spot inoculated accordingly. The plates were dried and incubated at 37^{0}C for 18 hrs.

3.7.5.3 Pigment production in *Pseudomonas aeruginosa*

Overnight culture of *Pseudomonas aeruginosa* isolates and the reference strains was prepared in sterile falcon tubes. After 18 hrs of incubation at 37^0C, the tubes were centrifuged at 4000rpm for 10 minutes at room temperature. The supernatant was discarded, and the cells washed x3 using sterile 100% PBS. Finally, a 10mls cell suspension was made using the sterile 100% PBS. 5µl of this suspension was spot inoculated into Pseudomonas Isolation Agar (PIA) ((Acumedia, US), taking care to match each corresponding sample ID. The plates were dried and incubated at 37^0C for 18 hours.

3.7.5.4 Exoenzyme Assays: Protease production

Skim milk agar plates were used for the detection of the ability of the isolates to produce protease. The appearance of a cleared hydrolysis zone indicated a positive test. The test was done using 25ml of 30g/l tryptic soy agar containing 2%(w/v) skimmed milk. The medium was heated with frequent agitation to dissolve the powders completely. Sterilization was done at 121°C for 15 minutes. The medium was cooled to 45-50°C and dispensed into plates. An overnight PA culture, 5µl was spot inoculated into the agar plates and incubated at 37^0C for 18 hrs after which caseinase activity was visualized through a proteolytic halo around the bacterial culture.

3.7.5.5 Trichloroacetic Acid (TCA) precipitation for Proteins

An overnight culture was prepared in sterile tubes. 9.5 ml of fresh sterile LB broth was added into each 25ml sterile tube. 0.5mls of the overnight culture was added into each tube of the corresponding sample ID and the tubes incubated at 37^0C for 5 hours to get the cells to an exponential stage of their growth. The cultures were centrifuged at 4000rpm at 20ºC for 10 minutes. The supernatant was harvested from each tube and filter sterilized using 0.45µm filter membranes in another row of clean tubes labeled with the sample IDs. 100% Trichloroacetic acid was prepared by adding10g of TCA into a graduated tube of 50mls capacity and adding distilled water to 20mls mark. Eppendorf tubes were labeled with sample IDs and added 1ml of the sterilized supernatants. 150µl of 100% TCA was added to make a final concentration of 13%. The tubes were kept in a cold room for 12 hours to precipitate. The samples were centrifuged in microcentrifuge at a high speed of 20,000rpm for 15 minutes at 5^0C. The supernatants were discarded leaving protein pellet intact. The pellets were washed x3 using cold acetone at 5^0C and finally the pellets were dried completely by placing tubes in 95°C heat block for 5-10 min to evaporate acetone. Bromophenol blue (loading dye), 30µl was added to the pellets and vortexed to dislodge the pellets and suspend them in the solution. Polyacrylamide gels were prepared by chemical copolymerization of acrylamide monomers with a crosslinking reagent, tetramethyl ethylenediamine (TEMED), required as an accelerator, in the presence of free radicals provided by ammonium persulfate (APS) as catalyst. The complete gel cassettes were placed into the buffer tanks; the gels being in direct contact with the electrode buffers. TEMED and APS were added to the SDS-PAGE separation gel solution when ready to pour. The gel was poured, leaving ~2 cm below the bottom of the comb for the stacking gel. The top of the gel was layered with isopropanol to help to remove bubbles at the top of the gel and also keep the polymerized gel from drying out. The gel was left in ~30 min to completely polymerize. The isopropanol was removed and washed out the remaining traces with distilled water. The stacking gel was poured on top of the separation gel. Combs were added to make wells. In ~30 min, the stacking gel had become completely polymerized. The gels were clamped into apparatus, and both buffer chambers were filled with gel running buffer. Prepared PA samples were loaded along with molecular mass protein markers into wells for separation by electrophoresis which was run at 10V cm^{-1} for 1.5hrs. Gels were stained with Coomassie stain overnight and visualized under UV light. Protein sizes were determined by comparing migration against a Precision Plus Protein Standard (Bio Rad) with a range of 10-250KD.

Bioluminescence assay was performed using biosensors in triplicates for each and the optical density readings were documented after 3hrs incubation at $37^{o}C$ on a shaker at 100rpm. This method used induction of QS system promoters by their cognate QS molecules as a proxy measurement (secretome titres). Quorum sensing reporter strains used are shown in **Appendix III.** Reporter strains were cultured in LB medium overnight at $37^{0}C$ on a roller drum. 75µl of the LB culture was added into the 96 well microtiter plate **(Appendix IV)** and 75µl of the thawed supernatant was also added into the wells. The plate was incubated at $37^{0}C$ for 3 hours with 100rpm shaking. The luminescence of the cultures was measured using Lucy1(Anthos Labtec instruments, Austria) for single point luminometry **(Appendix V).** This study used two types of reference strains. PA14 which is a highly virulent strain that causes disease in a wide range of organisms and PAO1 which is moderately virulent.

3.8 Quality assurance

All the standard operating procedures (SOPs) were strictly followed at all stages of microbiological analysis. American Type Culture Collection (ATCC) standard reference strains (*P. aeruginosa* ATCC27853 and *Escherichia. coli* ATCC 25922) were used for quality control of antimicrobial susceptibility testing. MDR *P. aeruginosa* isolates were confirmed by using standard biochemical tests. A standardized bacteriological procedure was followed to maintain correct laboratory results. At regular intervals and whenever a new batch of strain or reagent was prepared, standard strains of *P. aeruginosa* ATCC27853 and *E. coli* ATCC 25922 were used as positive controls. The sterility of the media was checked by incubating the media overnight before its use. The data was checked for completeness and representativeness prior to entry.

3.9 Data analysis

The data analysis in this study involved several statistical and computational methods to comprehensively assess the prevalence, antibiotic resistance, genotypic diversity, and physiological traits of *P. aeruginosa* isolates from surgical sites. For categorical variables, such as the distribution of bacterial isolates by patient characteristics (e.g., gender, age, wards, surgical sites), Chi-square analysis was used to determine the statistical significance of observed differences. A *P*-value of less than 0.05 was considered statistically significant, indicating that the observed distribution was unlikely to be due to chance. This analysis helped to identify any associations between patient demographics and the presence of *P. aeruginosa* in surgical site infections. Principal Component Analysis (PCA) was employed to explore the clustering patterns of the *P. aeruginosa* isolates based on their production of quorum sensing (QS) molecules and biofilm formation capabilities. PCA is a dimensionality reduction technique that identifies the principal components (key variables) that explain the most variance in the data. This analysis allowed the visualization of relationships between different isolates and the identification of groups of isolates with similar phenotypic traits, such as QS activity and biofilm formation. Sequence typing was conducted to determine the genetic diversity among the *P. aeruginosa* isolates. This involved analyzing the sequences of specific housekeeping genes to assign sequence types (STs) to each isolate. The sequence typing data were then used to construct phylogenetic trees, which depicted the genetic relationships among the isolates. Phylogenetic analysis involved aligning the sequences and using algorithms such as neighbor-joining or maximum likelihood to infer the evolutionary relationships between isolates, revealing clonal spreads and genetic variability. Mutation analysis focused on identifying specific genetic alterations associated with antibiotic resistance. The study examined known resistance genes, such as gyrA (conferring fluoroquinolone resistance), and analyzed their sequences for mutations. The presence and distribution of these mutations among the isolates were assessed to determine their contribution to the observed resistance profiles.

3.10 Logistical and ethical considerations

This study was approved by the the Directorate of Postgraduate Studies (DPS) 0f Masinde Muliro University of Science and Technology (MMUST). The study was also approved by the Institutional Scientific and Ethics Review Committee (ISERC) of MMUST (approval No.MMUST/IERC/176/2023) and the National Commission for Science, Technology, and Innovation (NACOSTI) Licence No. NACOSTI/P/23/28712. Confidentiality was maintained by coding samples and securely storing data. Ethical considerations for human participants were observed, and voluntary participation was emphasized. For incompetent individuals and minors, consent was obtained from parents or legal guardians. Older children or teenagers under 18 provided consent or through court order emancipation. Findings were presented anonymously, and measures were taken to minimize the risks of data breaches during information transmission. Authorization for data collection was obtained from the Kakamega county director of medical health services (No. ERC/226-11/2023) and the study was part of a larger project.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents the research outcomes addressing each study's objectives. Importantly, it gives comprehensive findings on various antimicrobial sensitivity patterns of the isolated strains of *P. aeruginosa,* while breaking down their genomes for drug-resistant genes (DRGs) and virulence genes. Noteworthy, it delves deeper into the identified sequence types (STs) and the determination of the genetic relatedness of these sequence types, in line with their evolutionary patterns. Physiological traits and quorum sensing mechanisms are also presented. Here the results are developed according to the set study objectives.

4.2 Determination of the prevalence of *Pseudomonas aeruginosa* isolates from surgical sites among patients attending KCGH

4.2.1 Patient demographics

A total of 128 (male, n=46 and female, n=82) patients attending Kakamega County General Hospital in the year 2022 were evaluated as summarized in **Table 4.1.** The median patient age, height and weight were 67 years (range 2-97), 152.7cm (range 111-172) and 61kg (range 47-72), respectively. Majority of the patients were from the female and male surgical wards.

Table 4 1 Characteristics of study participants

The Characteristics of participants who were positive for SSIs.

Characteristics	n =128 (%)	X^2	*P*
Gender			
Male	46 (64.0)	10.125	**0.002**
Female	82 (36.0)		
Age, yrs			
≤ 20	19 (14.8)		
21-40	45 (35.2)	0.007	0.998
41-60	35 (27.3)		
>60	29 (22.7)		
Wards			
Burns unit	2 (1.7)		
Female surgical	46 (35.9)		
ICU	6 (4.7)		
Male surgical	26 (20.3)		
Obs/gyn	12 (9.3)	-	-
Oncology	3 (2.3)		
Orthorpedic	6 (4.7)		
Pediatric	1 (0.8)		
Post-natal unit	21 (18.6)		
Renal unit	2 (1.7)		
Surgical sites			
Abdomen	32 (25.0)		
Cardiac	1 (0.8)		
Head	3 (2.3)		
Limb	30 (23.4)	-	-
Reproductive	50 (39.1)		
Respiratory	1 (0.8)		
Skeletal	4 (3.1)		
Skin	7 (5.5)		

This table presents the patient characteristics as frequencies and percentages in brackets. Abbreviations: ICU-intensive care unit. *P*-values obtained from a Chi-square analysis of the categorical variables: gender, age, wards, and surgical sites.

The table 4.1 displays data on a sample of 128 individuals, emphasising critical attributes including gender, age, ward assignment, and surgical site. The sample is composed of 64.0% males (46 individuals) and 36.0% females (82 individuals) in terms of gender distribution. The chi-square value of 10.125 and a *P*-value of 0.002

in the statistical analysis suggest that the proportions of males and females are significantly different. The age distribution of the sample is as per the following: 14.8% of the population is under the age of 20, 35.2% are between the ages of 21 and 40, 27.3% are between the ages of 41 and 60, and 22.7% are over 60 years old. The chi-square test for age distribution produced a *P*-value of 0.998 and a Chi-square value of 0.007, indicating that the age distribution is not substantially different across the categories. The patients were admitted to a variety of wards. The female surgical ward was home to the majority, with 35.9% of patients. The male surgical ward followed, with 20.3% of patients. The post-natal unit (18.6%), obstetrics/gynecology (9.3%), orthopaedic (4.7%), ICU (4.7%), oncology (2.3%), burns unit (1.7%), renal unit (1.7%), and paediatric (0.8%) were the other wards. The reproductive system was the most frequently reported surgical site, accounting for 39.1% of the total. The abdomen (25.0%) and extremities (23.4%) followed. The epidermis (5.5%), skeletal system (3.1%), head (2.3%), respiratory system (0.8%), and cardiac (0.8%) were the other surgical sites.

4.2.2 Recovery of microbes from surgical wounds

Figure 4.1 presents data on the prevalence of bacteria isolated from surgical sites and the burden of individual microbes per gender. Panel A illustrates the prevalence of different bacteria isolated from surgical sites as a percentage of the total cases. The most prevalent bacterium is Pseudomonas aeruginosa, accounting for 38.9% of the isolates. Following this, Gram-negative rods represent 25.8% of the bacterial isolates. *Staphylococcus aureus* and *Klebsiella spp*. are also notable, with lower prevalence rates, while *Escherichia coli* and *Klebsiella oxytoca* are the least prevalent among the bacteria identified. Panel B depicts the frequency of individual microbes according to gender. The graph shows that both males and females are affected by similar types of bacteria, with *P. aeruginosa* being the most frequent in both genders. Gram-negative rods, *Staphylococcus aureus*, and *Klebsiella spp*. also have notable frequencies. The frequencies are represented by different colors for males (yellow) and females (red), with the height of the bars indicating the number of cases. The statistical analysis reveals no significant difference in microbial burden between males and females, as indicated by a *P*-value of 0.942. Overall, *P aeruginosa* is the most common bacterium isolated from surgical sites and that there is no significant difference in the distribution of bacterial infections between males and females.

Figure 4.1

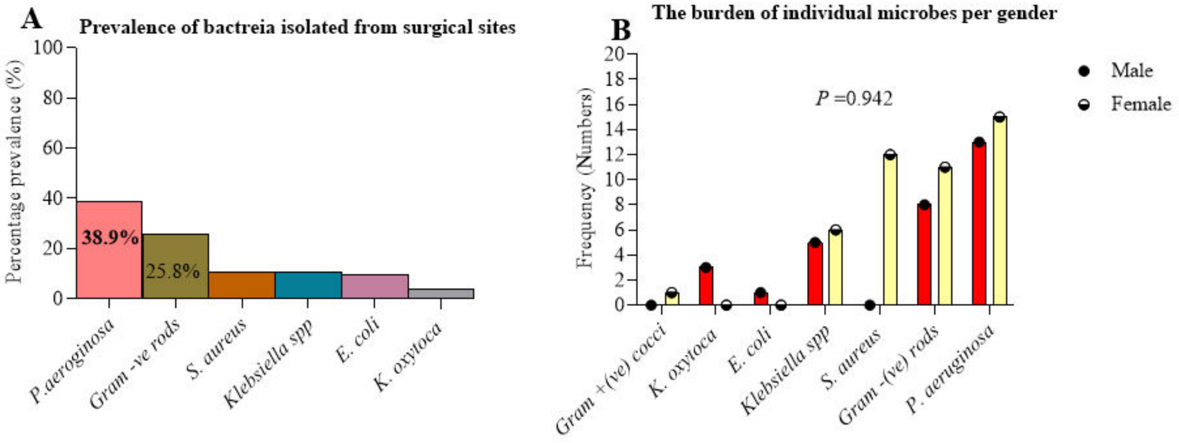


Figure 4 1 Bacterial isolates from surgical site infections. (A). The prevalence of bacteria isolated from surgical site infections. (B). The burden of individual microbes per gender.

4.3 Evaluation of the antibiotic resistance profiles of *Pseudomonas aeruginosa* isolates from surgical sites among patients attending KCGH

Summary results of antimicrobial sensitivity tests are summarized in **Table 4.2.** The table presents the antimicrobial sensitivity levels of *P. aeruginosa* isolates to various antibiotics across different classes. The results indicate a significant degree of resistance among the tested isolates, with some variation in sensitivity across different antibiotic classes. For the Cephalosporin class, Ceftazidime was tested, revealing that 60.7% of the isolates were resistant, with only 21.4% showing sensitivity. This suggests that Ceftazidime may not be highly effective against these *P. aeruginosa* isolates.

In the Penicillin class, Piperacillin/Tazobactam and Piperacillin were evaluated. Piperacillin/Tazobactam showed a more balanced distribution, with 39.3% of the isolates being sensitive and an equal percentage being in the intermediate category. In contrast, Piperacillin alone had a high resistance rate of 67.9%, with only 7.1% of isolates being sensitive, indicating that Piperacillin alone is likely less effective in treating infections caused by these isolates. The Aminoglycosides class, represented by Amikacin and Tobramycin, showed concerning results. Amikacin had no sensitive isolates, with 71.4% being resistant, while Tobramycin showed a better profile, with 35.7% of isolates being sensitive, although 64.3% were still resistant. In the Fluoroquinolones class, Ciprofloxacin was tested and found to have a resistance rate of 60.7%, with only 17.9% sensitivity, suggesting limited effectiveness in treating these isolates. Meropenem, a Carbapenem, demonstrated the highest sensitivity rate at 53.6%, with only 17.9% of isolates being resistant. This suggests that Meropenem might be the most effective antibiotic against the Pseudomonas aeruginosa isolates in this study. Overall, the results underscore a high prevalence of multidrug resistance among the *P. aeruginosa* isolates, with Meropenem standing out as the most effective antibiotic among those tested. These data revealed a statistically significant difference in antimicrobial sensitivity levels (Intermediate, Resistant, and Sensitive) across the different antibiotics X^2=51.72, P<0.0001.

Table 4 2 Antimicrobial sensitivity levels of the *P. aeruginosa* isolates.

Antibiotic Class	**Antibiotic**	**Antimicrobial Sensitivity Level (n=28)**		
		Intermediate (I) (%)	**Resistant (R) (%)**	**Sensitive (S) (%)**
Cephalosporins	Ceftazidime (30µg)	17.9	60.7	21.4
Penicillin	Piperacillin/Tazobactam (100µg /10µg)	39.3	21.4	39.3
	Piperacillin (100µg)	25	67.9	7.1
Aminoglycosides	Amikacin (30µg)	28.9	71.4	0
	Tobramycin (10µg)	0	64.3	35.7
Fluoroquinolones	Ciprofloxacin (5µg)	21.4	60.7	17.9
Carbapenem	Meropenem (10µg)	21.4	17.9	53.6

Table 4.2 showing antimicrobial sensitivity levels of the *P. aeruginosa* isolates. Micrograms (µg), I-intermediate resistance, R-resistant strains, S-sensitive strains.

From the preliminary antimicrobial sensitivity test results in table 4.2 above, it was critical to test for any multi-drug resistant strains among the tested *P. aeruginosa* isolates. The figure 4.2 shows a detailed comparison of the antimicrobial sensitivity levels of *P. aeruginosa* isolates against multiple antibiotics. The sensitivity levels are classified into three categories: Resistant, Intermediate, and Sensitive. The data reveals a concerning trend of high resistance across many of the isolates. Several isolates, such as ID004, ID006, ID013, ID014, and ID016, exhibit very high resistance levels, with some reaching 100%. This indicates that these isolates are largely unaffected by the antibiotics tested, suggesting they are multidrug-resistant (MDR).

Intermediate sensitivity is less common and is observed inconsistently among the isolates. Isolate ID009 stands out with a relatively high percentage of intermediate sensitivity, contrasting with many other isolates that show little to no intermediate response. Other isolates, such as ID004, ID010, and ID020, also demonstrate moderate levels of intermediate sensitivity.

Sensitivity to the antibiotics is generally low across most of the isolates, meaning that few isolates are highly responsive to the treatments. Isolates like ID003, ID008, and ID017 have slightly higher sensitivity levels, though these still remain below 50%. In contrast, isolates such as ID013 and ID014 show almost no sensitivity, reinforcing the dominance of resistance among these strains.

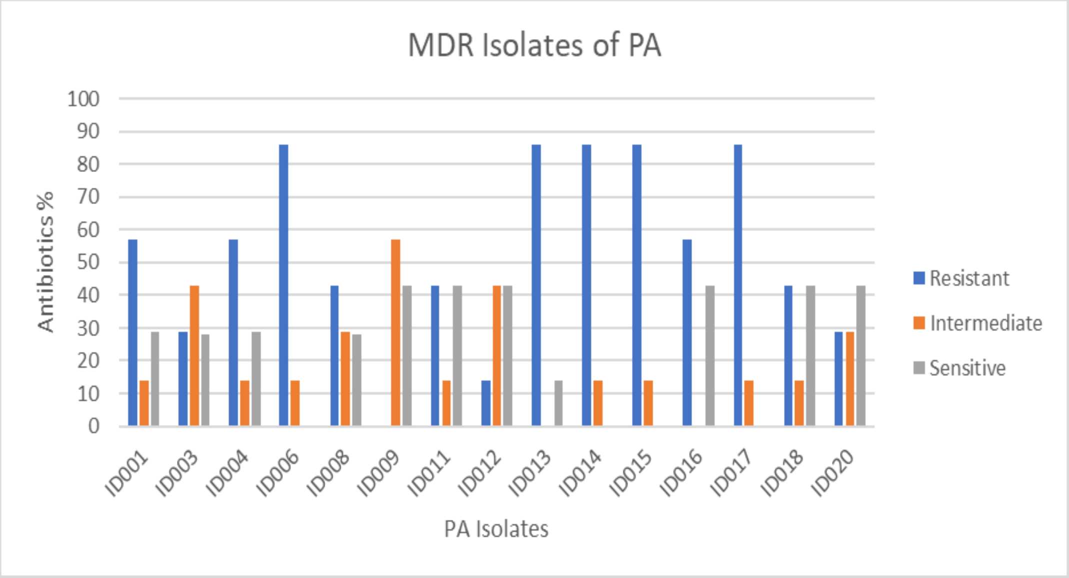


Figure 4 2 Percentages of sensitivity levels of strains of *P. aeruginosa* against antibiotic drugs.

4.3 Assessment of genotypic diversity in *Pseudomonas aeruginosa* isolates obtained from surgical sites among patients attending KCGH

4.3.1 DNA isolation, sequencing, and Genome Annotation of the selected isolates

In this study, representative 14 strains were sequenced, 13 belong to *P. aeruginosa,* and one, ID019 is *Stenotrophomonas maltophilia* and was discontinued from the analysis (Table 4.3). The genomic data provided for the *P. aeruginosa* isolates reveals a fascinating range of variability in their genetic makeup, with implications for their adaptability, pathogenicity, and potential resistance to treatments. Each isolate possesses a chromosome length that falls within a relatively narrow range, from about 6.2 million to 6.65 million base pairs (bp). Alongside this, the number of coding sequences (CDS) present on these chromosomes is also quite consistent, with counts varying from approximately 5,672 to 6,061 CDS. This suggests that while the overall size of the chromosomes is similar across the isolates, the number of genes they carry is also fairly uniform, indicating a stable core genome among these isolates.

Most of the isolates lacked plasmids entirely, which are extrachromosomal DNA elements often associated with horizontal gene transfer and antibiotic resistance. However, two isolates—ID008 and ID015—do carry plasmids. Isolate ID008, in particular, contains a relatively large plasmid of 46,344 bp, housing 48 CDS. In contrast, ID015 has a much smaller plasmid of just 2,259 bp, with only 3 CDS. The presence of these plasmids, especially in ID008, could potentially confer additional advantages, such as antibiotic resistance or enhanced virulence, which are often associated with genes found on plasmids.

The number and content of genomic islands, which are segments of DNA acquired through horizontal gene transfer and often contain genes beneficial for survival in specific environments, vary significantly among the isolates. The number of GIs ranges from 6 to 23 across the isolates, with the number of CDS on these GIs varying accordingly. Isolate ID011 stands out with the most extensive GI content, both in terms of length (650,092 bp) and the number of CDS (585). This isolate also has the largest proportion of its chromosome made up of GIs, at 9.7%, suggesting a high potential for adaptability and pathogenicity due to the acquisition of foreign genes. In contrast, isolate ID004 has the smallest GI content, with GIs comprising only 3.8% of its chromosome and containing just 247 CDS. This reduced GI content could imply a lesser capacity for adaptation compared to other isolates. The data suggests that while the *P. aeruginosa* isolates share a core set of genes reflected in their similar chromosome lengths and CDS numbers, there is significant variability in their accessory genomes, particularly in the presence of plasmids and the content of genomic islands. This variability could lead to differences in how these isolates respond to environmental pressures, including the presence of antibiotics. Isolates like ID011, with their extensive GI content, may possess a greater ability to adapt and survive under various conditions, potentially making them more resilient and harder to treat. On the other hand, isolates like ID004, with fewer GIs, may have less genetic flexibility. The presence of plasmids in some isolates further underscores the genetic diversity within these populations, highlighting the complexity of managing infections caused by *P. aeruginosa*. Additional genomic features for the other isolates are in highlighted in Figure 4.3.

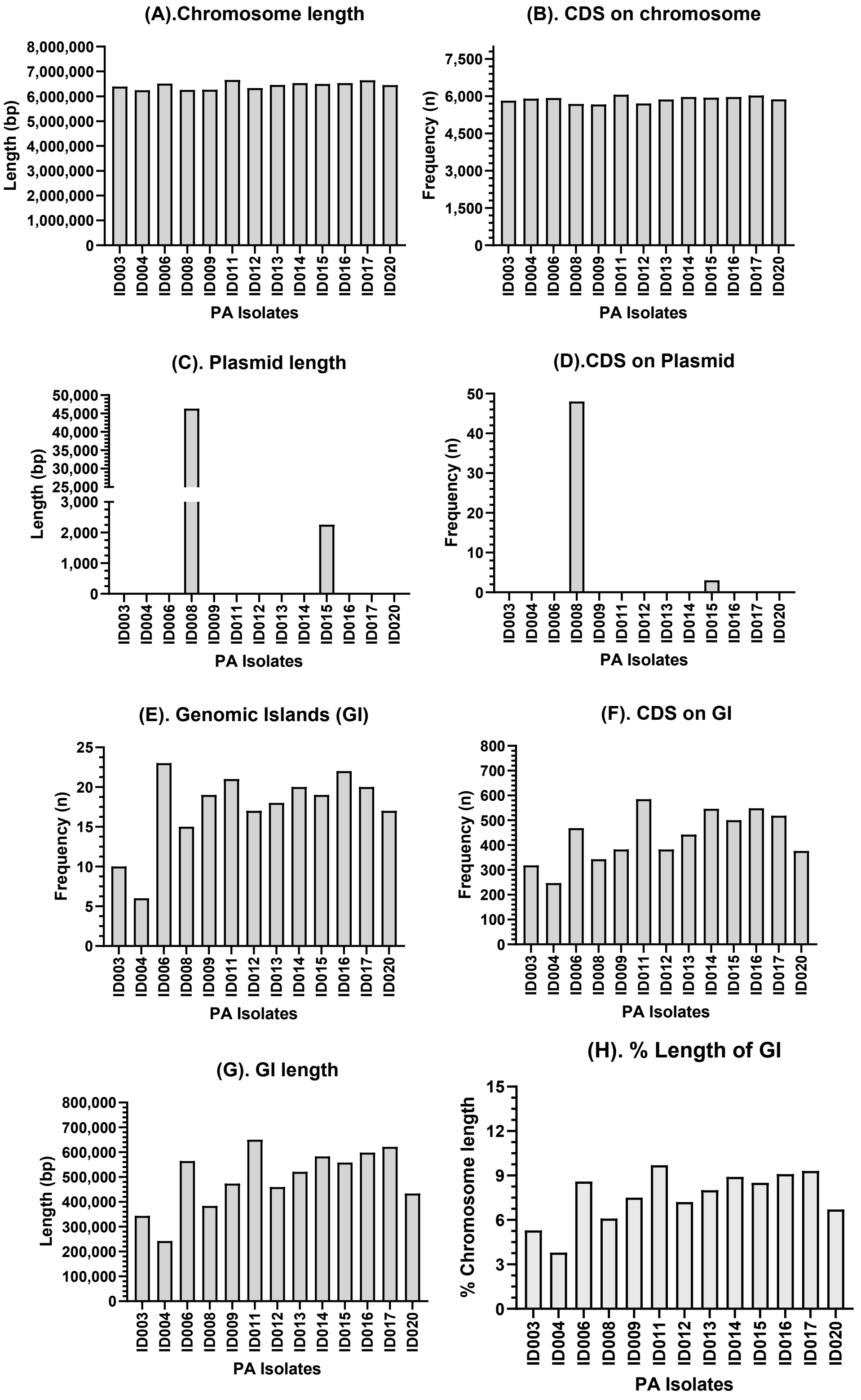


Figure 4 3 Further genomic features for the other isolates.

Table 4 3 Genome characteristics of *Pseudomonas aeruginosa* study isolates.

Isolate identity number	**Species**	**Chromosome Length (bp)**	**CDS on chromosome**	**Plasmid length**	**CDS on plasmid**	**Genomic islands (GIs)**	**CDS on GIs**	**Total length of GIs**	**% length GIs**
ID003	*P. aeruginosa*	6,392,455	5,820	No	No	10	318	343,523	5.3
ID004	*P. aeruginosa*	6,241,720	5,905	No	No	6	247	242,666	3.8
ID006	*P. aeruginosa*	6,510,369	5,933	No	No	23	468	564,147	8.6
ID008	*P. aeruginosa*	6,254,301	5,690	46,344	48	15	343	383,607	6.1
ID009	*P. aeruginosa*	6,265,160	5,672	No	No	19	382	473,580	7.5
ID011	*P. aeruginosa*	6,655,210	6,061	No	No	21	585	650,092	9.7
ID012	*P. aeruginosa*	6,329,299	5,712	No	No	17	382	459,891	7.2
ID013	*P. aeruginosa*	6,459,610	5,869	No	No	18	442	520,882	8.0
ID014	*P. aeruginosa*	6,528,294	5,973	No	No	20	546	583,118	8.9
ID015	*P. aeruginosa*	6,494,022	5,941	2,259	3	19	500	558,032	8.5
ID016	*P. aeruginosa*	6,529,781	5,973	No	No	22	548	598,406	9.1
ID017	*P. aeruginosa*	6,644,873	6,028	No	No	20	518	621,893	9.3
ID020	*P. aeruginosa*	6,452,400	5,879	No	No	17	376	433,834	6.7

Table 4.3 showing genome characteristics of *P. aeruginosa* isolates. CDS- Coding sequence, GIs- Genomic islands. Genomic islands were observed in ID011 (585) length of 650092 bp and lowest in ID004 (247) length of 242,666 bp.

4.3.2 Drug-resistant genes in chromosomes and plasmids

The data presented in figure and table 4.4 below represents the antibiotic resistance gene profiles in various *P.aeruginosa* isolates, highlighting the potential for resistance across different classes of antibiotics. Each value in the table represents the count of resistance genes associated with a specific antibiotic class within each isolate, reflecting the complex and varied resistance mechanisms these bacteria possess. Importantly, a single resistance gene may confer resistance to multiple antibiotics, leading to totals that exceed the actual number of distinct resistance genes in the genome. The isolates exhibit a strong presence of genes conferring resistance to aminocoumarins and carbapenems, with most carrying 14 to 15 aminocoumarin resistance genes and 15 to 17 carbapenem resistance genes. Isolate ID017 stands out with the highest number of these resistance genes, suggesting a particularly robust resistance profile. Resistance to aminoglycosides is also widespread, with most isolates harboring 11 to 12 resistance genes. However, isolate ID017 again distinguishes itself with 16 aminoglycoside resistance genes, indicating a heightened ability to resist this class of antibiotics.

In the case of cephalosporins and cephamycins, the isolates show a significant number of resistance genes, typically ranging from 18 to 20 for cephalosporins and 10 to 12 for cephamycins. This indicates a strong and consistent resistance to these beta-lactam antibiotics across the isolates. The resistance to diaminopyrimidines is similarly high, with most isolates carrying 19 to 20 resistance genes, suggesting that this antibiotic class may be less effective against these strains of *P. aeruginosa*. Fluoroquinolone resistance is particularly prevalent, with gene counts ranging from 29 to 31, underscoring the difficulty in treating these bacteria with this class of antibiotics. Macrolide resistance is also significant, with most isolates carrying 26 to 28 resistance genes. Notably, isolate ID015 shows the highest resistance to macrolides, with 28 genes identified. Some antibiotic classes, such as oxazolidinones, bicyclomycin-like agents, glycopeptides, and glycylcyclines, show consistently low resistance gene counts, typically just 1 to 2 genes per isolate. This suggests that these antibiotic classes might either be less frequently resisted by these isolates or target more specific pathways that fewer resistance genes can overcome.

Additionally, the isolates demonstrate a notable resistance to disinfectants and antiseptics, with 22 to 24 resistance genes identified across the board. This resistance highlights the potential for these bacteria to persist in environments where disinfection is routinely practiced, complicating efforts to control their spread and posing a significant threat in healthcare settings.

The *P.aeruginosa* isolates exhibit a broad and varied resistance profile across multiple antibiotic classes. High levels of resistance are especially evident against aminoglycosides, carbapenems, fluoroquinolones, macrolides, and tetracyclines, indicating that these bacteria are particularly challenging to treat. The consistent number of resistance genes across these major antibiotic classes suggests significant selective pressure, leading to the development of a diverse array of resistance mechanisms. The presence of resistance genes against disinfectants and antiseptics further underscores the resilience of these isolates, making them formidable adversaries in both clinical and environmental settings.

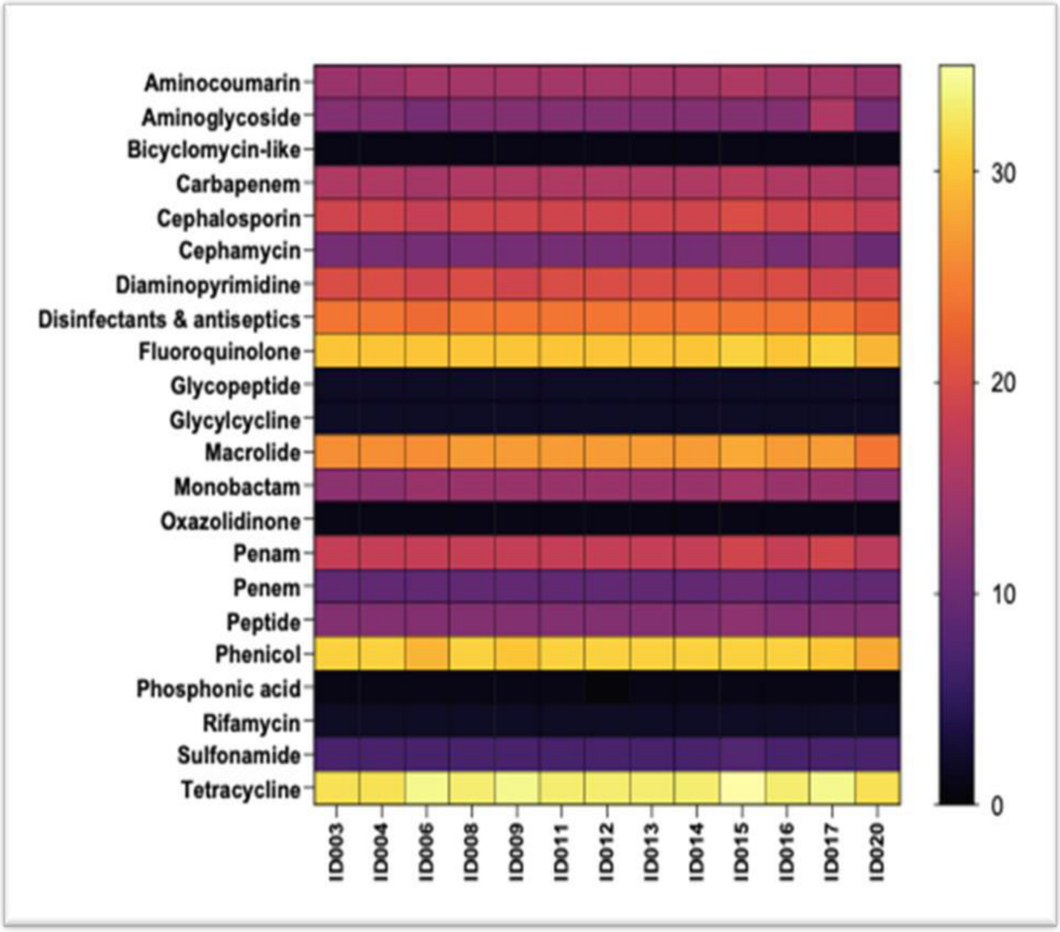


Figure 4 3 Chart of Resistance of study isolates to several antibiotics

Table 4 4 Antibiotic resistance gene spectra of *Pseudomonas aeruginosa* isolates.

Antibiotic	**ID003**	**ID004**	**ID006**	**ID008**	**ID009**	**ID011**	**ID012**	**ID013**	**ID014**	**ID015**	**ID016**	**ID017**	**ID020**
Aminocoumarin	14	14	15	15	15	15	15	15	15	16	15	15	14
Aminoglycoside	12	12	11	12	12	12	12	12	12	12	12	16	11
Bicyclomycin-like	1	1	1	1	1	1	1	1	1	1	1	1	1
Carbapenem	16	16	15	16	16	16	16	16	16	17	16	16	15
Cephalosporin	19	19	18	19	19	19	19	19	19	20	19	19	18
Cephamycin	11	11	11	11	11	11	11	11	11	12	11	12	10
Diaminopyrimidine	20	20	19	20	19	20	20	20	20	20	20	19	19
Disinfectants & antiseptics	24	24	23	24	24	24	24	24	24	24	24	24	22
Fluoroquinolone	30	30	30	30	30	30	30	30	30	31	30	31	29
Glycopeptide	2	2	2	2	2	2	2	2	2	2	2	2	2
Glycylcycline	2	2	2	2	2	2	2	2	2	2	2	2	2
Macrolide	26	26	26	27	27	27	27	27	27	28	27	27	24
Monobactam	13	13	14	14	14	14	14	14	14	15	14	14	13
Oxazolidinone	1	1	1	1	1	1	1	1	1	1	1	1	1
Penam	18	18	18	18	18	18	18	18	18	19	18	19	17
Penem	9	9	9	9	9	9	9	9	9	10	9	9	9
Peptide	12	12	12	12	12	12	12	12	12	13	12	12	12
Phenicol	31	31	29	31	30	31	31	31	31	31	31	30	28
Phosphonic acid	1	1	1	1	1	1	0	1	1	1	1	1	1
Rifamycin	2	2	2	2	2	2	2	2	2	2	2	2	2
Sulfonamide	7	7	7	7	7	7	7	7	7	8	7	7	7
Tetracycline	32	32	34	33	34	33	33	33	33	35	33	34	32

From Table 4.4, the total numbers are bigger than the numbers of antibiotic resistance genes as one gene may render resistance to several antibiotics

4.3.3 Mutational factors in drug resistance genes

Genes conferring mutation sites were determined for the selected *P. aeruginosa* isolates as shown in Table 4.5. The table shows the mutations present in specific drug-resistant genes across various *P. aeruginosa* isolates. The genes examined include *P. aeruginosa CpxR*, *catB7*, *emrE*, *gyrA* (which confers resistance to fluoroquinolones), *soxR*. In all the isolates (ID003 to ID020), mutations are consistently found in the *CpxR*, *catB7*, *emrE* and *soxR* genes. The uniform presence of these mutations across all isolates suggests that these genetic alterations are common in these *P. aeruginosa* strains and likely play a crucial role in their resistance mechanisms.

In contrast, the *gyrA* gene, which is associated with fluoroquinolone resistance, shows a different pattern. Most of the isolates (such as ID003, ID004, ID006, ID008, ID012, ID013, ID015, and ID017) did not have mutations in this gene. Only a few isolates, including ID009, ID011, ID014, ID016, and ID020, exhibited mutations in *gyrA*. This suggests that fluoroquinolone resistance is not as uniformly distributed among these isolates as the other resistance genes and may be more dependent on specific selective pressures or environmental factors.

Generally, while mutations in *CpxR*, *catB7*, *emrE*, and *soxR* are widespread and consistently present across all the *P. aeruginosa* isolates, mutations in *gyrA*, conferring resistance to fluoroquinolones, are more selective and present only in certain isolates. This variability in the *gyrA* mutations points to a more complex and potentially environment-specific mechanism of resistance to fluoroquinolones, contrasting with the more uniform mutation patterns observed in the other genes.

Table 4 5 : Mutations in drug-resistant genes identified.

Isolate ID	**Genes with mutations**				
	CpxR	***catB7***	***emrE***	***gyrA* conferring resistance to fluoroquinolones**	***soxR***
ID003	1	1	1	0	1
ID004	1	1	1	0	1
ID006	1	1	1	0	1
ID008	1	1	1	0	1
ID009	1	1	1	0	1
ID011	1	1	1	0	1
ID012	1	1	1	0	1
ID013	1	1	1	0	1
ID014	1	1	1	0	1
ID015	1	1	1	0	1
ID016	1	1	1	0	1
ID017	1	1	1	1	1
ID020	1	1	1	0	1

Table 4.5 showing mutations identified in the drug resistant gene from *P. aeruginosa* isolates '1' indicates the presence of a mutation, while a '0' indicates its absence.

The data in Table 4.6 illustrate the mechanisms of action for drug resistance genes across various *P. aeruginosa* isolates. The mechanisms are categorized into three primary modes: the efflux system, antibiotic inactivation, and target alteration. The table is divided into two sections: the first section quantifies the total number of resistance genes involved in each mechanism, while the second section indicates the presence of specific resistance gene categories. The efflux system is shown to be the predominant mechanism across all isolates, with each isolate having between 44 and 47 genes involved in this process. Isolates ID015 and ID008 exhibit the highest numbers (47 and 46 genes, respectively), suggesting a particularly robust efflux system in these strains. The efflux system is a key resistance mechanism where bacteria actively pump out antibiotics, thereby reducing drug accumulation and efficacy.

The second mechanism, antibiotic inactivation, is less prevalent but still present in all isolates. The number of genes involved in antibiotic inactivation ranges from 4 to 9 across the isolates. Notably, isolate ID017 has the highest count with 9 genes, indicating a stronger capacity for enzymatic degradation or modification of antibiotics, rendering them ineffective.

The third mechanism, target alteration, is consistently represented across the isolates, with 8 or 9 genes involved in modifying bacterial targets, preventing antibiotics from binding effectively. Isolate ID017, again, shows the highest count with 9 genes, suggesting a more pronounced ability to evade antibiotic action through this mechanism. In the second section of the table, specific resistance gene categories are identified for each mechanism. For the efflux system, most isolates have no additional gene categories noted. However, isolates ID004, ID012, and ID013 stand out, with ID012 showing the highest number (6) of additional efflux system-related genes, followed by ID013 with 3 and ID004 with 2. These additional genes may indicate more specialized or alternative efflux mechanisms contributing to the resistance profile.

Antibiotic inactivation genes are generally absent in this section, with a few exceptions. Isolate ID012 has 1 gene noted, while ID017, which has the highest overall count in this mechanism, shows 4 additional inactivation genes. This emphasizes ID017’s significant capacity for antibiotic inactivation as part of its resistance strategy. Target alteration genes are largely absent in the second section, with only isolate ID017 showing 1 gene in this category, reinforcing its higher capacity for resistance through multiple mechanisms. The efflux system is the most common resistance mechanism across these Pseudomonas aeruginosa isolates, with variations in the number of genes contributing to this and other mechanisms like antibiotic inactivation and target alteration. Certain isolates, such as ID017 and ID012, show a more complex resistance profile with additional resistance gene categories, particularly in antibiotic inactivation and efflux systems, suggesting that these isolates may have enhanced resistance capabilities.

Table 4 6 Drug resistance genes mechanism of action.

Mode of action	**ID003**	**ID004**	**ID006**	**ID008**	**ID009**	**ID011**	**ID012**	**ID013**	**ID014**	**ID015**	**ID016**	**ID017**	**ID020**
Efflux system	45	45	46	46	46	46	46	46	46	47	46	46	44
Antibiotic inactivation	5	5	5	5	5	5	4	5	5	5	5	9	5
Target alteration	8	8	8	8	8	8	8	8	8	8	8	9	8

Resistance gene categories

Mode of action	**ID003**	**ID004**	**ID006**	**ID008**	**ID009**	**ID011**	**ID012**	**ID013**	**ID014**	**ID015**	**ID016**	**ID017**	**ID020**
Efflux system	0	2	0	0	1	1	6	3	1	0	1	1	1
Antibiotic inactivation	0	0	0	0	0	0	1	0	0	0	0	4	0
Target alteration	0	0	0	0	0	0	0	0	0	0	0	1	0

Table 4.6 showing antibiotic resistance mechanism employed by the *P. aeruginosa* isolates in rendering antibiotics ineffective against them.

The Figure 4.5 illustrates the effectiveness of various antibiotic resistance mechanisms employed by *P. aeruginosa*. The most prominent resistance mechanism is antibiotic efflux. This mechanism plays a significant role in antibiotic resistance in *P. aeruginosa*, functioning by actively pumping antibiotics out of the bacterial cell, which reduces their intracellular concentration and thereby diminishes the antibiotics' effectiveness. The second most significant mechanism is antibiotic inactivation. This mechanism involves the enzymatic degradation or modification of the antibiotic, rendering it inactive and ineffective against the bacteria.

Antibiotic target alteration is another important mechanism. This involves mutations or modifications to the bacterial target of the antibiotic, preventing the antibiotic from binding effectively and thereby evading its inhibitory effects. A combination mechanism, antibiotic target alteration along with antibiotic efflux have also been utlized. In this strategy, the bacteria not only alter the target site of the antibiotic but also actively pump out the antibiotic, providing a dual defense against the drug.

A mechanism involving both antibiotic efflux and reduced permeability to antibiotics was found in the isolates. This combination decreases the influx of antibiotics into the bacterial cell and simultaneously increases their efflux, effectively lowering the intracellular concentration of the drug. Antibiotic target replacement, a less common mechanism was also found where the bacteria replace or bypass the original target of the antibiotic with an alternative target that the antibiotic cannot effectively bind to. *P. aeruginosa* primarily relies on the efflux system to resist antibiotics, followed by inactivation of antibiotics and target alteration. The bacteria also employ combination strategies, such as target alteration with efflux or reduced permeability with efflux, showcasing their versatility and adaptability in resisting various antimicrobial agents

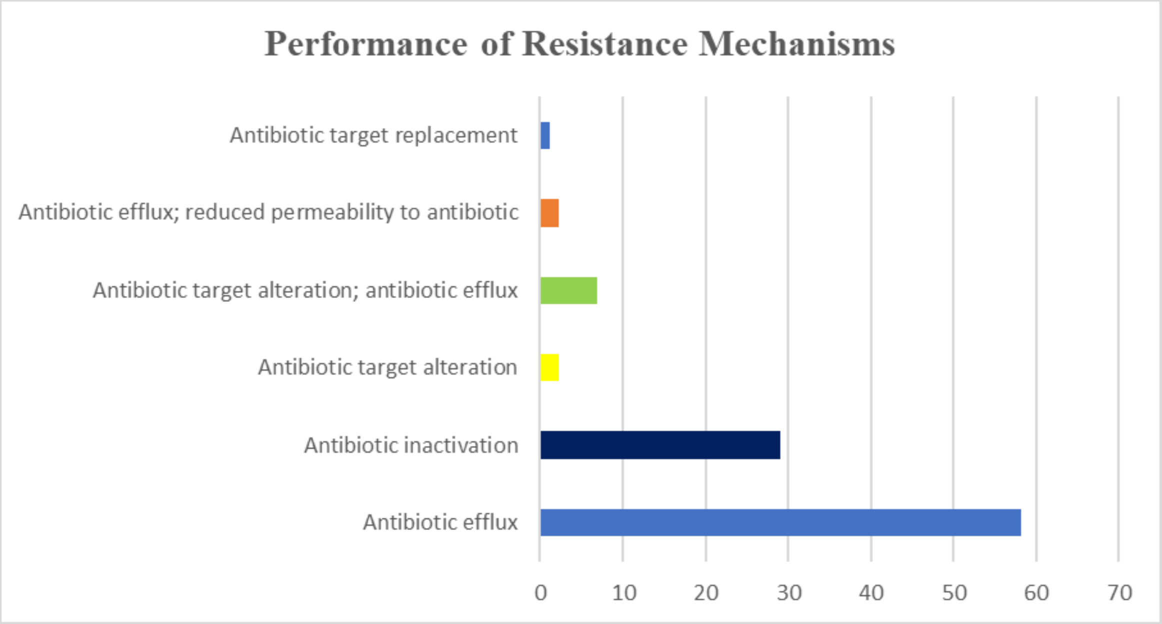


Figure 4 4 Showing different drug resistance mechanisms

4.3.4 Identification of virulence genes, present in *Pseudomonas aeruginosa* isolates

Many virulence genes were identified searching through VFDB database (**Appendix IX**). However, this database contains too many housekeeping genes of *P. aeruginosa* like chemotaxis proteins. Therefore, most of the identified genes are in the core part of the chromosome **(Table 4.7).** The most interesting virulence genes are those located in GIs and plasmids. The biggest horizontally acquired virulence genes were found in ID014 (31), ID016 (30) and ID015 (28) – all belong to ST274. ID008 has 2 virulence genes on its plasmid. The smallest number of virulence genes has ID004.

4.3.4.1 The distribution of virulence genes in Genomic islands

The distribution of virulence genes in genomic islands (GIs) across various *P. aeruginosa* isolates is shown in table 4.7 below. Each isolate is characterized by the number of virulence genes found within specific GIs and whether any virulence genes are present in plasmids.

In isolate ID003, there are 19 virulence genes distributed across several genomic islands, with significant contributions from GI#2 (8 genes), GI#5 (2 genes), GI#6 (1 gene), GI#7 (7 genes), and GI#9 (1 gene). This isolate has no virulence genes found in plasmids. Isolate ID004 contains 4 virulence genes, all of which are located in GI#2 (2 genes) and GI#4 (2 genes), with no plasmid-associated virulence genes. Isolate ID006 shows a broader distribution with 23 virulence genes. Key contributors include GI#4 (14 genes), GI#11 (1 gene), GI#13 (1 gene), GI#16 (1 gene), GI#20 (3 genes), and GI#21 (2 genes), with no virulence genes found in plasmids. Isolate ID008 has 20 virulence genes distributed across various GIs, with GI#1, GI#3, GI#5, GI#9, GI#10, and GI#13 each contributing several genes. This isolate uniquely has 2 virulence genes present in plasmids. Isolate ID009 has 27 virulence genes, spread across multiple GIs. Notable clusters include GI#10 (10 genes), GI#11 (2 genes), GI#12 (3 genes), GI#13 (9 genes), GI#16 (5 genes), and GI#17 (3 genes), with no plasmid-associated virulence genes. Isolate ID011 carries 26 virulence genes with significant contributions from GI#4 (1 gene), GI#5 (14 genes), GI#12 (1 gene), GI#13 (3 genes), and GI#14 (7 genes), with no virulence genes found in plasmids.

Isolate ID012 has 13 virulence genes distributed primarily in GI#7 (1 gene), GI#2 (1 gene), GI#8 (2 genes), GI#11 (1 gene), and GI#12 (2 genes), with no virulence genes found in plasmids. Isolate ID013 shows a total of 19 virulence genes, primarily located in GI#4 (1 gene), GI#5 (14 genes), GI#14 (1 gene), and GI#15 (3 genes), with no plasmid-associated virulence genes. Isolate ID014 has the highest number of virulence genes, totaling 31. Significant clusters are found in GI#4 (1 gene), GI#5 (3 genes), GI#9 (1 gene), GI#10 (1 gene), GI#12 (2 genes), GI#13 (1 gene), GI#14 (10 genes), GI#16 (3 genes), and GI#17 (9 genes), with no virulence genes found in plasmids. Isolate ID015 carries 28 virulence genes, spread across GIs such as GI#2 (9 genes), GI#3 (3 genes), GI#4 (6 genes), GI#5 (1 gene), GI#6 (2 genes), GI#9 (1 gene), GI#15 (5 genes), and GI#16 (1 gene), with no plasmid-associated virulence genes.

Isolate ID016 shows a broad distribution with 30 virulence genes. Notable contributors include GI#4 (1 gene), GI#5 (3 genes), GI#9 (1 gene), GI#10 (1 gene), GI#12 (2 genes), GI#13 (1 gene), GI#14 (7 genes), GI#15 (2 genes), GI#17 (3 genes), and GI#18 (9 genes), with no virulence genes found in plasmids. Isolate ID017 has a total of 22 virulence genes, primarily distributed in GI#2 (1 gene), GI#3 (7 genes), GI#12 (2 genes), GI#13 (2 genes), GI#15 (5 genes), GI#16 (1 gene), GI#17 (1 gene), and GI#19 (3 genes), with no plasmid-associated virulence genes. Isolate ID020 has the fewest virulence genes, with only 4 located in GI#3, and no plasmid-associated virulence genes.

Overall, the distribution of virulence genes within genomic islands varies significantly across the Pseudomonas aeruginosa isolates. While some isolates, like ID014 and ID016, have a large number of virulence genes spread across multiple GIs, others, like ID004 and ID020, have relatively few. The presence of virulence genes in plasmids is rare, with only isolate ID008 showing plasmid-associated virulence genes. This variability in the distribution and number of virulence genes suggests that different isolates may have varying levels of pathogenic potential, with some strains potentially being more virulent due to a higher number of these genes.

Table 4 7 Distribution of virulence genes in genomic islands.

Strain	**Core chromosome**	**Including virulence factors in GIs**	**In plasmids**
ID003	360	GI#2:8; GI#5:2; GI#6:1; GI#7:7; GI#9:1; **TOTAL:19**	0
ID004	356	GI#2:2; GI#4:2; **TOTAL:4**	0
ID006	359	GI#2:1; GI#4:14; GI#11:1; GI#13:1; GI#16:1; GI#20:3; GI#21:2; **TOTAL:23**	0
ID008	355	GI#1:5; GI#3:8; GI#5:1; GI#9:1; GI#10:2; GI#13:3; **TOTAL:20**	2
ID009	344	GI#2:1; GI#3:3; GI#10:1; GI#11:2; GI#12:3; GI#13:9; GI#16:5; GI#17:3; **TOTAL:27**	0
ID011	363	GI#4:1; GI#5:14; GI#12:1; GI#13:3; GI#14:7; **TOTAL:26**	0
ID012	363	GI#1:7; GI#2:1; GI#8:2; GI#11:1; GI#12:2; **TOTAL:13**	0
ID013	364	GI#4:1; GI#5:14; GI#14:1; GI#15:3; **TOTAL:19**	0
ID014	345	GI#4:1; GI#5:3; GI#9:1; GI#10:1; GI#12:2; GI#13:1; GI#14:10; GI#16:3; GI#17:9; **TOTAL:31**	0
ID015	347	GI#2:9; GI#3:3; GI#4:6; GI#5:1; GI#6:2; GI#9:1; GI#15:5; GI#16:1; **TOTAL:28**	0
ID016	346	GI#4:1; GI#5:3; GI#9:1; GI#10:1; GI#12:2; GI#13:1; GI#14:7; GI#15:2; GI#17:3; GI#18:9; **TOTAL:30**	0
ID017	361	GI#2:1; GI#3:7; GI#12:2; GI#13:2; GI#15:5; GI#16:1; GI#17:1; GI#19:3; **TOTAL:22**	0
ID020	367	GI#3:4; **TOTAL:4**	0

Table 4.7 showing virulence genes in genomic islands (GIs). GI# - refers to the genomic island number.

4.3.5 Phylogenetic relatedness of *Pseudomonas aeruginosa* isolates recovered from surgical wounds

4.3.5.1 Sequence typing

The PubMLST analysis was based on the seven house-keeping genes. Based on 7 housekeeping genes *acsA, aroE, guaA, mutL, nuoD, ppsA* and *trpE.* Ten sequence types were observed **(Table 4.8),** with two isolates ID006 and ID020 returning no ST hits. Sequence typing method used to categorize bacterial isolates based on the sequences of specific genes thus identifying and comparing of different strains. Table 4.8 represents sequence typing data on the sequence types (STs) associated with various the *P. aeruginosa* isolates.

The data reveal that ID001 is identified as ST245, ID003 corresponds to ST645, and ID004 is associated with ST870. ID006 did not have an assigned sequence type,. ID008 is classified as ST16, while ID009 is identified as ST2132. Isolates ID011 and ID013 share the same sequence type, ST244, and isolates ID014, ID015, and ID016 are all classified under ST247. ID012 is associated with ST856, ID017 corresponds to ST2305, and ID018 is identified as ST2021. ID020 also does not have an assigned sequence type, indicated as N/A.

Certain sequence types were shared among multiple isolates, suggesting possible relatedness or common ancestry between these strains. For example, ST244 is shared by isolates ID011 and ID013, while ST247 is shared by isolates ID014, ID015, and ID016. The presence of shared sequence types among different isolates could indicate the spread of specific clones within a population or across different environments. On the other hand, some isolates, like ID006 and ID020, do not have an assigned sequence type, which might be due to incomplete data or the presence of novel or uncommon sequence types that were not identified in the typing process.

The sequence typing data highlight the genetic diversity among the *P. aeruginosa* isolates, with several distinct sequence types represented in the dataset.

Table 4 8 Sequence typing *Pseudomonas aeruginosa* isolates summary.

Isolates	**ST**
ID001	ST245
ID003	ST645
ID004	ST870
ID006	N/A
ID008	ST16
ID009	ST2132
ID011, ID013	ST244
ID014, ID015, ID016	ST247
ID012	ST856
ID017	ST2305
ID018	ST2021
ID020	N/A

From table 4.8, 10 sequence types were identified in the PA isolates, where three isolates and two isolates were grouped into one sequence types each. However, isolate of ID020 remained a novel isolate, with no sequence type hits.

The phylogenetic tree in Figure 4.6 provides a comparative analysis of *P. aeruginosa* isolates based on their sequence types as determined by multilocus sequence typing (MLST). The tree shows that isolates ID014, ID015, and ID016, all identified as ST274, are closely related and form a tight cluster, indicating they share a recent common ancestor and are genetically similar. Isolate ID012 (ST856) is closely related to ID008 (ST16), suggesting a closer genetic relationship between these two isolates despite belonging to different sequence types. Isolate ID017, identified as ST2305, branches off slightly earlier than the ST856 and ST16 cluster, indicating a more distant relationship with these isolates, but still closer compared to other strains in the tree.

Isolate ID003, identified as ST645, forms its own branch, indicating it is genetically distinct from the other isolates in the tree. Isolates ID011 and ID013, both classified as ST244, cluster together, reflecting their genetic similarity and common lineage. Isolate ID009, identified as ST2132, is closely related to the ST244 cluster, suggesting some degree of genetic overlap or shared ancestry despite belonging to a different sequence type. Isolate ID004, identified as ST870, branches off independently, showing a more distinct genetic profile compared to the other isolates. Isolates ID006 and ID020, which do not have an assigned sequence type, are positioned further from the other *P. aeruginosa* isolates. ID020 is closely related to a reference strain of *P. aeruginosa* (NC 008463), while ID006 is more distantly related and clusters closer to *Pseudomonas fluorescens* (NC 004129), which is used as an outgroup in this analysis.

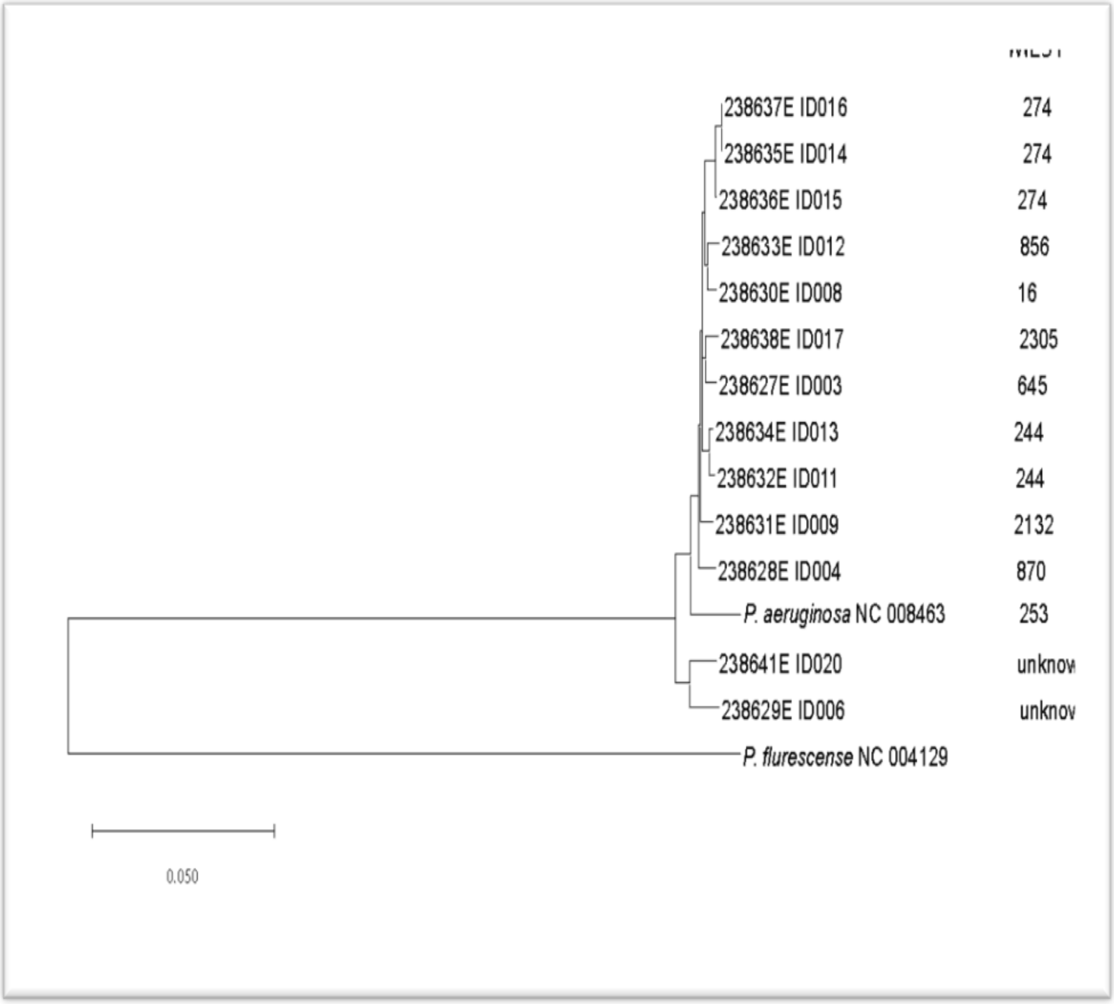


Figure 4 5 Phylogenetic consistency between STs and evolution of the isolates.

The phylogenetic tree reveals the genetic diversity among the Pseudomonas aeruginosa isolates. The clustering of certain isolates by sequence type, such as those belonging to ST274 and ST244, suggests these groups of isolates share a common evolutionary history. In contrast, isolates like ID003 (ST645) and ID004 (ST870) appear to be more genetically distinct from the others. The placement of isolates with unknown sequence types (ID006 and ID020) on separate branches further emphasizes their genetic divergence from the other isolates.

4.3.5.2 Phylogenetic relationships

The phylogenetic tree in Figure 4.7 presents the genetic relationships among various *P. aeruginosa* isolates, alongside comparisons to reference strains, including *P. fluorescens* and *Stenotrophomonas maltophilia*. Isolates ID016, ID014, and ID015 form a very tight cluster, indicating they are genetically very similar. This suggests they may share a recent common ancestor or could be closely related strains with minimal genetic variation. Isolates ID012 and ID008 are also closely related, forming a cluster that suggests a closer genetic relationship between these two isolates compared to others in the tree.

Isolates ID017, ID003, ID013, and ID011 are grouped together, indicating they share some genetic similarities, though not as closely related as the pairs of isolates in the previous clusters. Isolates ID009 and ID004 are placed together, suggesting they are genetically closer to each other compared to the other isolates in the tree but are more distinct compared to the tight clusters observed earlier.

The reference strain *P. aeruginosa* NC 008463 clusters with ID020 and ID006, indicating that these isolates share some genetic features with the reference strain but are more distantly related to the main group of clinical isolates. *P. fluorescens* NC 00412 and *Stenotrophomonas maltophilia* are included as outgroups in this analysis. These species are placed on branches that are significantly more distant from the *P.*

aeruginosa isolates, indicating substantial genetic divergence, highlighting their distinct evolutionary paths compared to *P. aeruginosa*.

The tree shows that most of the *P. aeruginosa* isolates are relatively closely related, clustering together within a few branches. However, there is some genetic diversity among these isolates, as indicated by the different branches they occupy. Isolates ID016, ID014, and ID015 form the most tightly clustered group, suggesting they might be closely related strains or clones. In contrast, isolates like ID020 and ID006 are more distantly related to the other clinical isolates and are closer to the reference strain NC 008463.These two unknown organisms in line with sequence typing (ID020 and ID006) seemed to be genetically related and distinct. The latter two were also related, owing to their similar sets of drug resistant genes.

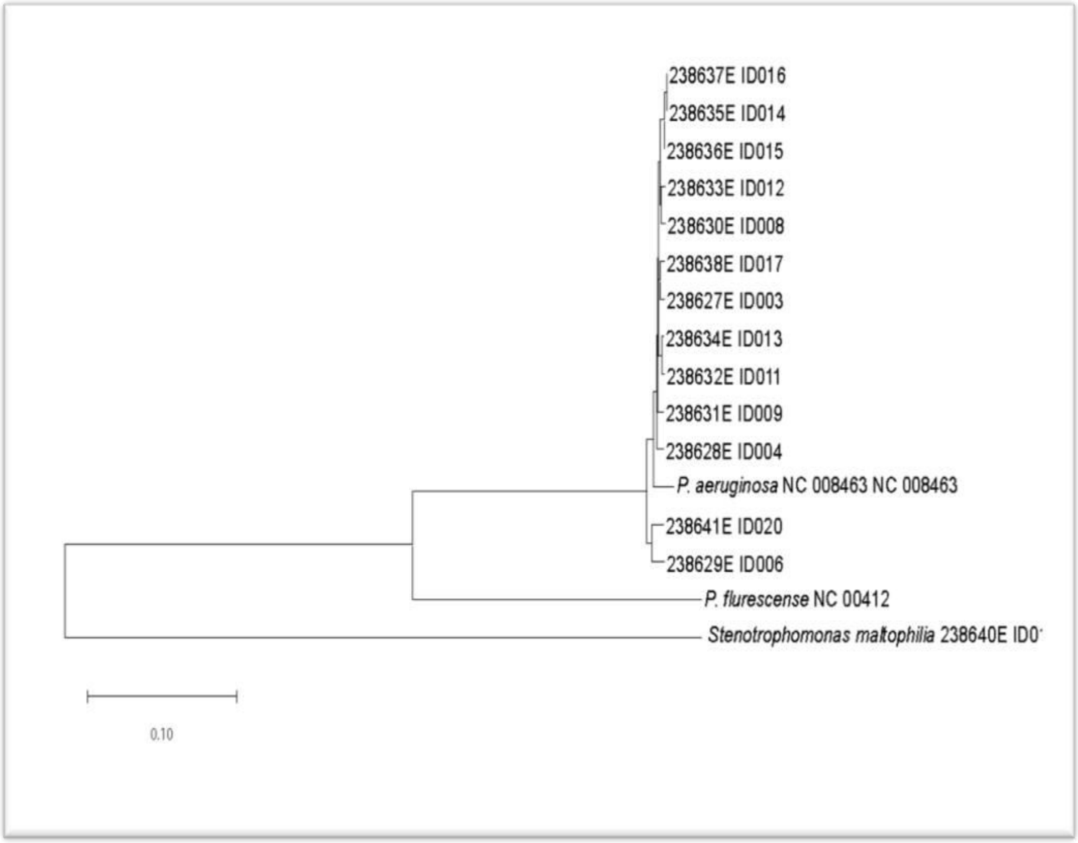


Figure 4 6 Genetic relatedness among the study isolates.

4.4 Physiological traits of *Pseudomonas aeruginosa* isolates from surgical sites

P. aeruginosa quorum sensing (QS)--activated virulence factors which included proteases, pigment, swarming, and motility were determined. Biofilm formation, which is QS-controlled activity in *P. aeruginosa* was also detected.

4.4.1 Protease secretion in *P. aeruginosa isolates*

The proteolytic halo around the control bacterial culture, PA14 generally was larger as compared to PA01 suggesting that PA14 produced more protease than PA01. It is important to note that across all the plates the pattern of halo formation was uniform **(Figure 4.8).** There was a lot of variation between tested isolates. ID001, ID018 and ID020 had a similar colonial characteristic of spread on the milk agar plate which was typical to the control bacterial culture, PA01 while ID008's colonial characteristic of spread resembled the control bacteria PA14. ID004 had the largest spread on the milk agar plate as compared to all tested bacteria. The halos around isolates ID001, ID004, ID009, ID012, ID014, ID015, ID016, ID017, and ID020 were like the ones formed by PA01, those around ID008, ID018 were similar to those formed by PA14. ID003 ID006, ID011, and ID013 had the least protease production as compared to all isolates. ID019 had a unique growth characteristic from all other isolates with the smallest growth.

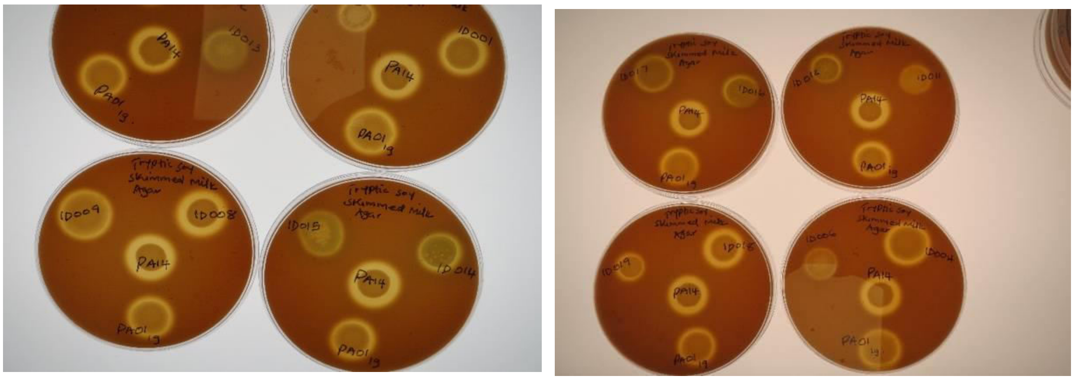


Figure 4 7 Plates showing caseinase activity through a proteolytic halo around the bacterial culture.

4.4.2 Motility and swarming in *P. aeruginosa isolates*

In PA motility analysis, the motility pattern around the control bacterial stabs was uniform in all the plates **(figure 4.9).** All stabbed isolates were motile and none of the growth remained confined to the stab line. However, there were differences in their swarming patterns as compared to the controls (wild type, PA14 and PA01) and variations in the abundance of secreted proteins across the different isolates.

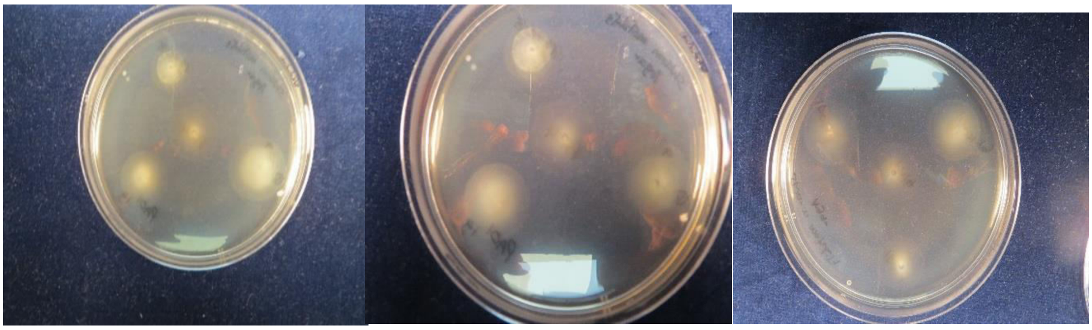


Figure 4 8 The motility pattern around the study isolates.

4.4.3 Exoprotein secretion in *P. aeruginosa* isolates

The Western blot image presented in Figure 4.10 focuses on the detection and characterization of secreted virulence factors in *P. aeruginosa* isolates. The pathogenicity of *P. aeruginosa* is largely attributed to its ability to produce a diverse array of virulence factors that facilitate infection, immune evasion, and tissue damage in the host. This analysis provides valuable insights into the expression and regulation of these factors.

In the Western blot, protein bands are observed between the molecular weights of approximately 25 kDa and 75 kDa, consistent with the known sizes of several P. aeruginosa virulence factors. The red arrows highlight specific bands detected in the isolates, which correspond to secreted proteins within this molecular weight range.

The presence of these bands indicates that these proteins are being expressed and secreted by the bacterial isolates.

A key virulence factor, Toxin A, is specifically mentioned. Toxin A is initially synthesized as a 71 kDa precursor protein, which includes a prokaryotic leader peptide (LP) that facilitates its secretion. Upon secretion, the leader peptide is typically cleaved, resulting in the mature form of Toxin A, which is approximately 68 kDa. In the Western blot, bands around the 68-71 kDa range likely correspond to this mature Toxin A protein, confirming its expression in some of the *P. aeruginosa* isolates.

The presence and intensity of these bands across different isolates suggest variability in the expression levels of Toxin A and potentially other virulence factors. For instance, stronger bands may indicate higher levels of Toxin A production, while weaker or absent bands could reflect lower expression or differences in regulation.

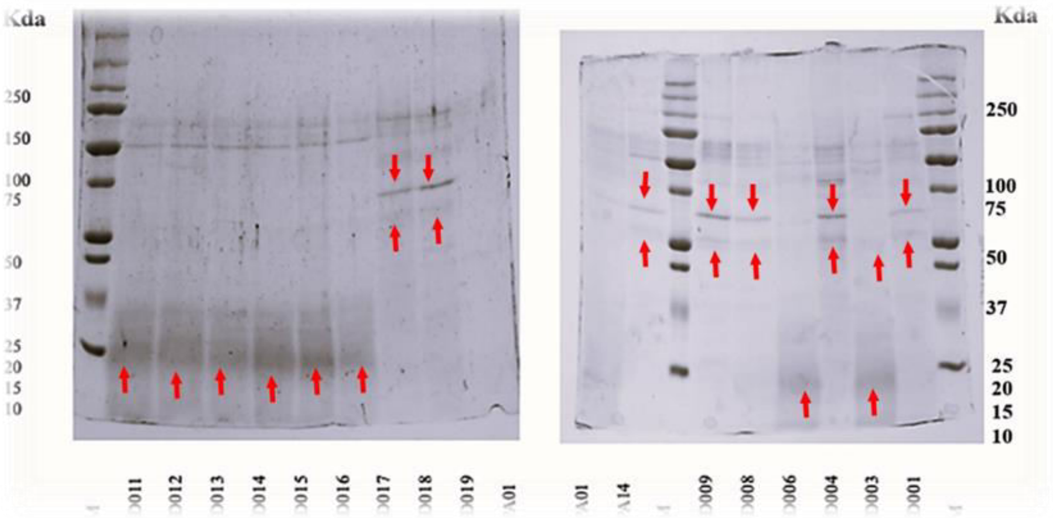


Figure 4 9 Secreted proteins of *P. aeruginosa* isolates separated by SDS-PAGE

4.4.4 Auxotrophy in *Pseudomonas aeruginosa*

The auxotrophic growth characteristics of the wild-type strains were uniform in all the plates supplemented with glucose only and in plates supplemented with glucose + amino acids **(Figure 13).** Among the tested isolates, 87.5% (14) had varied growth characteristics with basically smaller colonies as compared to the wild types and as compared to their growth in PIA. All the *P. aeruginosa* isolates seemed to be prototrophic but completely repressed the ability to swarm over semi-solid surfaces of the media indicated by the formation of tiny colonies. 5% (1) isolate ID017 produced a green pigment in plates supplemented with glucose only which was not observed in plates supplemented with glucose + amino acids and in the wild-type strains. 5%. ID009 produced shinny colonies in plates supplemented with glucose + amino acids which was a characteristic lacking in plates supplemented with glucose only.

Among the tested isolates, 27.7% (5) of isolates of *P. aeruginosa* (ID012, ID013, ID014, ID015 and ID016) produced a green pigment (pyocyanin) on the media as shown in **Figure 4.11.** 5% (1) isolate ID006 produced a brown pigment (pyorubin). The two reference strains PA14 and PA01(ig) did not seem to produce any pigment. Other growth characteristics on PIA included the appearance of dry shiny colonies which was observed in 38.8% (7) ID003, ID006, ID012, ID013, ID014, ID015 and ID016 of the tested organisms and was different from the reference strains. In most of the isolates 50% (9) produced colonies that were morphologically identical to the reference strains, with 5% (1) ID019 having a unique characteristic of smaller colonies and being rather more confined to the center as compared to the reference strains.

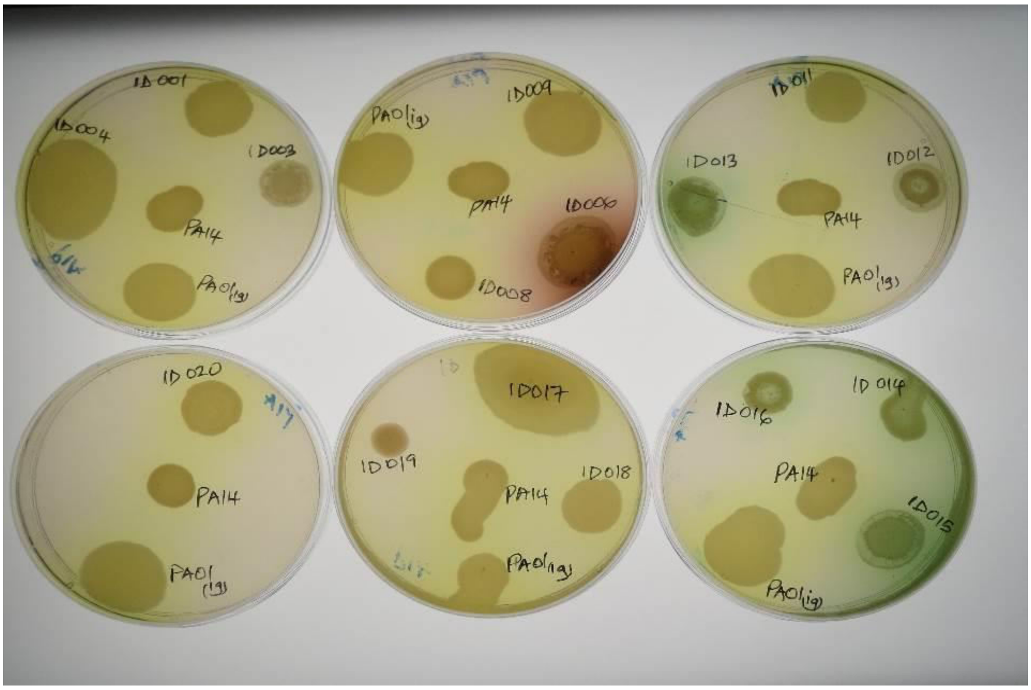


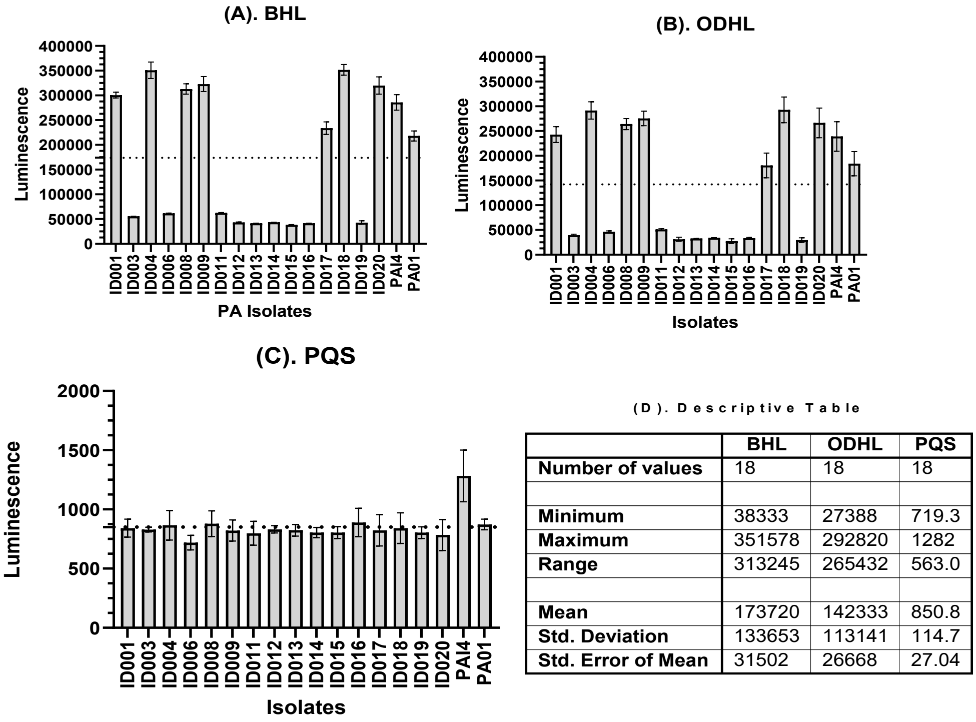
Figure 4 10 *P. aeruginosa* growth on PIA showing production of green pigment(pyocyanin) and brown pigment (pyorubin)

4.4.5 Quorum sensing in *P. aeruginosa* isolates

Figure 4.12 presents luminescence data from biosensors measuring the levels of three major quorum sensing (QS) signaling molecules in *P. aeruginosa* isolates: N-butanoyl-L-homoserine lactone (BHL), N-(3-oxododecanoyl)-L-homoserine lactone (OdDHL), and Pseudomonas quinolone signal (PQS). The data shows considerable variation in BHL levels among the isolates. Isolates ID001, ID004, ID008, ID009, ID017, ID018, and ID020 exhibit the highest luminescence readings, indicating elevated BHL levels compared to other isolates. This suggests that these isolates have more active BHL-mediated quorum sensing, which may influence their pathogenic behavior, such as virulence factor production and biofilm formation. The remaining isolates have relatively lower BHL levels, with some showing minimal luminescence, indicating lower activity of BHL-mediated QS in these strains.

The OdDHL luminescence data also show variability across the isolates. Isolates ID001, ID004, ID008, ID009, ID017, and ID018 display the highest luminescence levels, which correspond to elevated OdDHL signaling activity. These isolates likely have enhanced QS signaling via OdDHL, potentially leading to increased virulence and biofilm formation. Other isolates exhibit lower OdDHL levels, indicating less active QS signaling through this molecule. The luminescence readings for PQS show much less variability among the isolates compared to BHL and OdDHL. The levels of PQS are relatively consistent across all isolates, indicating that PQS-mediated quorum sensing is more uniformly active in these P. aeruginosa strains. This uniformity suggests that PQS signaling might play a more consistent role across different strains in regulating gene expression related to QS, regardless of the variations in BHL and OdDHL levels.

The descriptive statistics provided in the table summarize the luminescence data, showing that BHL and OdDHL exhibit significant variability among the isolates, with high standard deviations reflecting this variation. In contrast, PQS has a much lower mean luminescence and a smaller standard deviation, highlighting its uniform expression across the isolates. Overall, the results reveal significant variability in BHL and OdDHL quorum sensing activity among the *P. aeruginosa* isolates, with certain isolates showing particularly high levels. This suggests that these isolates may have enhanced QS signaling, which could contribute to increased virulence and biofilm formation. In contrast, PQS levels are relatively consistent across all isolates, indicating more uniform regulation of PQS-mediated quorum sensing among the strains. The differences in QS molecule levels highlight the diversity in quorum sensing activity within P. aeruginosa populations, which may influence their pathogenic behavior and response to environmental conditions.



	BHL	ODHL	PQS
Number of values	18	18	18
Minimum	38333	27388	719.3
Maximum	351578	292820	1282
Range	313245	265432	563.0
Mean	173720	142333	850.8
Std. Deviation	133653	113141	114.7
Std. Error of Mean	31502	26668	27.04

Figure 4 11 showing luminescence data from biosensors measuring the levels of three major quorum sensing (QS) signaling molecules in *P. aeruginosa* isolates. BHL; N-butanoyl-L-homoserine lactone, OdDHL; N-(3-oxododecanoyl)-L-homoserine lactone, PQS; Pseudomonas quinolone signal.

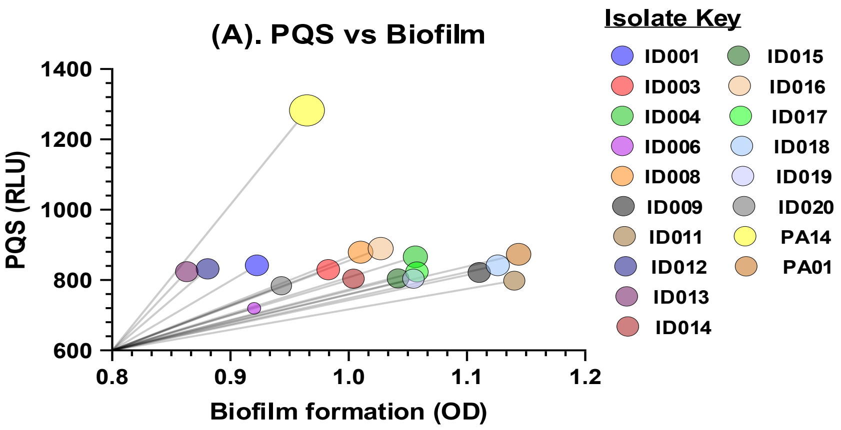
4.4.6 Clustering patterns of *Pseudomonas aeruginosa*

Figure 4.13 provides Principal Component Analysis (PCA) plots that illustrate the clustering patterns of *P. aeruginosa* isolates (ID001 to ID020) and wild-type strains (PA01 and PA14) concerning the production of quorum sensing (QS) molecules and biofilm formation. The relationship between the PQS (production and biofilm formation are shown, with isolates and wild-type strains distributed across the plot, indicating varying levels of PQS and biofilm formation. Some isolates cluster together, suggesting similar PQS production and biofilm formation characteristics.

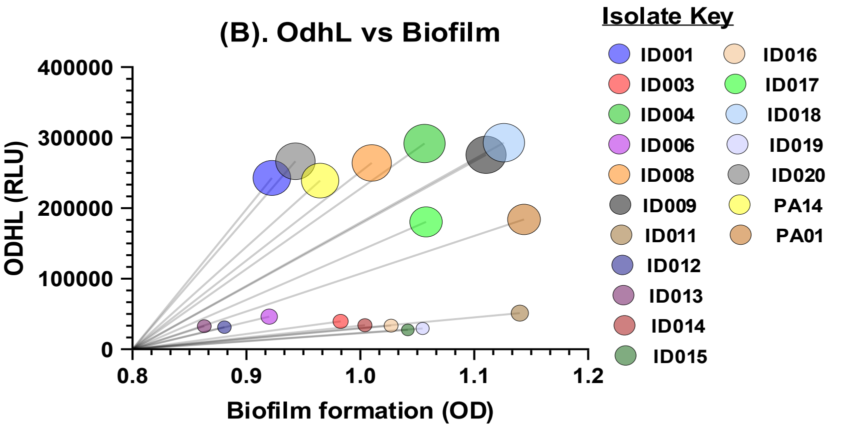
The relationship between the production of OdDHL (and biofilm formation is presented next, where the clustering pattern reveals that certain isolates share similar characteristics regarding OdDHL production and biofilm formation capacity. The relationship between BHL production and biofilm formation is also depicted, with the clustering reflecting a correlation between these two variables. Some isolates and wild-type strains show high levels of both BHL production and biofilm formation.

The data reveals the relationship between OdDHL and PQS production. The distribution of isolates and wild-type strains across the plot, with some clustering together, suggests similar production levels of these two QS molecules. The relationship between OdDHL and BHL production is highlighted, with the clustering pattern indicating that certain isolates produce similar levels of both OdDHL and BHL, potentially pointing to co-regulation or coordinated production of these QS molecules.

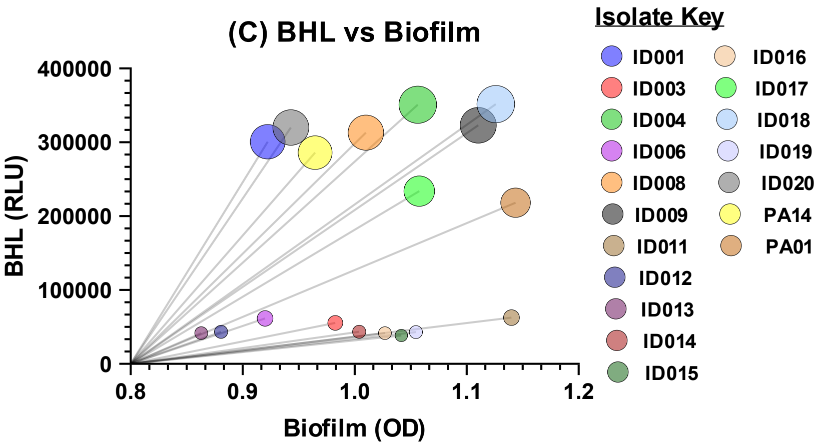
Also, the relationship between BHL and PQS production is shown, where the clustering of isolates suggests that some have similar levels of BHL and PQS production. Overall, *P. aeruginosa* isolates and wild-type strains cluster based on their production of QS molecules (PQS, OdDHL, BHL) and their biofilm formation capabilities. The clustering patterns observed in these PCA plots suggest that specific groups of isolates may share similar quorum sensing and biofilm formation characteristics, potentially indicating underlying genetic or regulatory similarities.



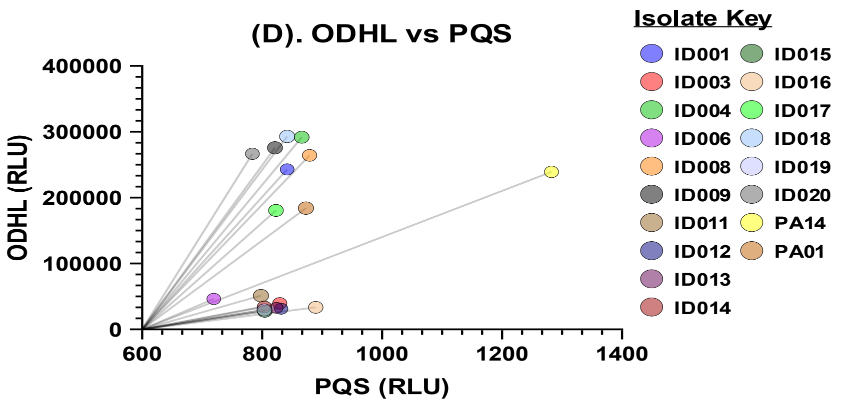
(A). PQS vs Biofilm



(B). OdhL vs Biofilm



(C) BHL vs Biofilm



(D). ODHL vs PQS

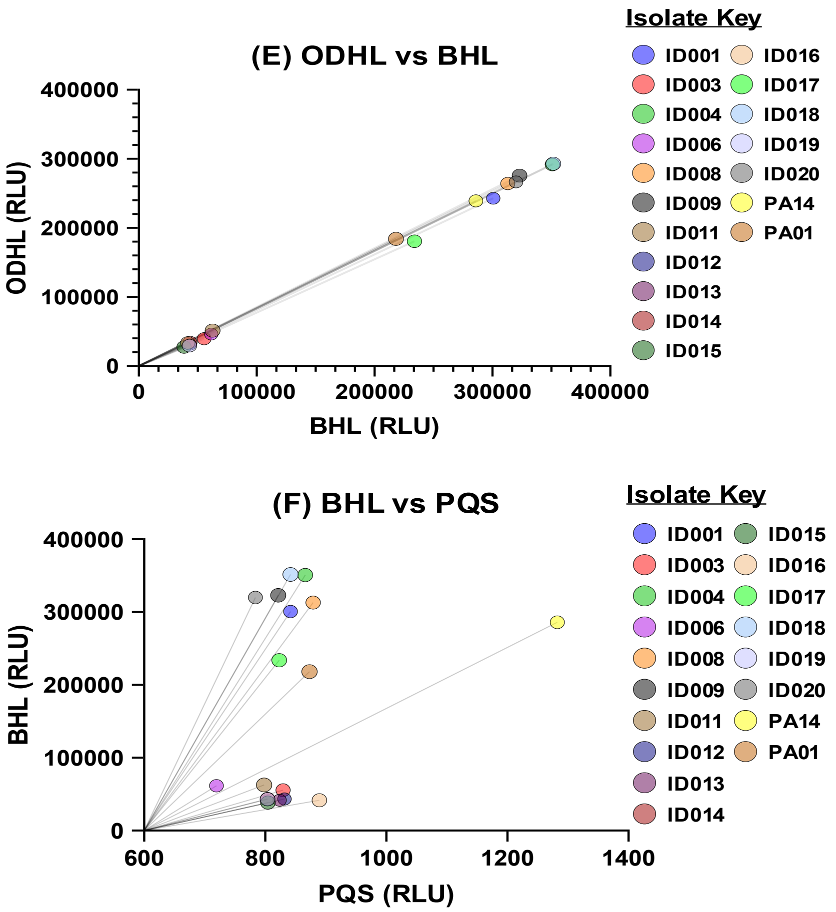


Figure 4 12 Principal component analysis plots showing the clustering patterns of *P. aeruginosa* isolates and wild-type strains.

CHAPTER FIVE

DISCUSSION

5.1 Prevalence of *Pseudomonas aeruginosa* among bacterial isolates from surgical sites

In accordance with other studies (Hafiz *et al*., 2023; Yang *et al*., 2023), this study identified *P. aeruginosa* as the most prevalent bacterium isolated from surgical sites, comprising 38.9% of the isolates. This finding is particularly relevant in hospital contexts with elevated levels of multidrug-resistant organisms. *P. aeruginosa* has been identified as a prevalent pathogen in SSIs, particularly in patients with compromised immune systems or those in intensive care units (Dong *et al*., 2024; Qin *et al*., 2022; Wood *et al*., 2023). However, certain studies, notably those conducted in low-resource settings, report lower prevalence rates of *P. aeruginosa*, frequently identifying *Staphylococcus aureus* or *Escherichia coli* as the more prevalent pathogens in SSIs (Ali *et al*., 2023; Dinda *et al*., 2013). This discrepancy may be attributable to variations in the patient populations studied, antibiotic utilisation patterns, or infection control practices.

This study demonstrates that males are considerably more likely to develop SSIs than females in terms of gender distribution ($X^2 = 10.125$, $P = 0.002$). This discovery is corroborated by numerous studies, which indicate that males may have a higher incidence of SSIs as a result of factors such as increased body hair, which can increase the likelihood of wound contamination (Lyu *et al*., 2023; Vlassoff, 2007). Nevertheless, other studies have not observed any substantial gender disparities in SSI rates, indicating that gender may not be a reliable predictor across various surgical types and populations (Cohen *et al*., 2013; Langelotz *et al*., 2014). The study does not identify any significant differences in age distribution (*P* = 0.998), suggesting that SSIs are distributed relatively evenly across various age groups. This is in contrast to certain studies that have identified age as a significant risk factor for SSIs, with aged patients often having higher SSI rates due to comorbidities and decreased immune function (Liou *et al*., 2022; Meng *et al*., 2024). Nevertheless, the relationship between age and SSI rates is not statistically significant in other studies, particularly when control is made for other characteristics such as the type of surgery or the patient's overall health (Bischoff *et al*., 2023; Bucataru *et al*., 2023). The distribution of wards in this study indicates that the female surgical ward (35.9%) and the male surgical ward (20.3%) are the most prevalent in terms of SSIs. This is consistent with research that indicates that surgical facilities that perform more complex or invasive procedures have higher rates of SSI. However, other studies may indicate that specific wards are more susceptible to the problem, contingent upon the hospital's specialisation. For instance, orthopaedic wards may experience elevated SSI rates as a result of the character of orthopaedic surgeries (Mohan *et al*., 2023; Mukagendaneza *et al*., 2019). The reproductive system is the most frequently reported site for SSIs (39.1%), followed by the abdomen (25.0%) and extremities (23.4%), according to this study. This is consistent with the results of previous research, which has consistently shown that abdominal and gynaecological interventions are frequently linked to increased SSI risks as a result of the inherent contamination risk associated with these procedures (Bucataru *et al*., 2023; Kulkarni & Kothari, 2023). In contrast, certain studies may emphasise distinct sites based on the population under investigation. For instance, orthopaedic surgeries are more prevalent in trauma centres, where limb surgeries are more frequent (Hou *et al*., 2022; Skender *et al*., 2022). The absence of significant differences in the microbial burden between males and females (P = 0.942) in this study is consistent with the results of other research, which frequently demonstrate that there is no significant gender difference in the type or burden of microbial pathogens isolated from SSIs (Langelotz *et al*., 2014; Mukagendaneza *et al*., 2019). This implies that, despite the fact that males may have a higher overall risk of SSIs, the varieties of bacteria responsible for these infections are comparable between the two sexes. However, the results indicate that the type of bacterial pathogens isolated and their distribution across various patient groups are consistent with those reported in the broader literature, despite the fact that certain demographic factors, such as gender, may influence the likelihood of developing SSIs. The significance of effective infection control measures, particularly in surgical wards, to prevent and manage these infections is underscored by the significant presence of *P. aeruginosa*. Additionally, the absence of age-related disparities in SSI rates may suggest that hospital protocols, patient care, and surgical techniques are equally effective across various age groups, potentially mitigate age-related vulnerabilities. These findings underscore the necessity of customised infection prevention strategies and ongoing surveillance that account for the unique risks associated with various patient demographics and surgical procedures.

5.2 Antibiotic resistance patterns of *P. aeruginosa* isolates to key classes of antibiotics

Global trends in multidrug-resistant *P. aeruginosa* are consistent with the antibiotic resistance patterns of *P. aeruginosa* isolates from surgical sites, which demonstrate a substantial degree of resistance across several important classes of antibiotics. Ceftazidime, a cephalosporin, exhibited a resistance rate of 60.7%, with only 21.4%

of isolates being sensitive. This high resistance is in accordance with studies that have reported significant resistance to cephalosporins, particularly in hospital settings (Nasser et al., 2020; Ramsay et al., 2023). This resistance is frequently ascribed to the production of beta-lactamases, which degrade the beta-lactam ring, rendering the antibiotic ineffective (Narendrakumar et al., 2023; Pandey & Cascella, 2024).

Piperacillin/Tazobactam exhibited a more balanced profile in terms of penicillin sensitivity, with a 39.3% sensitivity, whereas Piperacillin alone exhibited a significant resistance rate of 67.9%. These findings are consistent with the fact that, despite the fact that beta-lactam/beta-lactamase inhibitor combinations such as Piperacillin/Tazobactam can maintain some effectiveness, there is an increasing resistance to Piperacillin alone (Duske et al., 2023; Perry & Markham, 1999). This resistance is frequently associated with the overexpression of efflux pumps or mutations in porin channels, which inhibit the drug's penetration into the bacterial cell (Fernández & Hancock, 2012). Amikacin exhibited a resistance rate of 71.4% in the aminoglycosides class, with no isolates exhibiting sensitivity. Tobramycin demonstrated a marginally higher sensitivity of 35.7%, but it maintained a resistance rate of 64.3%. These results agree with reports that have shown high levels of resistance to aminoglycosides in P. aeruginosa, frequently as a result of enzymatic modification of the drugs, which renders them inactive (Khoshnood *et al*., 2023; Krause *et al*., 2016). The resistance observed in Amikacin is of particular concern, as this antibiotic is frequently employed as a last resort to treat severe infections caused by multidrug-resistant organisms.

Ciprofloxacin, a fluoroquinolone, demonstrated a resistance rate of 60.7% and a sensitivity of only 17.9%. *P. aeruginosa* develops resistance to Ciprofloxacin through mutations in target enzymes and efflux pumps that expel the antibiotic from the cell, a common trend worldwide (Shariati *et al*., 2022). The extensive use of fluoroquinolones in both hospital and community contexts is underscored by the high resistance rate observed in this study, which is indicative of global trends. Meropenem, a carbapenem, exhibited the maximum sensitivity rate at 53.6%, with a resistance rate of 17.9%. Carbapenems are frequently considered the final line of defence against multidrug-resistant *P. aeruginosa* (Buehrle *et al*., 2016). The relatively reduced resistance observed in this study is encouraging, but it is not uncommon. Conversely, there is an increasing apprehension regarding the proliferation of carbapenem-resistant strains and the production of carbapenemases, which are contributing to the rise in carbapenem resistance.

The resistance gene profiles of the *P. aeruginosa* isolates demonstrate a diverse and extensive resistance to a variety of antibiotic classes. The intricate nature of resistance mechanisms in these bacteria is emphasised by the abundance of resistance genes for carbapenems, aminoglycosides, and fluoroquinolones. Similar discoveries have been documented in *P. aeruginosa*, where multiple resistance genes are frequently present, simultaneously conferring resistance to multiple antibiotics (Michalska *et al*., 2015; Muteeb *et al*., 2023). This polyresistance is particularly problematic because it restricts the number of treatment options and increases the probability of treatment failure. The study also brings to light a concerning trend: a substantial number of resistance genes against antiseptics and disinfectants. Persistent environmental contamination in healthcare environments may result from this resistance. The capacity of *P. aeruginosa* to resist disinfection efforts further complicates infection control and contributes to the spread of resistant strains.

The antibiotic resistance patterns observed in this study are in accordance with the global trend of progressive multidrug resistance in *P. aeruginosa*. The imperative necessity for alternative treatment strategies and the development of new antibiotics is emphasised by the high resistance rates, particularly to commonly used antibiotics such as Ceftazidime, Ciprofloxacin, and Amikacin (Kariuki *et al*., 2022; Morales-Durán *et al*., 2024). The relatively higher sensitivity to Meropenem implies that carbapenems may still be effective in treating infections caused by these isolates. However, the presence of carbapenem resistance genes serves as a cautionary tale that this efficacy may not be sustained. The adaptive nature of *P. aeruginosa* and its capacity to persist in a variety of environments, including those with consistent disinfection practices, are underscored by the broad resistance gene profiles. This underscores the necessity of rigorous infection control measures, consistent resistance pattern surveillance, and the prudent application of antibiotics to prevent the further escalation of resistance.

These results contribute to the expanding body of evidence regarding the multidrug-resistant nature of *P. aeruginosa* and underscore the necessity of comprehensive strategies for managing infections caused by this formidable pathogen. The diminished efficacy of these commonly used drugs is underscored by the high resistance rates observed in antibiotics such as Ceftazidime, Ciprofloxacin, and Amikacin. Consequently, the use of last-resort antibiotics, such as carbapenems, is necessary. Nevertheless, the presence of carbapenem resistance genes in certain isolates indicates that even these potent medicines may not be effective for an extended period of time. This could result in a situation in which there are no effective antibiotics available to treat these infections.

5.3 Genotypic diversity in *P. aeruginosa* isolates obtained from surgical sites

The significant genetic variation among the *P. aeruginosa* isolates obtained from surgical sites in this study is consistent with the findings of other research on *P. aeruginosa* in various clinical settings. The genotypic diversity of *P. aeruginosa* isolates is illustrative of this. This diversity has significant implications for the comprehension of the adaptability, virulence, and resistance patterns of these bacteria.

Sequence typing was employed in this investigation to identify numerous sequence types (STs) among the isolates. Some STs were shared among various isolates, suggesting the possibility of clonal propagation or a shared ancestry. For instance, ST247 was shared by isolates ID014, ID015, and ID016, indicating that these isolates may have originated from a common source or have undergone limited genetic divergence. This discovery is consistent with other investigations that have documented the clonal dissemination of particular *P. aeruginosa* strains in hospital settings, particularly during epidemics of multidrug-resistant strains (Chichón *et al*., 2023; Silveira *et al*., 2020).

Conversely, certain isolates, including ID006 and ID020, lacked an ascribed sequence type, suggesting the existence of unusual or novel sequence types that had not been previously documented. This emphasises the genetic heterogeneity of *P.*

aeruginosa populations, a phenomenon that is frequently observed in studies that identify novel or rare STs, further emphasising the pathogen's adaptability. For instance, research has also documented a diverse array of STs in *P. aeruginosa*, underscoring its genetic diversity and the potential for continuous evolution in various environments (Elfadadny *et al*., 2024; Winstanley *et al*., 2016).

Various degrees of genetic relatedness among the isolates are indicated by the phylogenetic analysis conducted in this study, which demonstrates clustering of isolates based on their sequence types. A recent common ancestor or minimal genetic variation among these strains is suggested by the close clustering of isolates ID014, ID015, and ID016, which could be indicative of a clonal outbreak. This pattern is frequently observed in research that concentrates on *P. aeruginosa* strains associated with hospitals, where specific clones are accountable for persistent infections or outbreaks (Balaban *et al*., 2019).

Conversely, the placement of isolates such as ID003 (ST645) and ID004 (ST870) on more distinct branches of the phylogenetic tree implies that these isolates are genetically distinct from others, potentially reflecting divergent evolutionary trajectories or sources of infection. This discovery is in accordance with research that has also identified substantial genetic diversity among clinical *P. aeruginosa* isolates, with certain strains being more genetically distinct and presumably originating from distinct sources (Kiyaga *et al*., 2022; Klockgether *et al*., 2011).

The study also reveals a diverse distribution of virulence genes across genomic islands and plasmids among the isolates, with some isolates containing a high number of virulence genes, indicating a higher pathogenic potential. It is probable that isolates such as ID014 and ID016, which possess a substantial number of virulence genes, are more virulent, which could potentially contribute to their ability to establish infections in surgical sites. This observation is corroborated by research indicating that *P. aeruginosa* isolates with a higher number of virulence genes are frequently linked to more severe infections and worse clinical outcomes (Shanks *et al.*, 2023).

The adaptability of *P. aeruginosa* in surviving antibiotic treatment is evident in the variability of resistance genes, particularly the extensive presence of efflux system genes. This is in accordance with the recognition of efflux pumps as a significant mechanism of resistance in *P. aeruginosa*, which contributes to its capacity to resist multiple antibiotic classes (Lorusso *et al.*, 2022; Pang *et al.*, 2019).

These results are consistent with those of other investigations that have investigated the genetic diversity of *P. aeruginosa* in various environments. Significant genetic diversity has also been reported in research on clinical *P. aeruginosa* isolates, with the identification of multiple STs and a wide variety of virulence and resistance genes (Subedi *et al.*, 2018; Kiyaga *et al.*, 2022). In the same vein, research conducted in Europe on MDR *P. aeruginosa* has revealed a high level of genetic diversity and the presence of a variety of virulence and resistance genes. This indicates that *P. aeruginosa* strains are capable of rapidly evolving in response to environmental pressures and are highly adaptable (Elfadadny *et al.*, 2024; Pachori *et al.*, 2019; Pang *et al.*, 2019).

The complex and adaptable nature of *P. aeruginosa*, as reported in other research, is consistent with the genotypic diversity observed in this study. The unique phylogenetic relationships among the isolates, the variability in virulence and resistance genes, and the presence of multiple sequence types all emphasise the difficulties associated with managing infections caused by this pathogen. The findings underscore the necessity of continuous surveillance and a more comprehensive comprehension of the genetic mechanisms that underlie *P. aeruginosa* virulence and resistance in order to create more effective strategies for infection control and treatment.

5.4 Physiological traits of *P. aeruginosa* isolates from surgical sites

The physiological characteristics of *P. aeruginosa* isolates from surgical sites, as demonstrated in this study, underscore critical components of the bacterium's virulence, motility, biofilm formation, and quorum sensing (QS) activity. The bacterium's capacity to cause infection, evade the host immune system, and resist antibiotic treatment is contingent upon these characteristics.

The protease secretion variation among the *P. aeruginosa* isolates, particularly the elevated protease production of certain strains such as ID004, is consistent with other research findings (Aqel *et al.*, 2023; Kamal *et al.*, 2019). Proteases, notably elastase, are essential virulence factors that facilitate immune evasion and tissue destruction. Studies have demonstrated that isolates with elevated protease activity are frequently linked to more severe infections (Sendra *et al.*, 2024). The protease production differences among the isolates in this study, with some resembling the high protease-producing control PA14, indicate that these isolates may have increased virulence, a phenomenon that has been observed in clinical strains associated with chronic and acute infections.

Additionally, the isolates exhibit variability in their motility and swarming behaviour, as all exhibit motility but differ in their swarming patterns and the prevalence of secreted proteins. These results agree with other research that has identified motility and swarming as critical factors in the pathogenesis of *P. aeruginosa* (Murray *et al*., 2010). Biofilm formation and the secretion of virulence factors are frequently linked to swarming motility, which enhances the bacterium's capacity to colonise and persist in host tissues. The variations observed in these isolates indicate that they have varying capacities for infection dissemination and colonisation, which could potentially affect the severity of the infections they cause.

The Western blot analysis of exoprotein secretion demonstrated that the expression of virulence factors, including Toxin A, varied among the isolates. This variability is crucial, as Toxin A is a virulence factor that is widely recognised for its ability to cause tissue harm and evade the immune system. Studies have similarly reported variations in the expression of these virulence factors among clinical isolates, suggesting that the pathogenic potential of *P. aeruginosa* can fluctuate considerably depending on the strain (Bogiel *et al*., 2023; Liao *et al*., 2022). The presence of significant bands for Toxin A in certain isolates indicates that these strains may be more virulent and capable of causing more severe infections.

The diversity in physiological traits is further exemplified by the auxotrophic growth characteristics and pigment production of the isolates. Virulence is linked to the production of carotenoids such as pyocyanin and pyorubin, which are involved in immune modulation and oxidative stress resistance. The discovery that 27.7% of isolates produced green pigment (pyocyanin) is consistent with other research that establishes a correlation between pyocyanin production and heightened virulence and biofilm formation. This variability in pigment production among these isolates suggests that they have varying capacities to endure hostile environments and resist host defences, which could potentially impact the course of infections.

The levels of QS signalling molecules, including BHL, OdDHL, and PQS, revealed substantial variability in the analysis of QS in these isolates. This variation in QS activity is essential, as QS regulates the expression of numerous virulence factors and the formation of biofilms in *P. aeruginosa*. Variations in pathogenicity and antibiotic resistance have been observed in other studies, which have also reported differences in QS activity among clinical isolates. The isolates in this study with elevated QS activity may have a greater capacity for coordinated virulence, which could make them more challenging to treat and control. The relatively consistent levels of PQS across isolates, in contrast to the variability in BHL and OdDHL, indicate that PQS signalling may be more consistent in the regulation of virulence across different strains. Conversely, BHL and OdDHL signalling may be more influenced by specific environmental or genetic factors.

The clustering patterns that are observed in the principal component analysis of QS molecules and biofilm formation offer additional insights into the relationships between these physiological characteristics. Certain isolates may be clustered based on their similar biofilm formation and QS profiles, which implies that they may share underlying genetic or regulatory mechanisms that increase their adaptability and virulence (Sharma *et al*., 2023; Sionov & Steinberg, 2022). This discovery is in accordance with research that has identified specific clonal lineages of *P. aeruginosa* that are related to increased virulence and antibiotic resistance, particularly in hospital environments. Protease secretion, motility, exoprotein production, auxotrophy, pigment production, and quorum sensing activity are all physiological properties that are consistent with the established virulence mechanisms of *P. aeruginosa*. The genetic and phenotypic diversity of *P. aeruginosa* is emphasised by the variability in these traits among the isolates, which is a contributing factor to its capacity to cause a diverse array of infections and resist treatment.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

P. aeruginosa was the most frequently isolated bacterium from surgical locations, comprising 38.9% of the isolates, according to the study. This high prevalence emphasises the importance of *P. aeruginosa* as a significant pathogen in surgical site infections. The findings indicate that *P. aeruginosa* is a critical factor in the aetiology of surgical site infections, necessitating that it be prioritised in infection control and treatment strategies in clinical environments.

The *P. aeruginosa* isolates exhibited a substantial degree of multidrug resistance, as indicated by the study. High resistance rates were observed for commonly used antibiotics, such as Amikacin (71.4%), Ciprofloxacin (60.7%), and Ceftazidime (60.7%). Meropenem demonstrated the greatest sensitivity rate at 53.6%; however, resistance was observed in certain isolates. These results suggest that *P. aeruginosa* from surgical sites are highly resistant to multiple antibiotics, which presents a challenge for effective treatment and emphasises the necessity of alternative therapeutic strategies and rigorous antibiotic stewardship.

The study revealed a significant amount of genotypic diversity among the *P. aeruginosa* isolates, with the identification of numerous sequence types, including some that were novel or uncommon. The phylogenetic analysis revealed distinct clustering patterns among the isolates, which implies that certain strains have a clonal distribution and that there is significant genetic variability. This genotypic diversity suggests that *P. aeruginosa* in surgical sites is highly adaptable, with each strain having the potential to contribute to various infection outcomes and resistance profiles. The significance of continuous surveillance to monitor the spread and evolution of *P. aeruginosa* strains in clinical environments is underscored by the results.

The investigation determined that *P. aeruginosa* isolates exhibited a variety of physiological characteristics, such as varying levels of motility, exoprotein production, auxotrophy, pigment production, and quorum sensing activity. The pathogen's virulence and capacity to induce persistent infections are significantly influenced by these characteristics. The variability observed among isolates implies that the severity of infections may be influenced by the pathogenic potential of individual strains. The complexity of *P. aeruginosa* as a pathogen and the necessity of targeted interventions to mitigate its impact on patient outcomes are underscored by the presence of highly virulent strains with enhanced quorum sensing activity and biofilm formation capacity.

6.2 Recommendations

To combat the high prevalence of *P. aeruginosa* in surgical site infections, hospitals and healthcare facilities should improve their infection control protocols, particularly in surgical wards. This encompasses the strict observance of hand hygiene practices by healthcare personnel, regular environmental cleaning, and rigorous sterilisation procedures. The implementation of these measures will contribute to the reduction of the incidence and dissemination of *P. aeruginosa* in clinical environments.

Healthcare facilities should prioritise antibiotic stewardship programs in order to address the substantial multidrug resistance observed among *P. aeruginosa* isolates. Culture and sensitivity testing should be employed to guide the judicious use of antibiotics in these programs, thereby reducing the development of resistance. In order to guarantee the ongoing efficacy of available remedies, it is imperative to conduct routine monitoring and adjustments to antibiotic prescribing practices.

The necessity of continuous surveillance and genetic monitoring of strains in clinical environments is underscored by the significant genotypic diversity of *P. aeruginosa* isolates. Routine genomic sequencing and molecular typing of *P. aeruginosa* isolates should be implemented by hospitals to monitor the prevalence of specific strains, identify emergent resistance patterns, and inform targeted infection control strategies. This will facilitate the more effective identification and response to prospective outbreaks.

In light of the diverse physiological traits and virulence factors of *P. aeruginosa* isolates, research and development efforts should concentrate on the development of targeted therapeutic interventions. This may involve the creation of new antibiotics, anti-virulence agents, or treatments that specifically impede biofilm formation and quorum sensing. In order to enhance patient outcomes and mitigate the burden of *P. aeruginosa* infections, it is imperative to implement personalised treatment strategies that are tailored to the unique genetic and phenotypic characteristics of the infecting strain.

6.3 Recommendations for further studies

Additional research should concentrate on investigating the molecular mechanisms that underlie the antibiotic resistance observed in *P. aeruginosa* isolates. This could encompass comprehensive investigations into the influence of horizontal gene transfer on the dissemination of resistance, regulatory networks, and specific resistance genes. Novel therapeutic strategies that are designed to overcome resistance could be informed by a more profound understanding of these mechanisms.

Future research should examine the evolutionary pressures that drive the significant genotypic diversity among the isolates in various clinical and environmental contexts. The genetic composition of *P. aeruginosa* populations could be monitored over time through longitudinal studies, which could assist in the identification of factors that contribute to the emergence of novel strains and resistance patterns. These studies would offer valuable insights into the manner in which *P. aeruginosa* adjusts to a variety of environments and treatments.

In order to evaluate the impact of the specific virulence factors identified in *P. aeruginosa* isolates on clinical outcomes in patients, additional research is required. The severity of infections, treatment responses, and patient recovery could be correlated with the presence of specific virulence traits in studies. This would assist in the identification of the most critical virulence factors that could be targeted for therapeutic intervention or used as biomarkers to predict infection outcomes.

In light of the obstacles presented by multidrug-resistant *P. aeruginosa* and its diverse physiological characteristics, it is imperative that additional research investigates innovative therapeutic strategies that extend beyond conventional antibiotics. This may involve the investigation of anti-virulence therapies that disrupt quorum sensing or biofilm formation, the utilisation of bacteriophages, or the creation of combination therapies that address multiple aspects of *P. aeruginosa* pathogenesis. It would be imperative to conduct preclinical and clinical studies to evaluate the efficacy and safety of these novel remedies in order to facilitate the management of *P. aeruginosa* infections.

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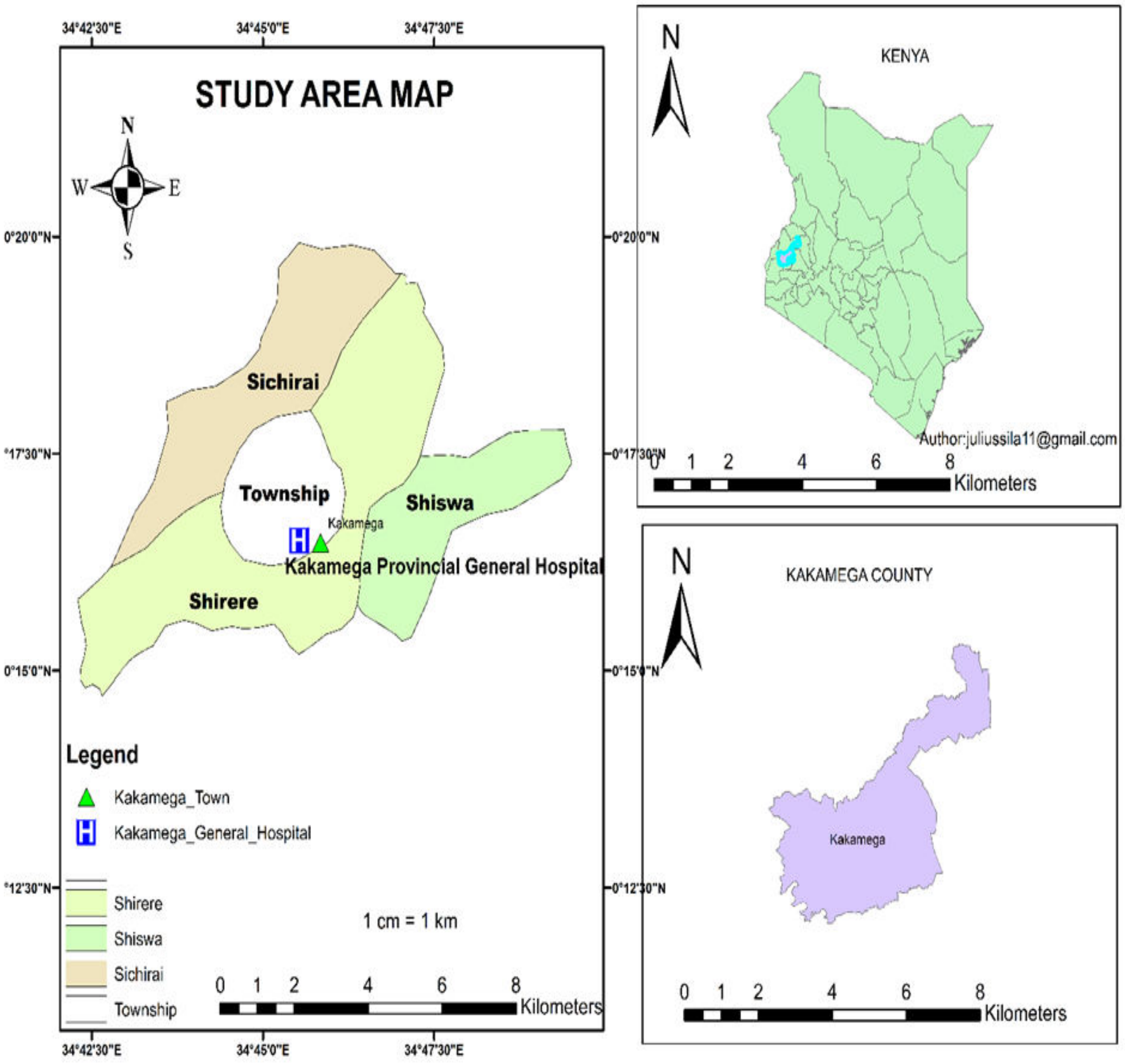
APPENDICES

Appendix I.

Map of Kenya showing the location of the Kakamega County General Teaching and Referral Hospital, Source: Google maps.



Appendix II.

Summary of Methods- Tools and Techniques used.

Analysis	Tool	Version	Description	Weblink	Citation
Annotation	KBase	v2 10.2022	Biology Knowledgebase for Predictive Biology KBase PROKKA annotation Pipeline v1.14.5	https://www.kbase.us	Arkin, A., Cottingham, R., Henry, C. *et al.* KBase: The United States Department of Energy Systems Biology Knowledgebase. *Nat Biotechnol* **36**, 566–569 (2018). https://doi.org/10.1038/nbt.4163
AMR genes determination	CARD-RGI	RGI 6.0.0, CARD 3.2.5	The Comprehensive Antibiotic Resistance Database-Resistant Gene Identifier	https://card.mcmaster.ca/analyze/rgi	Alcock *et al.* 2020. CARD 2020: antibiotic resistome surveillance with the Comprehensive Antibiotic Resistance Database. *Nucleic Acids Research*, **48**, D517-D525.
Virulence genes/factors determination	VDFB		The virulence factor database	http://www.mgc.ac.cn/VFs/	Lihong Chen, Zhaohui Xiong, Lilian Sun, Jian Yang, Qi Jin, VFDB 2012 update: toward the genetic diversity and molecular evolution of bacterial virulence factors, *Nucleic Acids Research*, Volume 40, Issue D1, 1 January 2012, Pages D641–D645, https://doi.org/10.1093/nar/gkr989
Sequence Types (ST)	PubMLST		Public databases for molecular typing and microbial genome diversity (Multi-Locus Sequence Typing).	https://pubmlst.org	Jolley, K. A., Bray, J. E., & Maiden, M. C. J. (2018). Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome open research*, *3*, 124. https://doi.org/10.12688/wellcomeopenres.14826.1
Plasmid Sequence identification	PLSDB	v. 2021_06_23_v2	Plasmid database. Searches for nucleotide	https://ccb-microbe.cs.uni-saarland.de/plsdb/plasmids/search_form/seq/	Georges P Schmartz, Anna Hartung, Pascal Hirsch, Fabian Kern, Tobias Fehlmann, Rolf Müller, Andreas Keller; PLSDB: advancing a

			sequences in plasmid		comprehensive database of bacterial plasmids, Nucleic Acids Res., 2021 Nov 25, doi: 10.1093/nar/gkab1111 Valentina Galata, Tobias Fehlmann, Christina Backes, Andreas Keller; PLSDB: a resource of complete bacterial plasmids, Nucleic Acids Res., 2018 Oct 31, doi: 10.1093/nar/gky1050
Phylogenetics	Mauve	(version 2.4.0)	Multiple genome alignments and Evolutionary Relationships	https://darlinglab.org/mauve/mauve.html	Darling, A. C., Mau, B., Blattner, F. R., & Perna, N. T. (2004). Mauve: multiple alignment of conserved genomic sequence with rearrangements. *Genome research*, *14*(7), 1394–1403. https://doi.org/10.1101/gr.2289704

Appendix III.

Quorum sensing reporter strains

JM109 (pSB1142)	odDHL biosensor strain. *Escherichia coli* JM109 containing pSB1142, tetracycline resistant (Winson et al 1998)
JM109 (pSB536)	BHL biosensor strain. *Escherichia coli* JM109 containing PSB536, carbecillin resistant (Winson et al 1998)
PA01 pqsA CTX-lux::pqsA	PQS biosensor strain. pqsA mutant of PA01containing a pqsA promoter:: luxCDABE fusion integrated at a neutral site in the chromosome

Appendix IV.

Gradient Gel electrophoresis set-up

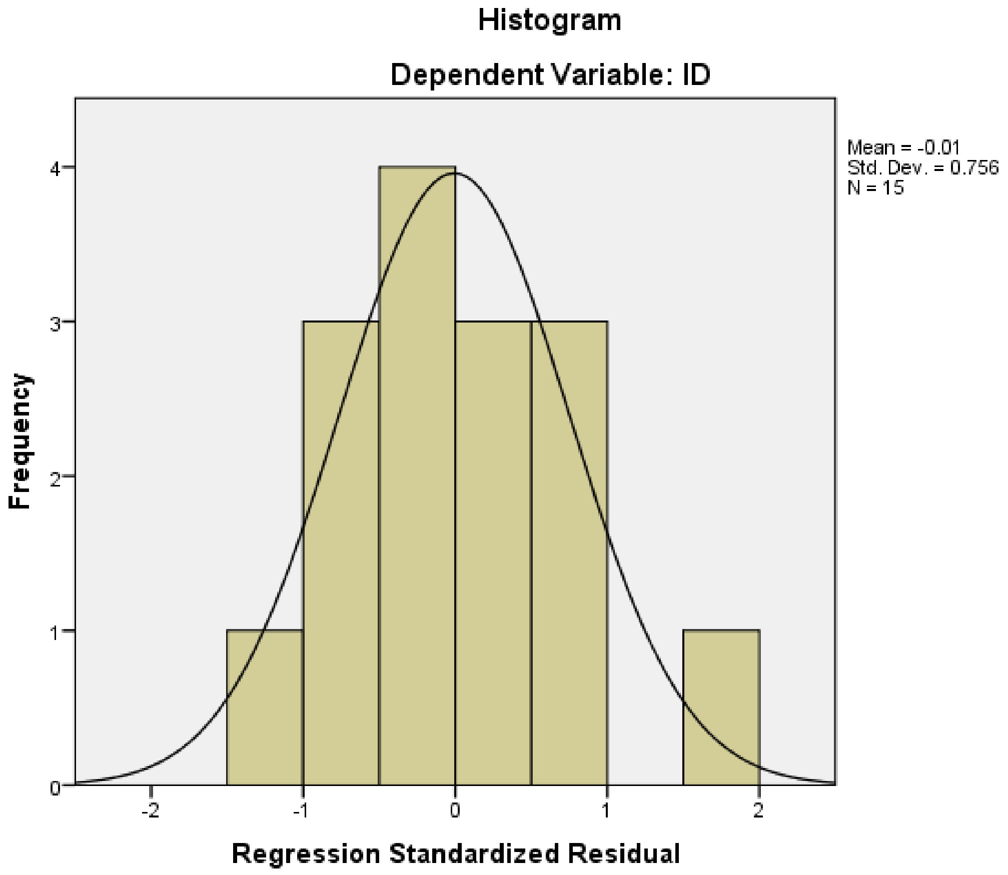
Lower gel/ separation gel	**10%**	**20%**	**Upper gel /stacking gel (5%)**	
30% Acrylamide	3.33 ml	6.66 ml	30% Acrylamide	1.5ml
1.5M Tris-HCl buffer PH 8.8	2.5 ml	2.5 ml	0.5M Tris -HCl buffer PH 6.8	1.14ml
10% SDS	100 µl	100 µl	10% SDS	90 µl
10%Ammonium persulfate (APS)	100 µl	100 µl	10%Ammonium persulfate (APS)	90 µl
TEMED	4 µl	4 µl	TEMED	10 µl
Water	4 ml	640 µl	Water	6.3ml

Appendix V. *Single point luminometry measurements of culture luminescence*

User: Meng												
Path: C:\Program Files\BMG\Omega\Meng\Data\												
Test ID: 161												
Test Name: luminescence												
Date: 09/03/2023												
Time: 16:50:25												
ID1: PQS biosensor												
Luminescence												
	Raw Data (Lens)											
	1	2	3	4	5	6	7	8	9	10	11	12
A	758	823	764	655	765	744	698	797	791	755	758	769
B	670	719	752	647	1042	832						
C	860	852	828	725	890	801	797	845	800	838	857	890
D	911	829	850	797	1337	921						
E	907	813	1006	778	982	919	899	853	880	818	798	1009
F	888	977	809	907	1468	866						
G	75	86	70	94	107	95	81	106	79	70	73	81
H	89	83	79	86	81	97						

Appendix VI.

Normal Probability Plot For PA AST Data



Appendix VII. Fisher's Exact test *P-value* significance for the antibiotics

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	105.000[a]	98	.296	1.000		
Likelihood Ratio	47.970	98	1.000	1.000		
Fisher's Exact Test	147.623			1.000		
Linear-by-Linear Association	.477[b]	1	.490	.513	.254	.003
N of Valid Cases	15					

a. 120 cells (100.0%) have expected count less than 5. The minimum expected count is .07.

b. The standardized statistic is -.690.

Appendix VIII. Drug Resistant Genes

ISOLATE	**Best_Hit (Gene)**	**Resistance Mechanism Encoded**	**Drug Class The gene encodes resistance against**
ID006, ID009, ID015, ID017, ID019, ID020	adeF	antibiotic efflux	fluoroquinolone antibiotic; tetracycline antibiotic
ID001	aadA	antibiotic inactivation	aminoglycoside antibiotic
All isolates	APH(3')-IIb	antibiotic inactivation	aminoglycoside antibiotic
ID017	APH(3")-Ib APH(3')-Ia APH(6)-Id	antibiotic inactivation	aminoglycoside antibiotic
ID003, ID008, ID011,	ArmR	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem
ALL ISOLATES	arnA	antibiotic target alteration	peptide antibiotic
	basR	antibiotic target alteration; antibiotic efflux	peptide antibiotic
	basS	antibiotic target alteration; antibiotic efflux	peptide antibiotic
	bcr-1	antibiotic efflux	bicyclomycin-like antibiotic
	cprR	antibiotic target alteration; antibiotic efflux	peptide antibiotic
	cprS	antibiotic target alteration; antibiotic efflux	peptide antibiotic

ID001, ID003	EreA2	antibiotic inactivation	macrolide antibiotic
ALL EXCEPT ID012	FosA	antibiotic inactivation	phosphonic acid antibiotic
ALL ISOLATES	MexA MexB	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem
	MexC MexD	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; aminoglycoside antibiotic; cephalosporin; penam; tetracycline antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
	MexE	antibiotic efflux	fluoroquinolone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
ID001, ID003, ID004, ID008, ID011, ID012, ID013, ID014, ID016, ID018	MexF	antibiotic efflux	fluoroquinolone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
ALL ISOLATES	MexG	antibiotic efflux	fluoroquinolone antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics
	MexH	antibiotic efflux	fluoroquinolone antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics
	MexI	antibiotic efflux	fluoroquinolone antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics
	MexJ	antibiotic efflux	macrolide antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics
	MexK	antibiotic efflux	macrolide antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics
	MexL	antibiotic efflux	macrolide antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics
	mexM	antibiotic efflux	phenicol antibiotic
	mexN	antibiotic efflux	phenicol antibiotic
	mexP	antibiotic efflux	macrolide antibiotic; carbapenem; tetracycline antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic;

			disinfecting agents and antiseptics
	mexQ	antibiotic efflux	macrolide antibiotic; carbapenem; tetracycline antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic; disinfecting agents and antiseptics
	MexR	antibiotic target alteration; antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem
	MexS	antibiotic efflux	fluoroquinolone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
	MexT	antibiotic efflux	fluoroquinolone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
	MexV	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; tetracycline antibiotic; phenicol antibiotic; disinfecting agents and antiseptics
	MexW	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; tetracycline antibiotic; phenicol antibiotic; disinfecting agents and antiseptics
ALL ISOLATES EXCEPT ID006	mexY	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; aminoglycoside antibiotic; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; phenicol antibiotic; disinfecting agents and antiseptics
ALL ISOLATES	MexZ	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; aminoglycoside antibiotic; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; phenicol antibiotic; disinfecting agents and antiseptics
ALL EXCEPT ID003	MuxA	antibiotic efflux	macrolide antibiotic; monobactam; tetracycline antibiotic; aminocoumarin antibiotic
ALL ISOLATES	MuxB	antibiotic efflux	macrolide antibiotic; monobactam; tetracycline antibiotic; aminocoumarin antibiotic
	MuxC	antibiotic efflux	macrolide antibiotic; monobactam; tetracycline antibiotic; aminocoumarin antibiotic
	nalC	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem
	nalD	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem
	OpmB	antibiotic efflux	macrolide antibiotic; monobactam; tetracycline antibiotic; aminocoumarin antibiotic

	OpmD	antibiotic efflux	fluoroquinolone antibiotic; tetracycline antibiotic; disinfecting agents and antiseptics
	opmE	antibiotic efflux	macrolide antibiotic; carbapenem; tetracycline antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic; disinfecting agents and antiseptics
	OpmH	antibiotic efflux	disinfecting agents and antiseptics
	OprJ	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; aminoglycoside antibiotic; cephalosporin; penam; tetracycline antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
	OprM	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; aminoglycoside antibiotic; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem; disinfecting agents and antiseptics
	OprN	antibiotic efflux	fluoroquinolone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
ID006, ID008	OXA-50	antibiotic inactivation	carbapenem; cephalosporin; penam
ID017	OXA-395	antibiotic inactivation	carbapenem; cephalosporin; penam
ID014, ID015, ID016	OXA-486	antibiotic inactivation	carbapenem; cephalosporin; penam
ID011, ID013	OXA-847	antibiotic inactivation	carbapenem; cephalosporin; penam
ID003	OXA-851	antibiotic inactivation	carbapenem; cephalosporin; penam
ID004, ID009, ID018	OXA-903	antibiotic inactivation	carbapenem; cephalosporin; penam
ID020	OXA-914	antibiotic inactivation	carbapenem; cephalosporin; penam
ID001, ID012	OXA-494	antibiotic inactivation	carbapenem; cephalosporin; penam
ALL ISOLATES	ParR	antibiotic efflux; reduced permeability to antibiotic	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; aminoglycoside antibiotic; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; phenicol antibiotic; penem; disinfecting agents and antiseptics
	ParS	antibiotic efflux; reduced permeability to antibiotic	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; aminoglycoside antibiotic; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; phenicol antibiotic; penem; disinfecting agents and antiseptics
ID011,	PDC-1	antibiotic	monobactam; carbapenem; cephalosporin

ID013		inactivation	
ID004, ID017	PDC-3	antibiotic inactivation	monobactam; carbapenem; cephalosporin; cephamycin; penam
ID001, ID006	PDC-5	antibiotic inactivation	monobactam; carbapenem; cephalosporin; cephamycin; penam
ID018	PDC-8	antibiotic inactivation	monobactam; carbapenem; cephalosporin
ID008, ID014, ID015, ID016	PDC-24	antibiotic inactivation	monobactam; carbapenem; cephalosporin
ID009	PDC-31	antibiotic inactivation	monobactam; carbapenem; cephalosporin
ID020	PDC-66	antibiotic inactivation	monobactam; carbapenem; cephalosporin
ID012	PDC-162	antibiotic inactivation	monobactam; carbapenem; cephalosporin
ALL ISOLATES	PmpM	antibiotic efflux	fluoroquinolone antibiotic; aminoglycoside antibiotic; disinfecting agents and antiseptics
	Pseudomonas aeruginosa catB7	antibiotic inactivation	phenicol antibiotic
	Pseudomonas aeruginosa CpxR	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; monobactam; aminoglycoside antibiotic; carbapenem; cephalosporin; cephamycin; penam; tetracycline antibiotic; peptide antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; sulfonamide antibiotic; phenicol antibiotic; penem
	Pseudomonas aeruginosa emrE	antibiotic efflux	aminoglycoside antibiotic
	Pseudomonas aeruginosa soxR	antibiotic target alteration; antibiotic efflux	fluoroquinolone antibiotic; cephalosporin; glycylcycline; penam; tetracycline antibiotic; rifamycin antibiotic; phenicol antibiotic; disinfecting agents and antiseptics
	rsmA	antibiotic efflux	fluoroquinolone antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
ID001	sul1	antibiotic target replacement	sulfonamide antibiotic

ALL ISOLATES	TriA	antibiotic efflux	disinfecting agents and antiseptics
	TriB	antibiotic efflux	disinfecting agents and antiseptics
	TriC	antibiotic efflux	disinfecting agents and antiseptics
	Type A NfxB	antibiotic efflux	macrolide antibiotic; fluoroquinolone antibiotic; cephalosporin; penam; tetracycline antibiotic; aminocoumarin antibiotic; diaminopyrimidine antibiotic; phenicol antibiotic
	vanW gene in vanG cluster	antibiotic target alteration	glycopeptide antibiotic
	YajC	antibiotic efflux	fluoroquinolone antibiotic; cephalosporin; glycylcycline; penam; tetracycline antibiotic; oxazolidinone antibiotic; glycopeptide antibiotic; rifamycin antibiotic; phenicol antibiotic; disinfecting agents and antiseptics

Appendix IX. Virulence genes

Gene	Description	ISOLATE
algB pilR fleQ	two-component response regulator AlgB [Alginate] [Pseudomonas aeruginosa PAO1] two-component response regulator PilR [Type IV pili] [Pseudomonas aeruginosa PAO1] transcriptional regulator FleQ [Flagella] [Pseudomonas aeruginosa PAO1]	ID001,ID004,ID008,ID009, ID011,ID012,ID015,ID018,ID020
fpvA fptA pchH phzA1	ferripyoverdine receptor FpvA [Pyoverdine] [Pseudomonas aeruginosa PAO1] Fe(III)-pyochelin receptor precursor [Pyochelin] [Pseudomonas aeruginosa PAO1] ABC transporter ATP-binding protein [Pyochelin] [Pseudomonas aeruginosa PAO1] phenazine biosynthesis protein PhzA [Phenazines biosynthesis] [Pseudomonas aeruginosa PAO1]	ID001,ID009,ID012,ID015,ID020
pchF pvdJ	pyochelin synthetase PchF [Pyochelin] [Pseudomonas aeruginosa PAO1] pyoverdine biosynthesis protein PvdJ [pyoverdine] [Pseudomonas aeruginosa PAO1]	ID001,ID009,ID012,ID013,ID015 ID018,ID020
pchE pvcC mucE	dihydroaeruginoic acid synthetase PchE [Pyochelin] [Pseudomonas aeruginosa PAO1] paerucumarin biosynthesis protein PvcC [pyoverdine] [Pseudomonas aeruginosa PAO1] small envelope protein MucE [Alginate regulation] [Pseudomonas aeruginosa PAO1]	ID001,ID009,ID012,ID015,ID018 ID020
phzM	phenazine-specific methyltransferase PhzM (adenosylmethionine dependent methyltransferase) [Pyocyanin] [Pseudomonas aeruginosa PAO1]	ID001,ID009,ID012,ID015
mbtB	Phenyloxazoline synthase MbtB (phenyloxazoline synthetase) [Mycobactin] [Mycobacterium tuberculosis H37Rv]	ID001,ID009,ID012,ID015,ID018
tagT	type six secretion associated protein TagT, ATP-binding component of ABC transporter [HSI-I] [Pseudomonas aeruginosa PAO1]	ID001, ID006,ID009,ID011,ID012 ID015,ID020
pvdL	peptide synthase PvdL [pyoverdine] [Pseudomonas aeruginosa PAO1]	ID001, ID003,ID009,ID012,ID013 ID015. ID018,ID020
(pilJ) **(fleR)**	twitching motility protein PilJ [Type IV pili] [Pseudomonas aeruginosa PAO1] two-component response regulator [Flagella] [Pseudomonas aeruginosa PAO1]	ID003, ID004,ID008,ID011
(phzC1)	phenazine biosynthesis protein PhzC [Phenazines biosynthesis] [Pseudomonas aeruginosa PAO1]	ID003,ID009, ID012,ID015
(ppkA) **(fbpC)**	serine/threonine protein kinase PpkA [HSI-I] [Pseudomonas aeruginosa PAO1] iron(III) ABC transporter, ATP-binding protein [FbpABC] [Neisseria meningitidis MC58]	ID003

(vgrG1b) **(vgrG1a)** **(tssH-5/clpV)**	type VI secretion system substrate VgrG1b [HSI-1 (Hcp-secretion island 1)] [Pseudomonas aeruginosa PAO1] type VI secretion system substrate VgrG1 [HSI-I] [Pseudomonas aeruginosa PAO1] Clp-type ATPase chaperone protein [T6SS-1] [Burkholderia pseudomallei K96243]	ID004, ID008
(tse2) **(cdpA)**	type VI secretion system effector Tse2 [HSI-1 (Hcp-secretion island 1)] [Pseudomonas aeruginosa PAO1] cyclic di-GMP phosphodiesterase [CdpA] [Burkholderia pseudomallei K96243]	ID004
(xcpR)	general secretion pathway protein E [xcp secretion system] [Pseudomonas aeruginosa PAO1]	ID004, ID006, ID008,ID013,ID017
(fepA) **(iroN)**	ferrienterobactin outer membrane transporter [Enterobactin] [Escherichia coli CFT073] salmochelin receptor IroN [IroN] [Escherichia coli CFT073]	ID004, ID008,ID013
(tsr)	methyl-accepting chemotaxis protein I [Flagella] [Burkholderia pseudomallei K96243]	ID004,ID008,ID011
(pilB)	type 4 fimbrial biogenesis protein PilB [Type IV pili] [Pseudomonas aeruginosa PAO1]	ID004, ID006,ID008,ID013
(pilB)	type IV pilus assembly protein [Type IV pili] [Burkholderia pseudomallei K96243]	ID004, ID006,ID008
(xcpQ) **(xcpS)** **(xcpT)**	general secretion pathway protein D [xcp secretion system] [Pseudomonas aeruginosa PAO1] general secretion pathway protein F [xcp secretion system] [Pseudomonas aeruginosa PAO1] general secretion pathway protein G [xcp secretion system] [Pseudomonas aeruginosa PAO1]	ID006,ID013
(wzy) **(xcpY)** **(wzz)** **(xcpX)** **(xcpW)** **(xcpP)** **(xcpU)** **(xcpZ)** **(fimV)** **(xcpV)** **(gspE)** **(bplB)** **(bplL)** **(bplC)** **(gspF)** **(ybtP)** **(cyaB)**	O-antigen chain length regulator [LPS] [Pseudomonas aeruginosa PAO1] general secretion pathway protein L [xcp secretion system] [Pseudomonas aeruginosa PAO1] positive regulator for alginate biosynthesis MucC [LPS] [Pseudomonas aeruginosa PAO1] general secretion pathway protein K [xcp secretion system] [Pseudomonas aeruginosa PAO1] general secretion pathway protein J [xcp secretion system] [Pseudomonas aeruginosa PAO1] secretion protein XcpP [xcp secretion system] [Pseudomonas aeruginosa PAO1] general secretion pathway protein H [xcp secretion system] [Pseudomonas aeruginosa PAO1] general secretion pathway protein M [xcp secretion system] [Pseudomonas aeruginosa PAO1] putative Type IV pili related protein [Type IV pili] [Pseudomonas aeruginosa PAO1] general secretion pathway protein I [xcp secretion system] [Pseudomonas aeruginosa PAO1] general secretion pathway protein E [T2SS] [Shigella dysenteriae Sd197] probable acetyltransferase [LPS] [Bordetella pertussis Tohama I] lipopolysaccharide biosynthesis protein [LPS] [Bordetella pertussis Tohama I] lipopolysaccharide biosynthesis protein [LPS] [Bordetella pertussis Tohama I] general secretion pathway protein F [T2SS] [Shigella dysenteriae Sd197] lipoprotein inner membrane ABC-transporter [Yersiniabactin] [Yersinia pestis CO92] cyclolysin secretion ATP-binding protein [Cya] [Bordetella pertussis Tohama I]	ID006
(mtrD)	multiple transferable resistance system protein MtrD [MtrCDE] [Neisseria meningitidis MC58]	ID008
(pilT)	twitching motility protein PilT [Type IV pili] [Pseudomonas aeruginosa PAO1]	ID006,ID013,ID017

(phzD1)	phenazine biosynthesis protein PhzD, isochorismatase [Phenazines biosynthesis] [Pseudomonas aeruginosa PAO1]	ID009,ID015

(phzB1)	phenazine biosynthesis protein PhzB [Phenazines biosynthesis] [Pseudomonas aeruginosa PAO1]	ID009,ID012,ID015,ID020
(flhA) **(aprA)** **(flhF)** **(fliK)** **(flhB)** **(fliM)** **(motD)** **(fleN)** **(fliR)** **(motC)** **(fliA)** **(fliP)** **(lasI)** **(fliL)** **(fliN)** **(fliO)** **(fliQ)** **(flhA)** **(fliP)** **(fliN)** **(pscR)** **(pcrD)** **(bcrD)** **(flhA)** **(fliM)** **(spaP)** **(ccmF)** **(cheZ)** **(flhB)** **(fliA)** **(bsaQ)**	flagellar biosynthesis protein FlhA [Flagella] [Pseudomonas aeruginosa PAO1] alkaline metalloproteinase precursor [Alkaline protease] [Pseudomonas aeruginosa PAO1] flagellar biosynthesis protein FlhF [Flagella] [Pseudomonas aeruginosa PAO1] flagellar hook-length control protein FliK [Deoxyhexose linking sugar, 209 Da capping structure] [Pseudomonas aeruginosa PAO1] flagellar biosynthetic protein FlhB [Flagella] [Pseudomonas aeruginosa PAO1] flagellar motor switch protein FliM [Flagella] [Pseudomonas aeruginosa PAO1] flagellar motor protein [Deoxyhexose linking sugar, 209 Da capping structure] [Pseudomonas aeruginosa PAO1] flagellar synthesis regulator FleN [Flagella] [Pseudomonas aeruginosa PAO1] flagellar biosynthetic protein FliR [Flagella] [Pseudomonas aeruginosa PAO1] flagellar motor protein [Deoxyhexose linking sugar, 209 Da capping structure] [Pseudomonas aeruginosa PAO1] flagellar biosynthesis sigma factor FliA [Deoxyhexose linking sugar, 209 Da capping structure] [Pseudomonas aeruginosa PAO1] flagellar biosynthetic protein FliP [Flagella] [Pseudomonas aeruginosa PAO1] autoinducer synthesis protein LasI [Quorum sensing] [Pseudomonas aeruginosa PAO1] flagellar basal body protein FliL [Deoxyhexose linking sugar, 209 Da capping structure] [Pseudomonas aeruginosa PAO1] flagellar motor switch protein FliN [Flagella] [Pseudomonas aeruginosa PAO1] flagellar protein FliO [Flagella] [Pseudomonas aeruginosa PAO1] flagellar biosynthetic protein FliQ [Flagella] [Pseudomonas aeruginosa PAO1] flagellar biosynthesis protein FlhA [Flagella] [Burkholderia pseudomallei K96243] flagellar biosynthesis protein FliP [Flagella] [Burkholderia pseudomallei K96243] flagellar motor switch protein FliN [Flagella] [Burkholderia pseudomallei K96243] type III secretion system protein PscR [TTSS] [Pseudomonas aeruginosa PAO1] type III secretion system protein PcrD [TTSS] [Pseudomonas aeruginosa PAO1] Type III secretion system LcrD homolog protein BcrD [TTSS] [Bordetella pertussis Tohama I] flagellar biosynthesis protein FlhA [Flagella] [Yersinia enterocolitica subsp. enterocolitica 8081] flagellar motor switch protein FliM [Flagella] [Burkholderia pseudomallei K96243] type III secretion system minor export apparatus protein SpaP [TTSS(SPI-1 encode)] [Salmonella enterica subsp. enterica serovar Typhimurium str. LT2] cytochrome c heme lyase subunit CcmF [Cytochrome c muturation (ccm) locus] [Legionella pneumophila subsp. pneumophila str. Philadelphia 1] chemotaxis regulator CheZ [Flagella] [Burkholderia pseudomallei K96243] flagellar biosynthesis protein FlhB [Flagella] [Burkholderia pseudomallei K96243] flagellar biosynthesis sigma factor [Flagella] [Burkholderia pseudomallei K96243] Type III secretion system protein BsaQ [Bsa T3SS] [Burkholderia pseudomallei K96243]	ID011

(chpA)	still frameshift probable component of chemotactic signal transduction system [Type IV pili] [Pseudomonas aeruginosa PAO1]	ID011,ID014,ID016,ID017
(pilJ)	twitching motility protein PilJ [Type IV pili] [Pseudomonas aeruginosa PAO1]	ID011,ID017
(plcH)	hemolytic phospholipase C precursor [PLC] [Pseudomonas aeruginosa PAO1]	ID013
(mucD)	serine protease MucD precursor [Alginate regulation] [Pseudomonas aeruginosa PAO1]	
(mucB)	anti-sigma factor MucA, inhibitor of alg gene expression [Alginate] [Pseudomonas aeruginosa PAO1]	
(mucA)	alkaline metalloproteinase precursor [Alginate] [Pseudomonas aeruginosa PAO1]	
(algU)	alginate biosynthesis protein AlgZ/FimS [Alginate] [Pseudomonas aeruginosa PAO1]	
(mucC)	negative regulator for alginate biosynthesis MucB [Alginate] [Pseudomonas aeruginosa PAO1]	
(pvdI)	peptide synthase [pyoverdine] [Pseudomonas aeruginosa PAO1]	
(fepA)	ferrienterobactin outer membrane transporter [Enterobactin] [Escherichia coli CFT073]	
(pvdN)	pyoverdine biosynthesis protein PvdN [pyoverdine] [Pseudomonas aeruginosa PAO1]	
(pilQ)	type IV pilus biosynthesis protein [Type IV pili] [Burkholderia pseudomallei K96243]	
(lasB)	elastase LasB [LasB] [Pseudomonas aeruginosa PAO1]	ID014,ID016,
(mucP)	metalloprotease protease [Alginate regulation] [Pseudomonas aeruginosa PAO1]	
(pilF) t	ype 4 fimbrial biogenesis protein PilF [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(fabZ)	(3R)-hydroxymyristoyl ACP dehydratase [LPS] [Brucella melitensis bv. 1 str. 16M]	
(phzE1)	phenazine biosynthesis protein PhzE [Phenazines biosynthesis] [Pseudomonas aeruginosa PAO1]	ID015
(pilU)	twitching motility protein PilU [Type IV pili] [Pseudomonas aeruginosa PAO1]	ID017
(chpB)	probable methylesterase [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(pilK)	methyltransferase PilK [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(chpD)	probable transcriptional regulator [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(chpE)	probable chemotaxis protein [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(pilI) t	witching motility protein PilI [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(chpC)	probable chemotaxis protein [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(pilG)	twitching motility protein PilG [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(pilH)	twitching motility protein PilH [Type IV pili] [Pseudomonas aeruginosa PAO1]	
(pilT)	twitching motility protein PilT [Type IV pili] [Neisseria meningitidis MC58]	
(tssH-5/clpV)	Clp-type ATPase chaperone protein [T6SS-1] [Burkholderia pseudomallei K96243]	
(cheA)	chemotaxis two-component sensor kinase CheA [Flagella] [Burkholderia pseudomallei K96243]	
(clpV1)	type VI secretion system AAA+ family ATPase [HSI-I] [Pseudomonas aeruginosa PAO1]	
(pilT2)	twitching motility protein PilT [Type IV pili] [Neisseria meningitidis MC58]	
(pvdH)	diaminobutyrate-2-oxoglutarate aminotransferase PvdH [pyoverdine] [Pseudomonas aeruginosa PAO1]	
(pilU)	twitching motility protein PilU [Type IV pili] [Neisseria meningitidis MC58]	