PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF *PSEUDOMONAS AERUGINOSA* IN CLINICAL ISOLATES: A CASE STUDY OF BULAWAYO METROPOLITAN PROVINCE.

BY

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ABSTRACT

Pseudomonas aeruginosa is gram negative bacterium which causes nosocomial infections in patients and is highly resistant to most antibiotics as it possess numerous mechanisms of resistance. These mechanisms possessed by *P. aeruginosa* make it difficult to treat in infections thus being of medical concern. A study was carried out to determine the prevalence and antibiotic susceptibility patterns of *P. aeruginosa* isolated from wound pus and urine samples. A total of 1200 primary cultured samples from patients with urinary tract and wound infections were screened for *P. aeruginosa* at two medical laboratories (Diagnostics Laboratory Services and Southern Pathology Laboratory) in Bulawayo from July 2015 to January 2016. *P. aeruginosa* was identified using standard biochemical tests according to Clinical Laboratory Standards Institute (CLSI) guidelines and its antibiotic susceptibility patterns to eight different antibiotics (Imipenem, Piperacillin, Piperacillin/Tazobactam, Ciprofloxacin, Norfloxacin, Gentamicin, Ceftriaxone, and Tetracycline) were determined using antibiotic zone diameters. A total of 78 samples tested positive for *P. aeruginosa* giving an overall prevalence of 6.5%. The prevalence of *P. aeruginosa* was higher in male patients (61.54%) than in female patients (38.46%). The eight antibiotics tested had significantly different efficacies against the *P. aeruginosa* isolates obtained from pus swab samples (Chi-square P=0.000). Imipenem was the most effective drug on the *P. aeruginosa* isolated from pus swabs (88.1%). Norfloxacin, Tetracycline, Gentamicin and Ceftriaxone were the least effective drugs on *P. aeruginosa* and had the same efficacy against the *P. aeruginosa* isolates obtained from pus swab samples (P=0.591). Therefore, the latter four antibiotics may not be considered as treatment options for *P. aeruginosa* mediated wound infections. However, Piperacillin, Piperacillin/Tazobactam and Ciprofloxacin can be used as alternative treatment options for *P. aeruginosa* mediated wound infections. In terms of their efficacy against *P. aeruginosa* isolated from urine samples, the eight different antibiotics could be placed in two major categories (P=0.000). Imipenem, Piperacillin, Ciprofloxacin and Piperacillin/Tazobactum were the antibiotics of choice and had equally the same efficacy against *P. aeruginosa* isolated from urine samples (P=0.120). These four antibiotics were significantly the most efficant antibiotics which can be used interchangeably in treating *P. aeruginosa* mediated urinary tract infections. The other four antibiotics (Norfloxacin, Gentamicin, Ceftriaxone and Tetracycline) were equally inferior to the latter antibiotics in terms of efficacy (Chi-square P=0.000). This study showed that *P. aeruginosa* was sensitive to Ciprofloxacin and resistant to Norfloxacin regardless of both belonging to the Quinolone class of drugs. Overall, all the isolates, irrespective of sample type were highly sensitive to β-lactams (Imipenem, Piperacillin and Piperacillin/Tazobactum). This study further showed that there were 32 antibiotypes for *P. aeruginosa* isolated from pus swab isolates and 29 antibiotypes for *P. aeruginosa* isolated from urine samples. This showed that different *P. aeruginosa* isolates respond differently to the same antibiotic. The most common antibiotype for pus swab *P. aeruginosa* isolates was $Nor^{R}Tet^{R}Gent^{R}$ $Ceft^{R}Cip^{R}$ with a frequency of 11.90%. The most common antibiotype for urine *P. aeruginosa* isolates was $Nor^{R}Tet^{R}Ceft^{R}Cip^{R}$ with a frequency of 8.34%. The rest of the antibiotypes for urine and pus swab isolates were unique and revealed high multi-drug resistance. The recommended effective drugs for treating *P. aeruginosa* mediated urinary tract infections in this study are Imipenem, Piperacillin/Tazobactam, Piperacillin and Ciprofloxacin respectively. Imipenem, Piperacillin, Piperacillin/Tazobactam and Ciprofloxacin respectively are recommended as

treatment choices for wound infections. Imipenem is recommended as the antibiotic of choice in treating *P. aeruginosa* mediated wound and urinary tract infections.

DEDICATION

To my parents (Mr & Mrs Chazanga), my grandmother (Jean), my siblings and aunt (Mrs B. Mukwashi).

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CHAPTER 1: INTRODUCTION

1. Background

Antibiotics are used to treat different types of infections thus being the core of medical health care. With time, the degree of effectiveness of antibiotics on infections decreased thereby increasing the costs of treatment and above all, the mortality rates in patients as a result of antibiotic resistance. The development of antibiotic resistance has become a major nightmare as many disease causing organisms have become resistant to at least one antibiotic (Gelband *et al.*, 2015).

1.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa (PSA) is a free-living, gram negative Gamma Proteobacteria of the family Pseudomonadaceae. It is a motile, aerobic, rod shaped bacterium and its size ranges from 0.5-0.8 µm by 1.5-3.0 µm. It is ubiquitous in nature and is found mostly in soil, water and on several other surfaces. PSA can survive in a wide range of physical conditions and even tolerates adverse conditions like temperature, pH, antiseptics and some antibiotics (Todar, 2009). PSA produces two distinct pigments which are pyoverdin and pyocyanin which give its colonies their blue-green colour (Gellatly and Hancock, 2013). Pyocyanin is a radio active pigment produced by *P. aeruginosa* (Jamunadevi, 2012) that interferes with host cells inhibiting phagocytosis as well as inducing apoptosis in neutrophils whilst pyoverdin is important in establishment of infection (Gellatly and Hancock, 2013). PSA is an opportunistic and nosocomial pathogen especially in immunocompromised people and. It causes bacteremia, urinary tract infections, wound infections and colonises individuals with cystic fibrosis amongst other infections (Jamunadevi, 2012).

Its ability to tolerate different environmental conditions makes it a nosocomial and opportunistic pathogen of high clinical relevance (Todar, 2009). Approximately 10-15% of the nosocomial infections worldwide are due to *Pseudomonas aeruginosa* (Strateva and Yordanov, 2009).

Despite the advances made in the medical and pharmaceutical industry through the introduction of antipseudomonal drugs, PSA infections remain a big threat in patients due to its resistance to antibiotics. Most drugs have lost their efficacy against PSA and the susceptibility patterns change with time and regions in the world. The increase in antibiotic resistance by PSA has been observed in several epidemiological studies carried out all over the world (Rajat, 2012). A study by Tam *et al.*, (2010) in USA showed that *P. aeruginosa* isolates were resistant to carbapenems and quinolones. In a separate study, a high resistance frequency of up to 60-83% was observed in Turkey (Savas *et al.*, 2005). The increase in antibiotic resistance in disease causing pathogens is not only a problem in America, Europe and Asia but also in Africa (Kimang'a, 2012). A study by Igumbor *et al.* (2000) at Parirenyatwa Hospital (Zimbabwe) revealed the emergence of multidrug resistant strains of *P. aeruginosa* and that an increase in antibiotic resistant strains. Therefore this is a major cause for concern as it inhibits the efficiency of therapeutic drugs.

Amongst prokaryotes, *Pseudomonas aeruginosa* has the largest genome with a high proportion of the genome encoding regulatory, transport and virulence proteins. About 0.3% of its genes code for antibiotic resistance proteins (Mesaros *et al*, 2007). Virulence factors make PSA very competitive against host defences and these have attributed the success of PSA as an opportunistic pathogen (Gellatly and Hancock, 2013). PSA also possesses enzymic and mutational mechanisms of drug resistance (Strateva and Yordanov, 2009) and it is resistant to many classes of drugs thus making it difficult to treat (Gellatly and Hancock, 2009).

The natural resistance to antibiotics and the ability to occupy different environments by *P. aeruginosa* is influenced by the gram negative outer membrane and as well as its ability to colonize in the form a biofilm (Todar, 2009). PSA has AmpC β-lactamase which is an enzyme that induces resistance to β-lactams through hydrolysis of the antibiotics. Its cells become impermeable and this excludes many antibiotics from entering the cell.

The outer membrane pores formed by the Outer membrane porin F (OprF porin) play a role in the exclusion of antibiotics by PSA. Efflux pumps in *P. aeruginosa* pump remove β-lactams, tetracyclines and fluoroquinolones amongst other drugs which lack effective antipseudomonal activity as well as detergents. Multiple drug resistance is owed to the combination of up-regulated efflux, loss of OprD and impermeability of the cell membrane (Livermore, 2002). Although drug combinations have been used to treat PSA, intrinsic, acquired and adaptive drug resistance in *Pseudomonas aeruginosa* has been shown to be on the increase (Gellatly and Hancock, 2013).

1.2 Problem statement

P. aeruginosa is a Multidrug resistant organism that causes nosocomial infections in hospital patients. It is difficult to treat especially with common antibiotics as it is resistant to a wide range of antibiotics. It has been noted that antibiotic sensitivity tests have to be repeated several times with drug replacements so as to get treatment options for infections as most of the strains are highly resistant to most antibiotics. Different anti-pseudomonal drugs e.g Tazobactum and Piperacillin have been developed so as to try and reduce the severity PSA infections. However, PSA still poses as a threat to clinicians and pharmacists due to its genetic complexity which attributes its resistance (Stover *et al.,* 2000). Resistance can be developed towards antibiotics during the course of treatment of infection (Lister *et al.,* 2009)

thus slowing down or reversing any possible progress made in terms of treatment. The possession of innate resistance by PSA is therefore a cause for concern needing investigation.

1.3 Justification of study

Bacteria that are resilient are difficult to treat and remove from hospital environments therefore there is need to know their prevalence so as to control their numbers to avoid outbreaks. Since PSA is a nosocomial infectious organism, it poses as a threat to patients especially in the Intensive care unit (ICU) and also in immunocompromised individuals.

Knowledge of the most effective drugs can help in treating PSA infections in patients effectively as their immune systems will be compromised. The study will also help to determine prevalence rates of *Pseudomonas aeruginosa* in urine and pus swab samples. This can therefore help in identifying changes in antibiotic patterns thus clinicians can get drug profiles for different samples depending on whether they are for urine or wound infections. Determination of drug efficacy against P. aeruginosa can help in the screening of antibiotics suitable in treating PSA mediated infections. Resistance patterns of microorganisms vary from place to place thus localised studies of urinary tract infections help in getting knowledge on the pathogens as well as the treatment options (Lisa *et al*., 2015). Antibiotic susceptibility tests are done repeatedly on single isolates in laboratories in a bid to establish treatment options and this is very costly and time consuming. Therefore, comparing the risk of emergence of resistance to anti-pseudomonals using zone diameters will help with information on possible useful drugs in PSA infections.

1.4 Objectives of the study

Main objective

To determine the prevalence of PSA and its antibiotic susceptibility patterns in clinical isolates.

Specific Objectives

- To isolate and identify PSA
- To determine the prevalence of PSA
- To determine the antibiotic sensitivity patterns of PSA clinical isolates
- To determine the drug profile for different samples that are more effective, ie. Drugs for urine infections and drugs for wound infections.

CHAPTER 2: LITERATURE REVIEW

2.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a rod-shaped, gram negative Gamma Proteobacteria which belongs to the family Pseudomonadaceae which has 12 members. *P. aeruginosa* has been regarded as a human pathogen since 1940 (Sadaf *et al*. 2016). It is the major cause of nosocomial infections (Mesaros *et al*., 2007) causing 10-15% of the worldwide nosocomial infections (Strateva and Yordanov., 2009). *P. aeruginosa* is a free-living, motile bacterium and measures 0.5 to 0.8μm by 1.5 to 3.0μm in size (Todar, 2008). It is ubiquitous in nature (Todar, 2008) and is highly adaptable thus it can survive in a variety of natural and artificial environments (Gellatly and Hancock, 2013). *P. aeruginosa* is a cosmopolitan aerobic bacillus and can be isolated from different places including soil and water (Gales *et al*., 2001). It is metabolically versatile and posses regulatory systems and other accessory elements (Frank, 2012) which allow it to be found in nutrient deficient environments (Gellatly and Hancock., 2013) as it has minimal nutritional needs (Gales *et al*., 2001). Reservoirs of PSA can form on water taps, mops, apparatus and surfaces in hospitals (Mansour *et al*., 2013). *P. aeruginosa* has the largest bacterial genome sequences with the genome being 6.264.403 base pairs. The size and complexity of the genome is an evolutionary adaptation allowing the adaptation to different environmental conditions (Stover *et al*., 2000). It is a facultative anaerobe which uses nitrogen as an acceptor in the absence of oxygen so as to achieve growth (Mesquita *et al*., 2013). The optimum growth temperature is 37^{o} C although *P. aeruginosa* can grow at 42^{o} C. The two pigments (Pyoverdin and pyocyanin) and odour produced by *P. aeruginosa* distinguish it from other bacteria (Todar, 2008). Pyocyanin gives the colonies their characteristic blue-green colour (Gellatly and Hancock, 2013) whilst the fluorescent pigment pyoverdin is produced in low nutrient concentrations and aids in the metabolism of iron (Jamunadevi *et al*., 2012).

P. aeruginosa is the epitome of opportunistic pathogens and causes diverse infections like respiratory, wound, urinary tract infections and bacteremia. It is also the second lead cause of sepsis in Intensive Care Unit (ICU) patients (Jamunadevi *et al*., 2012). Furthermore, PSA causes bacteremia and septicaemia in patients with AIDS, severe burn and diabetes related immunodeficiency (Mesaros *et al*., 2007). The bacterium initiates infection by exploiting and breaking host defences though hardly infecting uncompromised tissues. *P. aeruginosa* isolated from clinical specimens has two colony types. The first type colony has a fried-egg appearance which is large and has flat edges which look elevated. The second type of colonies are mucoid and are usually isolated from secretions from urinary and respiratory tract infections (Todar, 2008).

Pseudomonas aeruginosa has great antibiotic resistance and approximately 0.3% of the genes code for antibiotic resistance proteins. It has a high capacity to resist antibiotics intrinsically, or post acquisition of resistance gene, overexpression of efflux pumps, decreased expression of porin coding genes and through mutation. The mechanisms of resistance are expressed simultaneously and this results in multi-resistance (Mesaros *et al*., 2007).

Regardless of the advances made in medical and surgical care as well as improvement and introduction of antibiotics, *P. aeruginosa* still posses as a threat to patients as it has high antibiotic resistance (Bekele *et al*., 2015). Resistant strains cause a three-fold higher mortality rate and this is a cause for concern as healthcare costs are also increased (Mesaros *et al*., 2007). *P. aeruginosa* is highly resilient as it forms biofilms in the environment. Its resilience is greatly influenced by antibiotic and disinfectant resistance of the biofilms and this is a great medical challenge. Treatment options for *P. aeruginosa* are limited due to the emergence and spread of antibiotic resistant strains. Resistance to antibiotics is often exhibited and the treatment of persistent infections is hindered by adaptive resistance which results in mortality (Gellatly and Hancock., 2013).

2.2 Pathogenesis of *P. aeruginosa*

Sources of *P. aeruginosa* can be in two forms, endogenous where isolates have unique genotypes or exogenous whereby isolates will have the same genotypes with environmental or other patient samples (Mansour *et al*., 2013). Pathogenesis of *P. aeruginosa* infections is determined by a wide array of factors which are determinants of virulence. Most of the infections are invasive and toxinogenic with an infection being comprised of three major stages which are bacterial attachment and colonisation, invasion and finally dissemination (Todar, 2008).

2.2.1 Colonisation

Colonisation is the first stage of any infection post transmission of the pathogen. *P. aeruginosa* adheres to the epithelial cells using its pilli and produces target specific adhesins that aid in binding onto the cells (Todar, 2008). The flagellum binds onto glycolipids during infection (Gellatly and Hancock., 2013). Mucoid strains have additional adhesion due to the production of alginate. The alginate helps anchor the bacterial cells in biofilms to the surfaces of the site of infection and host tissues may be injured during colonisation (Todar, 2008).

2.2.2 Invasion

The production of enzymes and toxins which breakdown barriers and damage host cells plays an important role in the invasion of tissues by *P. aeruginosa*. Invasion is made successful by the ability to evade host immune defences and resistance to phagocytosis. This is because *P. aeruginosa* cells are protected from antibodies and phagocytes by the slime (mucoid) layer. Enzymes elastase and alkaline protease are highly active in the invasion stage by interfering with the epithelium as well as fibrin formation allowing for the attachment of the bacterium. Three soluble proteins also play a role in *P. aeruginosa* in invasion and these are a cytotoxin (leukocidin) and two haemolysins (phospholipase and lecithinase). The cytotoxin forms pores and affects neutrophils.

The haemolysins and cytotoxin have cytotoxic effects on host defence cells thus contributing to invasion (Todar, 2008). The integrity of physical barriers in the host is thus disturbed by bacterial invasion (Gales *et al*., 2001).

2.2.3 Dissemination

Dissemination of *P. aeruginosa* is mediated by cell associated and extracellular products of specific diseases/ infections. Resistance to antibiotics further promotes dissemination of *P. aeruginosa*. Some exotoxins lead to the symptoms of septicaemia through pathological activity (Todar, 2008).

2.3 Virulence factors and associated host responses

Different virulence factors interfere with host defences by damaging the tissues or enhancing the competitiveness of the bacterium (Gellatly and Hancock, 2013). Some of the virulence factors are cell associated whilst some are secreted after colonisation. These virulence factors make pathogenesis successful and include exoenzymes, proteases and pigments. The multifactorial virulence of *P. aeruginosa* differs from isolate to isolate and stages of infection (Mesquita *et al*., 2013). Many cells in the body of the host coordinate to prevent colonisation of *P. aeruginosa*. The different symptoms of infection are as a result of host responses and virulence factors of the bacterium (Gellatly and Hancock., 2013).

2.3.1 Flagella and Type 4 pilli

P. aeruginosa possesses a single flagellum and several Type 4 pilli which function as adhesins and also in motility. These structures cause inflammatory responses in infection (Gellatly and Hancock., 2013). The flagellum initiates host immune responses as it is very immunogenic (Mesquita *et al*., 2013). It adheres to the epithelial cells and also allows the bacterium to swim in aqueous environments thus playing an important role in the initiation of infection.

The type 4 pilli play the most important role in adhesion, motility and biofilm formation. The pilli functions in the aggregation of *P. aeruginosa* resulting in the formation of micro colonies on tissues. These micro colonies aggregate the bacteria in one place and protect *P. aeruginosa* from host immune responses and antibiotics. Antipseudomonal therapy targets the flagella and pilli though antigenic variability of the pilli in strains hinders their success (Gellatly and Hancock., 2013).

2.3.2 Type 3 secretion system (T3SS)

This is a property of many pathogenic gram negative bacteria which allows the direct secretion of toxins into the host's cells and is a major virulence factor in *P. aeruginosa*. Effector proteins are released into the host by the bacterium through pores formed in the cell membrane of the host. *P. aeruginosa* has 4 effector proteins/ exotoxins which have been identified and these are ExoY, ExoS, ExoT and ExoU. These effector proteins are enzymes. ExoU is a phospholipase which acts as a cytotoxin and causes death of host eukaryotic cells through the loss of plasma membrane integrity. *P. aeruginosa* exploits breaches in the epithelial barrier of the host using the T3SS thus preventing wound healing and promoting cell injury (Gellatly and Hancock., 2013).

2.3.3 Quorum sensing systems and biofilms

Quorum sensing is a mechanism which allows bacteria to adapt to environmental changes (Gellatly and Hancock, 2013). The systems allow the cell to cell communication of *P. aeruginosa* through the sensing of extracellular generated concentrations of signal molecules/ autoinducers (Mesquita *et al*., 2013). The autoinducers act as cofactors in transcriptional regulation of genes in response to environmental changes or population increase (Gellatly and Hancock, 2013) as well as synchronizing the behaviours of bacteria (Mesquita *et al*., 2013). The auto inducers determine the degree of pathogenicity of *P. aeruginosa* as they control cell survival, biofilm formation and virulence (Gellatly and Hancock., 2013).

Biofilms are highly organised bacterial communities encased in extracellular polymeric substances which are attached to each other and attached to a surface. The volume of the biofilm allows the community to resist mechanical forces as well as reducing or hindering the penetration of antibiotics and host cell defence molecules. Bacteria in biofilms may have increased antibiotic resistance as they are adapted to general stress since resource are limited in the biofilm (Gellatly and Hancock., 2013). Biofilms are therefore the other reason for persistent infection since they serve as environmental reservoirs of bacteria (Matz *et al*., 2008).

2.3.4 Proteases

P. aeruginosa secretes several proteases which play a role in the establishment of infection. They degrade immunoglobulins and cause the disruption of tight epithelial junctions. Host complement proteins and fibronectin are degraded by alkaline protease. Alkaline protease aids *P. aeruginosa* in avoiding detection by the host. LasA and LasB are elastases produced which have proteolytic effects on the host tissues (Gellatly and Hancock., 2013).

2.3.5 Pigments

Pyocyanin induces oxidative stress on host cells and disrupts the mitochondrial electron transport (Gellatly and Hancock., 2013). Catalase is a radioactive phenazine compound which generates reactive oxygen. It inhibits the multiplication of T-lymphocytes in patients (Jamunadevi *et al*., 2012) as well as inhibiting phagocytosis and induces apoptosis in neutrophils (Gellatly and Hancock, 2013). Pyoverdin aids in iron metabolism (Jamunadevi *et al*., 2012) as it plays a role in iron chelation which is important in the establishment of infection (Gellatly and Hancock., 2013).

2.3.6 Lipopolysaccharide and Exotoxin A

Lipopolysaccharide is a glycolipid which plays a role in antigeniety, inflammatory responses, exclusion of external molecules and in the interaction with antibiotics by *P. aeruginosa* thus contributing to antibiotic resistance. Exotoxin A inhibits protein synthesis in the host cells thus resulting in decrease in immune response and cell death (Gellatly and Hancock., 2013).

2.4 *Pseudomonas aeruginosa* related infections

P. aeruginosa causes nosocomial infections worldwide (Strateva and Yordanov, 2009) and has impacts on the medical costs, morbidity and mortality in hospitals and communities. Patients with underlying medical conditions like old age, immunosuppression and neutropenia are mostly affected (Gellatly and Hancock, 2013). Most of the infections are usually life threatening and difficult to treat (Anil and Shahid., 2013).

2.4.1 Diagnosis of *P. aeruginosa*

The gram morphology of *P. aeruginosa* is used in its basic identification. Inability to ferment lactose, production of a fruity odour as well as a positive oxidase reaction is also used in identification. The advanced method of distinguishing and identifying *P. aeruginosa* involves checking for florescence of colonies under ultraviolet light (Todar, 2008).

2.4.2 Urinary tract infection

The most common bacterial infections in developing countries are urinary tract infections with approximately 250million people diagnosed annually (Alo *et al*., 2015). *P. aeruginosa* causes about 12% of hospital acquired urinary tract infections (Mesaros *et al*., 2007) and most infections are related to catheterization (Todar ,2008). *P. aeruginosa* adheres to the bladder uroepithelium causing infection to occur either via the ascending or descending route. *P. aeruginosa* from the urinary tract can invade the bloodstream causing bacteremia (Todar, 2008).

Females are more prone to infections than males as their urethra is short and is located close to the anus. Female susceptibility to urinary tract infections is increased by pregnancy and sexual activity (Lisa *et al*., 2015). Urinary tract infection causing bacteria have been changing over the years and there has been a shift in treatment options (Joshi *et al*., 2011).

2.4.3 Wound infections

Wounds are as a result of disruption of the epithelial integrity of the skin and infection is due to colonisation of the wound by microbes (Shrestha & Sharma 2013). The integument of the skin is broken by burns, dermatitis and incisions and immunocompromised patients with AIDS are easily infected by *P*. *aeruginosa* (Todar, 2008). Increases in mortality and morbidity rates occur due to sepsis, amputation of limbs and other wound infections (Shrestha & Sharma 2013) and these sites can be easily colonized by microorganisms with PSA being of particular interest.

2.5 Antibiotic classes and action

Antibiotics are chemical compounds produced by microorganisms which are used to interfere with or stop the normal function and structure of bacteria (Modak *et al*., 2013). Different antibiotics have been discovered and produced since 1929 (Todar, 2008) and their discovery was a great medical achievement as lives could be saved (Modak, 2013). Antibiotics are categorized into classes according to their targets and mode of action (Todar, 2008). Major antibiotic classes interfere with four targets which can either be protein, cell wall, folate or RNA and DNA biosynthesis (Modak *et al.*, 2013).

2.5.1 β-lactams

All β-lactam antibiotics are structurally related as they have a β-lactam ring (Figure 2.1) which interferes with the cell wall synthesis in bacteria. Transpeptidases are inhibited by the action of β-lactam antibiotics (Lakshmi *et al*. 2014).

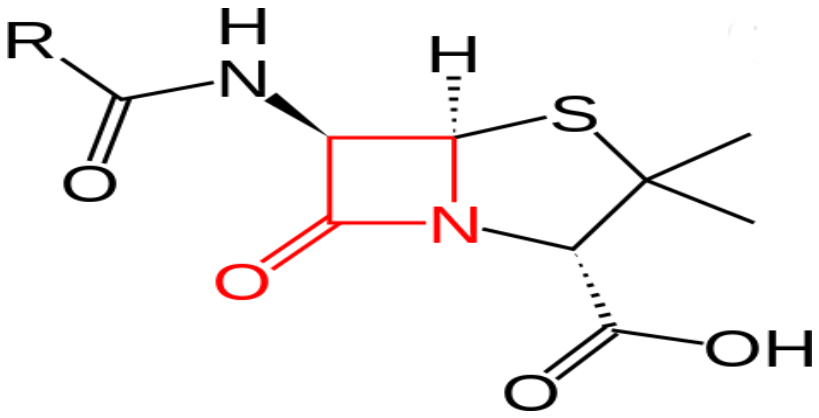

Figure 2.1: Structure of Beta lactam antibiotics

Piperacillin/Tazobactam is a Beta lactam/ penicillanic acid sulfone combination made up of Piperacillin and Tazobactam. Tazobactam inhibits beta lactamases produced by the microorganism whilst Piperacillin inhibits cell wall synthesis (Berger, 2014).

Imipenem is a carbapenem and has the same activity as β-lactams. It inactivates the penicillin binding proteins and cause lysis of the cell wall. It is has a wide spectrum of activity and is used when there are limited treatment options for infection treatment (Papich, 2015). The risk of Imipenem resistance is high in patients who would have previously used other antibiotics like Piperacillin/ Tazobactam and Vancomycin (Onguru *et al.*, 2008). Carbapenems are generally the main drugs used in the treatment of *P. aeruginosa* (Rizek *et al.*, 2014).

2.5.2 Quinolones

Ciprofloxacin is a Fluoroquinolone antibiotic which is used to treat diverse infections. It is mainly used to in treatment of infections caused by gram negative organisms. Quinolones (Ciprofloxacin and Norfloxacin) inhibit DNA gyrase and topoisomerase IV therefore DNA replication in the bacteria is inhibited by the inhibition of the two enzymes (Francis *et al.*, 2015). Fluoroquinolones are the only antibiotics available for oral treatment of *P. aeruginosa*.

resistance to these antibiotics has developed in the form of mutation of target genes (Jalal *et al.* 2000*).*

2.5.3 Cephalosporins

Ceftriaxone is a third generation Cephalosporin which interferes with the cell wall membrane by binding onto it and causing cell death. It has bactericidal effects against susceptible bacteria (Vallerand and Sanoski., 2014).

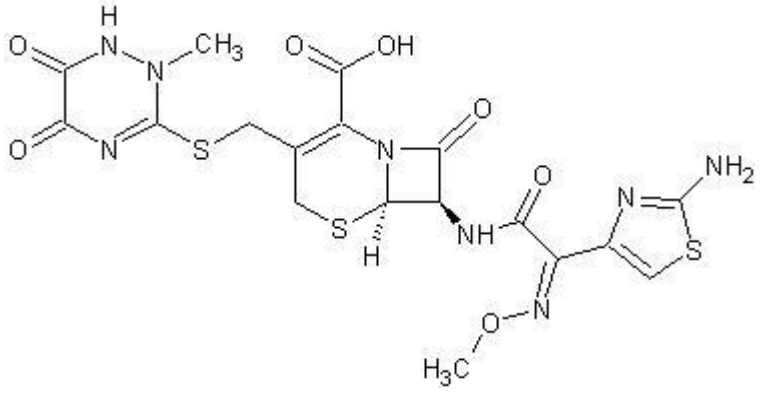

Figure 2.2: Structure of Ceftriaxone

2.5.4 Tetracycline

Tetracyclines are a class of antibiotics of broad spectrum antibiotics (Chopra and Roberts, 2001). The specific antibiotic Tetracycline is a 1st generation Tetracycline. Tetracycline antibiotics prevent the attaching of amino-acyl- Trna onto the ribosome thus inhibiting protein synthesis. They are effective against both gram positive and negative bacteria (Fuoco, 2012) though resistance in microorganisms has limited the efficacy of tetracyclines (Chopra and Roberts, 2001). The structure general structure of Tetracycline is shown in Figure

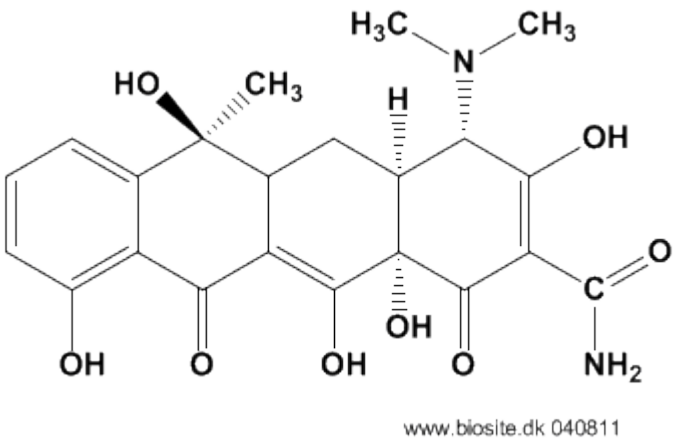

Figure 2.3: Structure of Tetracycline

2.5.5 Aminoglycosides

A typical example of an Aminoglycoside is gentamicin (Larson, 2015). Gentamicin is produced by the species Micromonospora and is active against both gram negative and positive bacteria. It has effects on the protein synthesis within the bacterium (Milanesi and Ciferri, 1966) through the misreading of mRNA (Tangy *et al*., 1985).

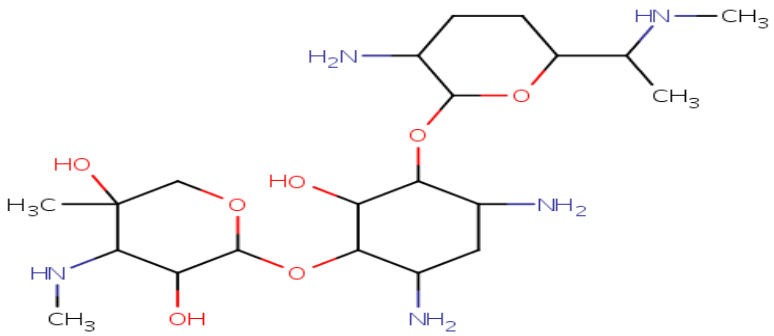

Figure 2.4: Structure of Gentamicin

Gentamicin is used as the first line of defense in infections caused by gram negative organisms (Larson, 2015).

2.6 Mechanisms of antibiotic resistance in *P. aeruginosa*

P. aeruginosa has the intrinsic ability to resist most classes of antibiotics. It can also acquire resistance and the combination of both intrinsic and acquired resistance make the treatment of *P. aeruginosa* infections difficult. Intrinsic resistance is encoded in the genome whilst acquired resistance is as a result of genetic transfer, mutation and expression of a resistance cassette taken up by *P. aeruginosa*. Adaptive resistance is due to adaptation to environmental stress which may cause changes in gene expression. All forms of resistance may be expressed within a single isolate and the rate of resistance is increasing regardless of the use of combination drug therapies. Older drugs like polymyxins are now being used due to limited availability of new drugs to prevent and treat PSA infections (Gellatly & Hancock 2013).

2.6.1 Outer membrane

The outer membrane forms a barrier which inhibits the penetration of hydrophilic molecules (Lambert 2002). The outer membrane restricts the uptake of β-lactam antibiotics to the porin proteins on the outer membrane (Gellatly & Hancock 2013). Resistance to aminoglycosides can be due to low accumulation as a result of reduced permeability of the outer membrane (Strateva & Yordanov 2009).

2.6.2 Over-expression of efflux pumps

P. aeruginosa has 12 efflux pumps which eject antibiotics (Gellatly & Hancock 2013) thus making it the microbe with the highest number of efflux pumps as compared to other infection causing pathogens (Stover et al. 2000).

An efficient extrusion system for toxic substances is formed by the tripartite arrangement of efflux pumps (Lambert 2002) as these systems have wide substrate specificity(Strateva & Yordanov 2009). Proteins located in the cytoplasmic membrane (MexB, MexF, MexD, MexY) which are energy dependent pumps that have wide substrate specificity. The gated

outer membrane protein (OprM, OprJ, OprN, OprM) are the second component whilst MexA, MexC, MexE and MexX which are located in the periplasm space form the third protein linking the other two proteins. The pumps are categorised into four genetically different systems which are MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM (Strateva & Yordanov 2009). Mex AB-OprM and MexXY-OprM efflux β-lactams, tetracycline and aminoglycosides whilst MexEF-OprN increases resistance to quinolones. The over production of efflux pumps result in increased antibiotic minimum inhibitory concentrations (Gellatly & Hancock 2013).

2.6.3 Secretion of enzymes

Enzyme inactivation of antibiotics has spread in strains and this is high in the case of β-lactams and aminoglycosides. Strains possessing extended-spectrum β-lactamases are resistant to all β-lactams except carbapenems. The enzymes are limited to strains found in certain geographic areas and the population of strains with extended-spectrum β-lactamases is increasing worldwide. AmpC plays a role in the hydrolysis of several β-lactam antibiotics. Some strains have carbapenem-hydrolysing metallo- β-lactamases which inactivate β-lactam subclasses except the monobactams. The genes encoding carbapenemases are located in isolation but with other resistance genes like those coding for aminoglycoside-inactivating enzymes. The isolates with different genes located close to each other show co-resistance in phenotypes (Mesaros et al. 2007).

2.6.4 Mutation, Target modification and loss of OprD

Mutation alters the antibiotic's target thus the antibiotic has no binding site (Gellatly & Hancock 2013). Resistance to Fluoroquinolones is through target mutation. Fluoroquinolones target topoisomerase IV and DNA Gyrase with DNA Gyrase being the primary target in *P. aeruginosa*. Ciprofloxacin targets Gyrase and in mutation, the efficacy is reduced (Mesaros et al. 2007). Resistance to Aminoglycosides is through target modification and this can be

through the methylation of 16S rRNA (Mesaros et al. 2007). The diverse substrate range and overexpression of efflux systems in *P. aeruginosa* are very important mutational resistance mechanisms (Strateva & Yordanov 2009). Resistance to Imipenem is due to the loss of outer membrane protein OprD (Hirsch & Tam 2010).

2.7 Multi-drug resistance (MDR)

Multi-drug resistance is the resistance to at least three antibiotics from different antibiotic classes (Ansari et al. 2015). *P. aeruginosa* has a multi-drug resistance phenomena and is usually as a result of a combination of mechanisms to overcome antibiotic stress (Mesquita et al. 2013)The mutational hyper expression of efflux genes increases acquired multi-drug resistance in *P. aeruginosa* (Poole, 2001). Multi-drug resistant *P. aeruginosa* increases both patient mortality and morbidity (Hirsch and Tam, 2010). It was found that the mortality rates rose from 25% to 60% in hospitals (Nathwani *et al*., 2014) Antibiotic resistant infections have an economic impact as the treatment costs increase due to hospitalization as well as antibiotic costs (Hirsch and Tam, 2010). Cost of treatment on MDR *P. aeruginosa* infection in the US ranges from $27.710 to $187,260 thus proving to be very expensive (Nathwani *et al*., 2014).

2.8 Treatment options

P. aeruginosa can develop resistance during the course of treatment and this posses as a great problem (Lister *et al*., 2009). The availability of therapeutic options is limited by the increase in MDR *P. aeruginosa* (Anil and Shahid, 2013) and the effect of anti-pseudomonals is unreliable (Carmeli *et al*. 1999). *P. aeruginosa* has become highly resistant to commonly used antibiotics and resistance to newer drugs is increasing (Parmar *et al*., 2013). Most of the cheaper antibiotics have lost their efficacy due to malpractices as a result, complicated and expensive drugs are being used to try and limit infections (Rajat *et al*., 2012). Therapeutic

options should limit antibiotic pressure so as to avoid selection which happens during treatment and may result in treatment failure (Mesaros *et al*., 2007). Aminoglycosides may be used in combination treatment of *P. aeruginosa* (Carmeli *et al*., 1999). Alternative use of oils extracted from plants like Thyme and marjoram may be useful in the treatment of *P. aeruginosa*. Plants may be useful as they are potentially useful in the treatment of infections as they produce compounds which have the potential of being used in combination with antibiotics (El-hosseiny *et al*., 2014).

CHAPTER 3: MATERIALS AND METHODS

3.1 Study site

The study was carried out at two private medical laboratories in Bulawayo over a period of seven months (July 2015-January 2016). The two laboratories were Diagnostics Laboratory services and Southern pathology.

3.2 Sample collection and Primary sample screening

The samples were screened from 1200 samples based on the presence of blue-green pigmentation due to pyocyanin and pyoverdin as well as a fruity odour on the culture plates. Wound pus swab samples cultured on MacConkey media were selected based on the fermentation of lactose and inhibition of proliferation if gram negative bacteria and β-haemolysis on Chocolate and Blood agar were also used in the selection/ screening process. B-haemolysis on Blood agar and growth on Cysteine Lactose Electrolyte Deficient (CLED) agar were used to selection of urine samples. The large flat and elevated morphology of colonies was also used to screening *P. aeruginosa* from the media plates. Pus swab samples were from wound infections including burns and post operative sites which were swabbed. The samples were grouped according to gender to determine the gender-based prevalence of *P. aeruginosa* in the urinary tract and wound infections.

3.3 Identification and Confirmation of *P. aeruginosa*

After selection and isolation of colonies, the oxidase test and the Gram's were carried out on the putative positive cultures to confirm *Pseudomonas aeruginosa.* Biochemical tests were also carried out and these include indole, motility and citrate tests.

3.3.1 Oxidase test

A single colony was picked from MacConkey and CLED media plates using a sterile loop and smeared on an oxidase test strip composed of filter paper containing oxidase reagent. The strip was observed for ten seconds and a colour change was recorded.

3.3.2 Gram stain

A single colony was smeared on a glass slide, air dried and heat fixed to avoid washing off of the smear. Crystal violet was flooded on the slide for one minute and washed off using distilled water. The slide was blot dried then flooded with Gram's iodine for one minute and washed. Acetone was flooded onto the slide to destain the stained smear and then washed off using distilled water. The distilled water was tipped off the slide and Safranin O was flooded onto the slide for one minute and then washed off using running water. The slide was air dried and viewed under a microscope at X 100 magnification for the general overview and distribution of cells and at X100 with oil immersion using the objective lens to ascertain the morphology and Gram status of the bacterial cells.

3.3.3 Indole test

Indole media was prepared according to manufacturer's guidelines. A single colony of the was inoculated in bijou bottles containing 4 ml sterile Tryptone water and incubated at 35-37^{o} C for 18- 48hrs. A volume of 0.5 ml Kovac's reagent was added to the bijou bottles and gently shaken so as to test for the production of indole.

3.3.4 Motility test

Motility test media was aseptically prepared according to manufacturer's guidelines in 8 ml bijou bottles and a single colony was inoculated (stabbed) into the media and incubated at 35-37^{o} C for 16-18 hrs. The media was observed for a distributed pink/ red colour which indicated the presence of a motile organism.

3.3.5 Citrate test

Simmon's citrate media was prepared according to the manufacturer's guidelines and poured into sterile petri dishes aseptically. A single colony was inoculated into the medium using a sterile loop and incubated at 35-37^{o} C for up to 48 hrs. The medium was observed for the presence of a bright blue colour.

3.4 Antibiotic Sensitivity Testing of *P. aeruginosa* isolates

3.4.1 Antibiotics used in sensitivity tests

Eight different drugs from five antibiotic categories/ classes (Table A.2, Appendix A).were tested using a modified Kirby-Bauer disc diffusion method (Section 3.4.2). The antibiotics tested were Imipenem, Piperacillin, Norfloxacin, Tetracycline, Gentamicin, Ceftriaxone, Ciprofloxacin and Piperacillin/Tazobactum.

3.4.2 Modified disc diffusion assay

Muller-Hinton media was prepared using standard procedures and stored at 4^{o} C. A volume of 2 ml normal saline was pipetted into a sterile aliquot tube. The bacterial sample was continuously inoculated into the saline using a sterile wire loop until a turbidity which matched that of 0.5 McFarland's solution (turbidity standard) was achieved. A sterile loop was dipped into the standardized suspension and used to streak over the surface of the Muller-Hinton medium. The plate was rotated during streaking to ensure even distribution of the sample. Drug discs of the eight mostly used antipseudomonal drugs were placed on the medium using sterile forceps and the plates were incubated under aerobic conditions at 35-37^{o} C for 16-18 hours. After incubation, the diameters of the zone of inhibition for each drug was measured on the underside of the plate using a ruler and recorded.

The results were interpreted using Clinical Laboratory Standards Institute (CLSI) tables which relate zone diameter to microbial resistance/sensitivity to specific drugs.

3.5 Data Analysis

The overall, sample specific and gender based prevalence of *P. aeruginosa* in the isolates was determined using the formula:

Prevalence=Number of positive *P. aeruginosa* isolates obtained ÷ Total number of isolates

Sample percentage susceptibility was calculated using the formula:

Susceptibility=Number of sensitive/ Resistant isolates ÷ Total Number of isolates X 100%

The isolate specific antibiotypes/ resistance patterns which is a collection of all the antibiotics a specific isolate was resistant to were determined.

The data obtained for the different antibiotics was analysed using the Chi-square test for Heterogeneity of variances in SPSS so as to determine the most effective drug/drugs for treating *P. aeruginosa* mediated wound and urinary tract infections.

CHAPTER 4: RESULTS

4.1 Prevalence and Distribution of PSA

Out of a total of 1200 clinical samples screened for *P. aeruginosa* using gram's and biochemical tests 426 samples were from wound swabs and 784 were urine samples. Out of the 78 samples that were positive for *P. aeruginosa,* 42 were from pus swabs and 36 were from urine. The overall prevalence of *P. aeruginosa* in this study was 6.5%. The prevalence of *P. aeruginosa* in pus swab samples was 9.8% whereas that of urine samples was 4.59 % (Table 4.1).

Table 4.1: Overall and sample specific prevalence

Type of prevalence	**Overall**	**pus swab samples**	**urine samples**
Percentage	6.5	9.86	4.59

The prevalence of *P. aeruginosa* in male patients was of 62% and the prevalence in females was 38%(Figure 4.1). *P. aeruginosa* was more prevalent in male patients than in female patients across the specimen types.

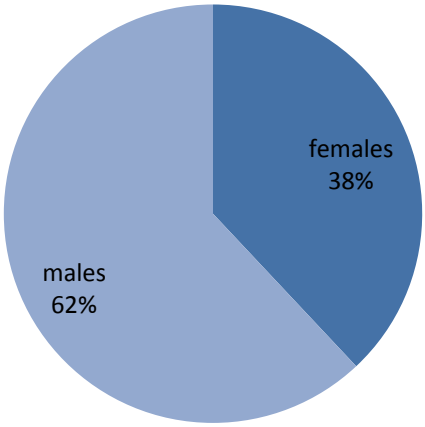

Figure 4.1: Gender based prevalence of *P. aeruginosa* isolates

4.2 Antibiotic Susceptibility Patterns of *P. aeruginosa*.

4.2.1 Antibiotic Sensitivity patterns of *P. aeruginosa* isolated from pus swab samples.

The antibiotic sensitivity patterns of *P. aeruginosa* in pus swabs differed across antibiotics as the percentage sensitivity values were different. *P. aeruginosa* isolates from pus swabs were highly sensitive to Imipenem with a percentage sesitivity value of 88.1%. The isolates were least sensitive to Tetracycline with a sensitivity value of 28.57% (Figure 4.2.1).

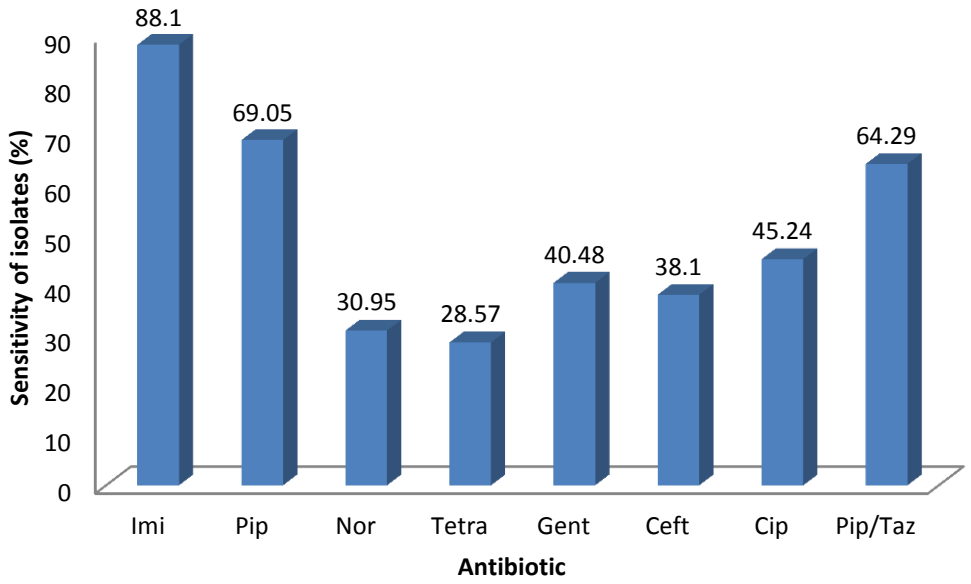

Figure 4.2.1: Percentage antibiotic sensitivity patterns of *P. aeruginosa* isolated from pus swab samples

Key- Imi- Imipenem, Pip- Piperacillin, Tetra- Tetracycline, Cip- Ciprofloxacin, Ceft- Ceftriaxone, Nor- Norfloxacin, Pip/Taz- Piperacillin-Tazobactum, Gent-Gentamicin

4.2.2 Antibiotic Sensitivity patterns of P. aeruginosa isolated from urine samples.

The *P. aeruginosa* isolates obtained from urine were most sensitive to Imipenem (83.33%). The least sensitivity in the isolates was confired towards Tetracycline and Gentamicin both with a percentage values of 36.11% (Figure 4.2.2).

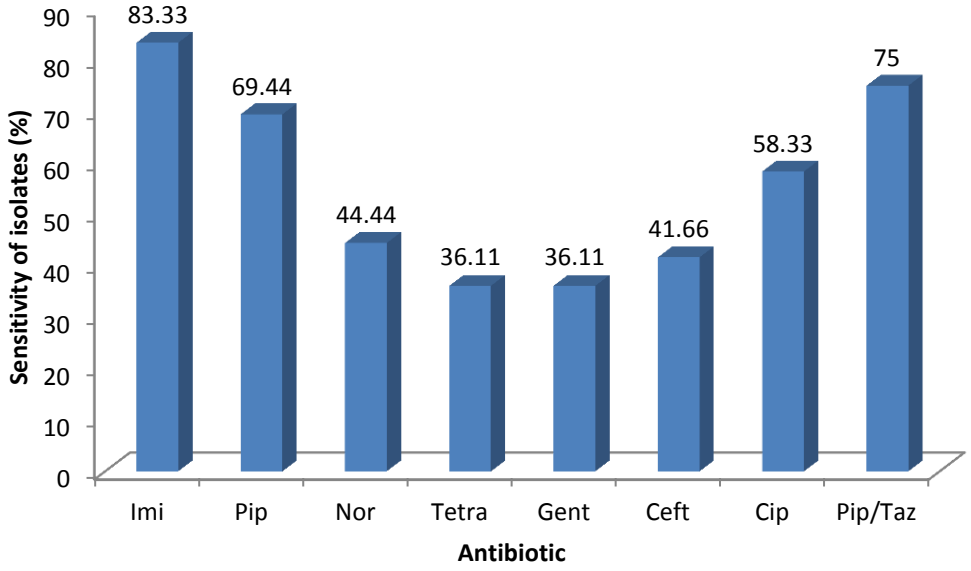

Figure 4.2.2: Percentage antibiotic sensitivity patterns of *P. aeriginosa* isolated from urine samples

Key- Imi- Imipenem, Pip- Piperacillin, Tetra- Tetracycline, Cip- Ciprofloxacin, Ceft- Ceftriaxone, Nor- Norfloxacin, Pip/Taz- Piperacillin-Tazobactum, Gent-Gentamicin

4.3 Antibiotic Resistance Patterns of *P. aeruginosa*.

4.3.1 Antibiotic Resistance patterns of *P. aeruginosa* isolated from pus swab samples.

The highest resistance was to Tetracycline with a value of 71.43%. The isolates were least resistanct to Imipenem with a percentage resistance of 11.9%. There was equal resistance towards Gentamicin and Ceftriaxone both with values of 59.52% (Figure 4.3.1).

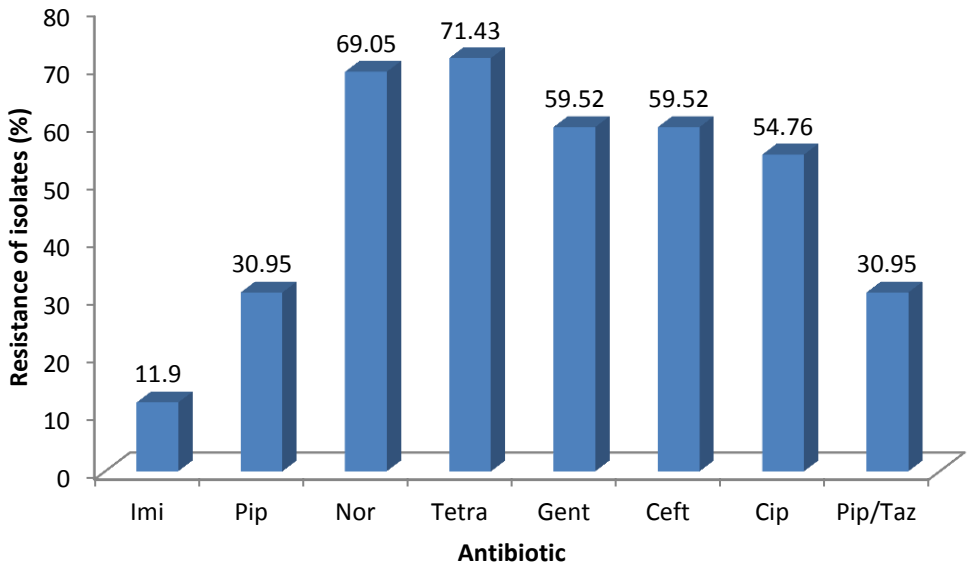

Figure 4.3.1: Percentage antibiotic resistance patterns of *P. aeruginosa* in pus swab samples

Key- Imi- Imipenem, Pip- Piperacillin, Tetra- Tetracycline, Cip- Ciprofloxacin, Ceft- Ceftriaxone, Nor- Norfloxacin, Pip/Taz- Piperacillin-Tazobactum, Gent-Gentamicin

4.3.2 Antibiotic Resistance patterns of *P. aeruginosa* isolated from urine samples

The highest antibiotic resistance in urine samples isolates was towards Tetracycline and Gentamicin (63.89%). Isolates from urine samples had least resistance towards Imipenem with a frequency of 16.67%. There was equal resistance towards Norfloxacin and Ceftriaxone as they both had values of 55.56% (Figure 4.3.2).

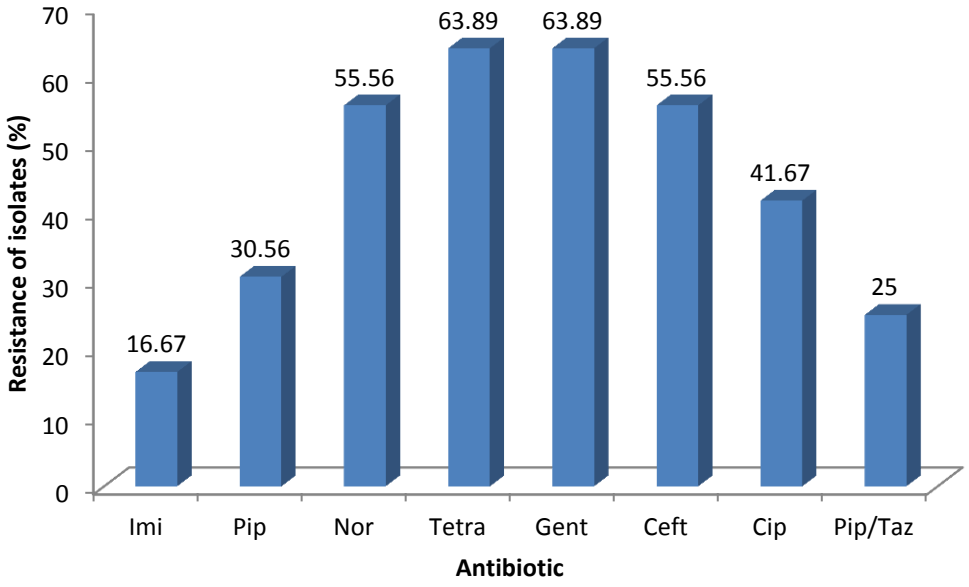

Figure 4.3.2: Percentage antibiotic resistance pattern of *P. aeruginosa* in urine samples

Key- Imi- Imipenem, Pip- Piperacillin, Tetra- Tetracycline, Cip- Ciprofloxacin, Ceft- Ceftriaxone, Nor- Norfloxacin, Pip/Taz- Piperacillin-Tazobactum, Gent-Gentamicin

4.4 Antibiotypes of *P. aeruginosa* isolates

4.4.1 Antibiotype of *P. aeruginosa* isolated from pus swabs

The antibiotype of *P. aeruginosa* isolated from pus swabs showed marked resistance to different antibiotics tested . Overall, a total of 32 resistance patterns were obtained amongst the pus swab isolates. The most common resistance pattern (Nor^R $Tetra^R$ $Genta^R$ $Ceft^R$ Cip^R)was obtained in five isolates with a percentage frequency value of 11.90%. Out of the 32 antibiotypes, 27 isolates had different antibiotypes/ resistance patterns which were unique and had percentage frequencies of 2.38% (Appendix B, Table B5). One isolate, A1 (Appendix B, Table B5) was resistant to one antibiotic (Tetracycline) with a frequeny of 2.38%. A total of

fifteen isolates (A1-A3, Appendix B, Table B5) showed resistance to up to three antibiotics with the antibiotypes having percentage frequencies of either 2.38% or 4.76%.

4.4.2 Antibiotypes of *P. aeruginosa* isolated from urine samples

A total of 29 antibiotypes/ resistance patterns were obtained for *P. aeruginosa* urine isolates. Three isolates had the most common resistance pattern (Nor^R $Tetra^R$ $Ceft^R$ Cip^R) with a frequency of 8.34%. Out of the 29 antibiotypes, 25 were unique to an individual isolate with frequencies of 2.78% each. Only one isolate was susceptible to all of the antibiotics tested (Appendix B, Table B6). A total of five isolates were resistant to single drugs each having a frequency of 2.78%. Most of the *P. aeruginosa* isolates (fourteen) were resistant to four antibiotics each (Appendix B, Table B6)

The antibiotypes A6 and A7 (Appendix B, Table B5, Table B6) showed Extremely Drug Resistant (XRD) isolates. These isolates were resistant to six or seven antibitics with each isolate having a unique resistance pattern and a frequency of 2.38%.

4.5 Drug efficacy on *P. aeruginosa* isolates

4.5.1 Drug efficacy on *P. aeruginosa* isolated from pus swabs

The Chi-square Heterogeneity of proportions showed that the proportions of sensitive *P. aeruginosa* isolates were significantly different among the drugs ($P<0.05$ refer to appendix C , table C1.1). Therefore the efficacy of the eight antibiotics against the isolates was therefore significantly different. The proportion of sensitive isolates for Imipenem was significantly different from the proportions of all the other antibiotics (88.1% refer to appendix C, table C1.5.1). Imipenem had the highest efficacy on *P. aeruginosa* isolates isolated from pus swabs. The proportions of sensitive isolates were unequal for Imipenem and Piperacillin ($P <0.05$ refer to appendix C, table C1.5). The multiple comparisons of proportions of the two antibiotics showed that there were significant differences in the efficacy of the antibiotics against *P. aeruginosa* isolated from pus swabs. The proportion of sensitive *P. aeruginosa*

isolates for Norfloxacin, Tetracycline, Gentamicin, Ceftriaxone, Ciprofloxacin and Piperacillin/Tazobactam were unequal (P<0.05 refer to appendix C, table C1.2). Therefore this showed that the latter antibiotics had significantly different effects on the isolates. The proportions of sensitive isolates were unequal for Imipenem, Piperacillin, Ciprofloxacin and Piperacillin/Tazobactum (P<0.05 refer to appendix C, table C1.4) showing that there were significant differences in the efficacy of these drugs against *P. aeruginosa* isolated from pus swabs. The four antibiotics were the most effective drugs against *P. aeruginosa* isolated from pus swabs. The proportions of sensitive *P. aeruginosa* isolates were equal for Norfloxacin, Tetracycline, Gentamicin and Ceftriaxone (P>0.05 refer to appendix C, table C1.3). Multiple comparisons of proportions of the four antibiotics showed that there were no significant differences in the efficacy of these antibiotics against *P. aeruginosa* isolated from pus swabs. The latter four antibiotics had the least efficacy against the *P. aeruginosa* isolated from pus swab samples.

4.5.2 Drug efficacy on *P. aeruginosa* isolated from urine samples

The Chi-square test for Heterogeneity of proportions showed that the proportions of sensitive *P. aeruginosa* isolates obtained from urine samples were unequal across all the eight antibiotics (P<0.05 refer to appendix C, table C 2.1) Therefore the eight drugs tested in this study had significantly different efficacies on the *P. aeruginosa* isolates. The proportions of sensitive *P. aeruginosa* isolates were unequal for Piperacillin, Norfloxacin, Tetracycline, Gentamicin, Ceftriaxone and Ciprofloxacin (P<0.05 refer to appendix C. table C 2.2). Therefore, the efficacy of the six antibiotics against the *P. aeruginosa* isolated from urine samples was significantly different. The proportions of sensitive isolates were equal for Imipenem, Piperacillin, Ciprofloxacin and Piperacillin/Tazobactam (P>0.05 refer to appendix C, table C 2.4) showing that there were no significant differences in the efficacy of the antibiotics against *P. aeruginosa* isolated from urine samples. Multiple comparisons of proportions showed that the latter four most performing antibiotics had the same effect on the *P. aeruginosa* isolates. The proportions of sensitive isolates were equal for Norfloxacin, Tetracycline, Gentamicin and Ceftriaxone (P>0.05 refer to appendix C, table C 2.3). The multiple comparisons of proportions for the latter four least performing antibiotics showed that the antibiotics had the same efficacy against *P. aeruginosa* isolates obtained from urine samples.

CHAPTER 5: DISCUSSION

5.1 Prevalence and distribution of *P. aeruginosa*

The objective of this part of study was to isolate *Pseudomonas aeruginosa* from clinical samples and this was successful as a total of 78 positive isolates were obtained. The overall prevalence of *P. aeruginosa* obtained in this study (6.5%) showed that *P. aeruginosa* could potentially be significant in causing urinary tract and wound infections. The highest prevalence of *P. aeruginosa* was in pus swab samples (9.8%) as compared to urine samples (4.59%) and the high prevalence relates to the results obtained in a study by Rajat *et al.,* (2012). Wounds (burns, post operation lacerations) are a problem in the medical field as they have a high surface area for colonisation by microorganisms and due to the disruption of the skin, microorganisms colonise the site and proliferate causing infection (Anguzu and Olila, 2007). The relatively high prevalence of *P. aeruginosa* in wound infections may be due to inefficient infection control through sanitation in hospitals as shown by Mansour *et al.,*(2013) thus resulting in the contamination of the wounds by *P. aeruginosa*.

The prevalence of *P. aeruginosa* in urine samples shows that it poses a lesser risk in causing urinary tract infections as compared to wound infections. The higher prevalence in pus swabs (9.8%) indicates the spread of *P. aeruginosa* may be through physical contact as patients share resources like bath rooms and bedding and in the case of wounds, they are exterior, exposed and bacteria may be easily transmitted from the surfaces onto the wounds. *P. aeruginosa* and other microorganisms such as Klebsiella and Staphylococcus come after *Escherichia coli* which is the most common causative agent of urinary tract infections causing approximately 75-90% of the infections (Asati, 2013).

P. aeruginosa is resistant to most detergents and disinfectants (Todar, 2008) such that due to lack of adequate resources in hospitals in Zimbabwe, reservoirs may be established in the

hospitals making patients more prone to infection especially if immunocompromised. Cross contamination with hospital acquired strains plays an important role in the prevalence of nosocomial infections(Rishi *et al*., 2013). The high prevalence of *P. aeruginosa* (6.5%) may be due to the presence of multi-drug resistant strains in the hospitals (Gellatly and Hancock, 2013) which are resilient and difficult to treat. A study by Gellatly and Hancock, (2013) suggests that use of broad-spectrum antibiotics may also contribute to the high prevalence as *P. aeruginosa* has developed resistance to most antimicrobials. As a means of reducing the prevalence rates of *P. aeruginosa* it may be essential to reduce the formation of reservoirs which may increase the prevalence of *P. aeruginosa.* There is need to develop antibiotics and disinfectants which may interfere with biofilm production so as to avoid attachment of *P. aeruginosa* onto surfaces, enhance penetration of the chemicals as well as to interfere with the maturation of the biofilm (Rasamiravaka *et al.*, 2015).

The relatively high *P. aeruginosa* prevalence in males (62%) obtained in this study as compared to females (38%) shows that males are more prone to contracting *P. aeruginosa*. A study conducted by Rajat *et al*., (2012) in Ahmadabad showed that the prevalence in males were higher than in females. The high prevalence in males could be due to males not being as particular as women in terms of hygiene.

5.2 Antibiotic Susceptibility Patterns of *P. aeruginosa*

The objective of this part of the study was to determine the antibiotic sensitivity pattern of *P. aeruginosa* isolated from pus swab and urine isolates. The varied responses to the eight different antibiotics tested by P. aeruginosa shows that the antibiotics had different efficacies against the isolates.

5.2.1 Antibiotic Sensitivity Patterns of *P. aeruginosa* isolated from pus swab samples

P. aeruginosa isolated from pus swab samples had different sensitivity towards the antibiotics. The isolates were mostly sensitive to Imipenem, Piperacillin and Piperacillin/ Tazobactum. The percentage sensitivity of the isolates to the three above mentioned antibiotics ranged from 64.24- 88.1% (Figure 4.2.1). The least percentage sensitivity was towards Tetracycline and Norfloxacin respectively, with Tetracycline having the least percentage sensitivity. This shows that the isolates were less sensitive to Tetracycline and Quinolone classes of antibiotics as compared to the other classes. Ceftriaxone, Gentamicin and Ciprofloxacin had intermediate percentage sensitivity with values ranging from 38.1-45.24%. These results showed that *P. aeruginosa* was neither highly nor less sensitive to Aminoglycosides (Gentamicin), Cephalosporin (Ceftriaxone) and some Quinolones (Norfloxacin, Ciprofloxacin). Norfloxacin and Ciprofloxacin are both Quinolones but their effects on *P. aeruginosa* in this study were different and this may show that the isolates had different responses to these drugs regardless of them belonging to the same class. The different responses may be due to genetic variation within the isolates. The different responses to antibiotics of the same class showed that alternative drugs from the same class may be used in treating infection though this may be limited by the fact that *P. aeruginosa* is highly resistant to most common antibiotics.

5.2.2 Antibiotic Sensitivity patterns of *P. aeruginosa* isolated from urine samples

P. aeruginosa isolated from urine samples was highly sensitive to Imipenem, Piperacillin/Tazobactum and Piperacillin respectively with the percentage values ranging from 69.44- 83.33%. The isolates were highly sensitive to the β-lactam class of antibiotics as all the three drugs with the highest sensitivity belong to that class. The isolates were least sensitive to Tetracycline and Gentamicin as both the drugs had the least and equal percentage sensitivity values.

This shows that the two antibiotics could have more or less the same effect on the isolates regardless of belonging to different antibiotic classes. Ceftriaxone, Norfloxacin and Ciprofloxacin had intermediate sensitivity with the percentage values ranging from 41.66-58.33%. This showed that the *P. aeruginosa* isolates from urine samples were highly sensitive to β-lactam drugs, less sensitive to tetracycline and Aminoglycoside drugs and averagely sensitive to Quinolone and Cephalosporin classes of drugs.

Comparatively, *P. aeruginosa* isolates from both urine and pus swab samples were highly sensitive to β-lactam antibiotics. The isolates showed the highest sensitivity towards Imipenem whilst Tetracycline was the main antibiotic to which the isolates across both sample types were least sensitive to. This shows that *P. aeruginosa* is less sensitive to the effect of Tetracyclines and this may be due to the possession of inhibitory factors. Regardless of having similarities in terms of most and least sensitive drugs, the hierarchy of sensitive drugs differed in the samples as all the antibiotics obtained different sensitivity values. This may be explained by the presence of genetic variation amongst the isolates. Conclusively without considering the type of specimen, *P. aeruginosa* isolates were generally highly sensitive to β-lactam and Quinolone classes of antibiotics though the percentage range of sensitivity or response to these drugs was different in the samples. *P. aeruginosa* isolates obtained from urine samples had a higher range of sensitivity values (Figure 4.2.2) showing that they were more sensitive to the antibiotics than isolates obtained from pus swabs. The difference in sensitivity shown by the isolates may have been influenced by the severity of the infections.

5.3 Antibiotic Resistance Patterns of *P. aeruginosa*

5.3.1 Antibiotic Resistance Patterns of *P. aeruginosa* isolated from pus swab samples

All the isolates from pus swabs had a degree of resistance to the antibiotics (Chi-square P< 0.05). The greatest resistance towards Tetracycline (Figure 4.3.1) showed that most of the *P. aeruginosa* isolates from pus swabs inhibited the functioning of Tetracycline. This study further showed that *P. aeruginosa* isolates obtained from pus swab samples was greatly resistant to Tetracycline, Quinolone, Aminoglycoside and Cephalosporin classes of antibiotics (Appendix C1, Table C 1. 3). The isolates were least resistant towards Imipenem, Piperacillin and Piperacillin/Tazobactum respectively. This could possibly mean that the isolates did not produce high proportions of β-lactam inhibitors/ β-lactamases towards these antibiotics.

5.3.2 Antibiotic Resistance Patterns of *P. aeruginosa* isolated from urine samples

This study showed that the greatest resistance in *P. aeruginosa* isolated from urine samples was towards four antibiotics (Tetracycline, Norfloxacin, Ceftriaxone and Gentamicin). The Chi-square (P>0.05) (Appendix C 2, Table C 2.3) showed that the way the isolates reacted to the antibiotics was approximately the same thus the four antibiotics had more or less the same effect on the isolates. Tetracycline and Gentamicin are commonly used broad spectrum antibiotics (Gellatly and Hancock, 2013) thus this could explain the high resistance to them by the isolates. The isolates could have been previously exposed to the antibiotics thus attaining resistance against them. The *P. aeruginosa* isolates showed least resistance to Imipenem, Piperacillin and Piperacillin/Tazobactum thus showing that the *P. aeruginosa* isolates had minimal inhibition of β-lactams. High resistance to Gentamicin was exhibited by isolates from urine samples (63.89%) (Figure 4.3.2) and this was different from the results

obtained in a study by Bekele *et* al., (2015) as most of the isolates in that study were sensitive to Gentamicin (86.12%).

Comparatively, isolates from both urine and pus swab samples had more or less similar resistance patterns as they were greatly resistant to tetracycline, Quinolone (Norfloxacin), Aminoglycoside (Gentamicin) and Cephalosporin (Ceftriaxone) classes of antibiotics (Appendix C, Table C1. 3 and Appendix C2, Table C 2.3).

This resistance could be due to the over use of antibiotics as they are more affordable as compared to the β-lactams (Imipenem, Piperacillin, Piperacillin/Tazobactum). Ciprofloxacin was considered to be the most effective Fluoroquinolone against *P. aeruginosa* which can be administered orally (Ansari *et al*., 2015) but resistance by *P. aeruginosa* to this antibiotic is increasing. This study showed a resistance towards Ciprofloxacin of 54.76% and 41.67% by *P. aeruginosa* isolates obtained from pus swab and urine samples respectively. This may have a great impact on healthcare as its effectiveness is decreasing thus there may be need to find alternative fluoroquinolones drugs. Due to the high cost of the antibiotics, medical practitioners tend to resort to the broad spectrum antibiotics or continuously use the same antibiotics and this may result in resistance towards them. *P. aeruginosa* has a core genome which has genes that are common to the species (Gellatly and Hancock, 2013) and this may explain the similarity in resistance to Tetracycline and Aminoglycosides regardless of the specimen of isolation.

Tetracycline is a commonly used antibiotic in Zimbabwe for a variety of infections and this may explain why it has the greatest resistance in isolates from both types of clinical specimens. Some individuals tend to abuse prescription drugs such that when used to treat infections, the drugs will not be as effective as they should be. The increase in self diagnosis of diseases without the help of medical practitioners and use of unprescribed antibiotics at

home to treat illnesses (Faiz and Basher, 2011) may be another explanation to the increase in antibiotic resistance by infection causing microorganisms and in this case *P. aeruginosa*. Another possible reason for the development of antibiotic resistance is the incomplete administration of antibiotic courses as patients have a tendency of terminating the intake of antibiotic courses when they feel better and this in turn results in disease causing organisms developing resistance towards the antimicrobials due to partial exposure. Resistance to Carbapenems (Imipenem) has developed in less than a decade of their use (Vaez *et al*., 2015) thus showing that P. aeruginosa has a high mechanism of developing resistance to antibiotics.

5.4 Antibiotypes of *P. aeruginosa* isolates

The objective of this section of the study was to determine the resistance patterns of the isolates to the eight antibiotics tested. The antibiotypes show the antibiotics to which individual isolates were resistant to.

5.4.1 Antibiotype of *P. aeruginosa* isolated from pus swabs

This study showed that the *P. aeruginosa* isolates from pus swabs had distinct resistance patterns. These patterns differed from one specimen to another. The different antibiotypes Appendix B, Table B5) showed that isolates had resistance to different combinations of drugs. This suggests that the *P. aeruginosa* isolates had different inhibitory effects on the antibiotics. The most common resistance pattern was in 5 isolates (Appendix B, Table B5) with the antibiotype being a combination of Tetracycline-Quinolone-Aminoglycoside-Cephalosporin resistance with 5 specific drugs. The unique patterns (27) in the other isolates (Appendix B, Table B5) showed that *P. aeruginosa* isolates possessed different resistance properties from other isolates and these could be in the form of intrinsic, acquired or adaptive resistance (Gellatly and Hancock, 2013). This property then makes the treatment of *P. aeruginosa* infections complicated as an isolate may carry inhibitory properties against all

classes of antibiotics. The isolates should therefore be treated independently from other isolates regardless of being *P. aeruginosa*. Some of the isolates had resistance profiles which included Imipenem, Piperacillin and Piperacillin/Tazobactum (β-lactams) to which in this study *P. aeruginosa* was most sensitive to. The incorporation of these drugs into the resistance profiles/ antibiotypes may then limit the treatment options as resistance to other classes of antibiotics was high. Due to high resistance to most drugs tested, alternative drugs which are not necessarily classified as anti-pseudomonals would therefore have to be used as treatment options.

5.4.2 Antibiotypes of *P. aeruginosa* isolated from urine samples

The *P. aeruginosa* isolates obtained from urine samples showed unique resistance profiles with a few exceptions where patterns were similar in two or more isolates (Appendix B, Table B6). The most common resistance pattern (8.34%) was a Quinolone, Tetracycline, Cephalosporin combination. This indicated that resistance to the above mentioned 3 antibiotic classes was high and to some extent resistance to the specific antibiotics (Norfloxacin, Tetracycline, Ceftriaxone, and Ciprofloxacin) belonging to those classes was also high. Only one isolate showed no resistance to any of the antibiotics and this is very rare amongst *P. aeruginosa* isolates. This response shown to all antibiotics by one isolate showed that there are some isolates that may respond to treatment very well and most probably they haven't been highly exposed to antibiotic treatment before to attain resistance.

Isolates from both pus swab and urine samples showed a high degree of multi drug resistance (MDR) whilst some isolates were extremely drug resistant (XRD) which limited the therapeutic options in treating the infections.

In general, irrespective of specimen type, the *P. aeruginosa* isolates possessed similar resistance trends as they were mainly resistant to drugs belonging to Quinolone, Tetracycline,

Cephalosporin and Aminoglycoside classes of drugs. Some of the isolates were resistant to single drugs (Appendix B, Table B5, and Table B6) and this however does not limit the treatment options as other antibiotics can be selected from the seven drugs to which were effective against the isolates. The isolates which were resistant to three to five antibiotics limited the treatment options as a few drugs remained as treatment options. The worst resistance was in isolates whose resistance profiles had combinations of six or seven drugs in antibiotypes A5 to A7 (Appendix B, Table B5, Table B6) as the isolates showed resistance to all classes of antibiotics used in this study. Such extreme resistance means there would be narrow treatment options. Narrow treatment options would pose as a challenge to medical practitioners as they would have few prescription options or they would have to resort to other treatment options which may not be successful.

The antibiotypes of great concern in this study were those of A6 and A7 (Appendix B, Table B5, Table B6) as these were extremely drug resistant isolates. With little to no infection control such strains may result in an increase rates due to *P. aeruginosa* related infections especially if such strains are found in the Intensive care unit (ICU) and theatre in reservoirs. There is need to ensure that transmission of such resistant strains is prevented through good and proper sanitation in the hospitals. The genes of *P. aeruginosa* are unique to each strain (Gellatly and Hancock, 2013) and this may explain the different resistance patterns in the isolates regardless of specimen type. It is recommended that each hospital should have its own antibiotic sensitivity pattern (antibiogram) which will help in the choice of antibiotics to be used in treatment since the sensitivity patterns differ across hospitals (Rajat *et al*., 2015).

5.5 Drug efficacy on *P. aeruginosa* isolates

The objective of this section was to determine the efficacy of different drugs on *P. aeruginosa* isolates so as to determine specific drugs for *P. aeruginosa* mediated urinary tract

and wound infections. The different antibiotics tested on the isolates *in vitro* showed different effects on the isolates.

5.5.1 Drug efficacy on *P. aeruginosa* isolated from pus swab samples

Testing the effect of antibiotics using the Chi-square test for heterogeneity of proportions allowed for the screening of antibiotics according to their efficacy. The eight antibiotics tested had statistically different effects on the isolates ($P<0.05$). This showed that the different classes of antibiotics had different effects on the isolates such that the responses to the antibiotics were different. Imipenem is the first line of defense in treating *P. aeruginosa* mediated wound infections. The second most effective drug was Piperacillin. Ciprofloxacin and Piperacillin/Tazobactum were in the intermediate zone between the most and least effective antibiotics.

Imipenem and Piperacillin can be considered to be the first treatment options for *P. aeruginosa* mediated wound infections as these showed to be the most efficant antibiotics. The most effective antibiotics (Imipenem, Piperacillin, Ciprofloxacin and Piperacillin/Tazobactam) had significantly different efficacy against *P. aeruginosa* though Imipenem showed the greatest efficacy against *P. aeruginosa*. Piperacillin, Ciprofloxacin and Piperacillin/Tazobactam can therefore be used as treatment options after Imipenem. In the case that Imipenem and Piperacillin are not available, the alternative treatment options may be Piperacillin/Tazobactum and Ciprofloxacin.

The four least efficant drugs (Norfloxacin, Tetracycline, Gentamicin and Ceftriaxone) to which the *P. aeruginosa* isolates showed the greatest resistance belonged to the Quinolone, Tetracycline, Aminoglycoside and Cephalosporin antibiotic classes respectively. These antibiotics had more or less the same effects on the *P. aeruginosa* isolated from pus swab samples. This suggests that the isolates were able to inhibit the functioning of these

antibiotics making them less viable as treatment options. The antibiotics with equal proportions of isolates (Chi-square P > 0.05) showed that the antibiotics had more or less the same effect on the isolates regardless of the isolates having different physiological responses owing to the different antibiotic classes. The isolates obtained from the pus swab samples showed equal percentage (59.52%) resistance to Gentamicin and Tetracycline (Figure 4.3.1) showing that these two antibiotics had the same proportion of isolates to which they were effective against. This similar effect of the antibiotics was also observed in the case of Norfloxacin and Ceftriaxone as well as Tetracycline and Gentamicin against *P. aeruginosa* isolated from urine samples (Figure 4.3.2). This suggests that *P. aeruginosa* isolates to some extent had equal reaction rates to the antibiotics such that the proportions were within the same range or equal.

5.5.2 Drug Efficacy on *P. aeruginosa* isolated from urine samples

The *P. aeruginosa* isolates obtained from urine samples significantly responded differently to all the antibiotics tested on them (Chi-square P<0.05). This was shown by the unequal proportions obtained using the Chi-square test for heterogeneity of proportions (Appendix C2, Table C 2.1). The differences were expected as the antibiotics tested belonged to different classes of antibiotics which have different properties and exhibit different effects.

Norfloxacin, Tetracycline, Gentamicin and Ceftriaxone were the four least effective drugs against *P. aeruginosa* isolates and this was shown to be statistically similar (Chi-square P>0.05). This showed that the resistance of the isolates to the antibiotics was more or less the same. The isolates showed the highest resistance to the latter named four antibiotics as compared to others. Due to the high resistance by *P. aeruginosa* isolates, Norfloxacin, Tetracycline, Gentamicin and Ceftriaxone may not be regarded as treatment options for *P.*

aeruginosa caused urinary tract infections. The resistance to these antibiotics may be due to their over use in the treatment in urinary tract infections as well as other infections. The isolates may have been exposed to these antibiotics before and developed resistance towards them. Resistance may have been acquired from other isolates as *P. aeruginosa* invades/ colonises environments in colonies and genetic material may be shared amongst the individuals in the colonies.

The four most effective drugs (Imipenem, Piperacillin/Tazobactam, Piperacillin and Ciprofloxacin) had significantly similar efficacy against *P. aeruginosa* isolated from the urine samples (Chi-square P>0.05) (Appendix C2, Table C 2.4). The latter four antibiotics can therefore be used interchangeably as treatment options *P. aeruginosa* mediated urinary tract infections. Alternative treatment options were found to be available and this was advantageous as clinicians would not be highly limited in terms of antibiotics to prescribe in the case of *P. aeruginosa* mediated urinary tract infections.

Isolates from both specimen types were resistant to the same antibiotics (Tetracycline and Gentamicin) showing that these antibiotics may be considered to be uneffective in treating any of the infections caused by *P. aeruginosa*. Due to the innate resistance shown by *P. aeruginosa* against these antibiotics, they may not be regarded as treatment options in *P. aeruginosa* caused infections. The results obtained in this study also showed that *P. aeruginosa* had inhibitory effects against Tetracycline, Cephalosporin and Aminoglycoside classes of antibiotics.

In the case of Quinolones, *P. aeruginosa* may have resistance to specific antibiotics and this study showed that *P. aeruginosa* was resistant to Norfloxacin but sensitive to Ciprofloxacin though they belonged to the same antibiotic class. Such differences in sensitivity may give room for the use of alternative antibiotics from the same antibiotic class as treatment options.

Clinicians may prescribe alternative drugs from drug classes to which resistance to a specific antibiotic would have been conferred. This helps in increasing treatment options though to some extent the isolates may confer total resistance to all potentially effective antibiotics belonging to a particular class.

The most effective drug against *P. aeruginosa* from both specimen types was Imipenem and this showed that majority of the isolates did not inhibit the effects of the antibiotic. This could be due to the fact that Imipenem is not a commonly used antibiotic due to its high cost and also considering the economic constrains being faced in Zimbabwe, most people would resort to cheaper antibiotics. The hierarchy/ order of the effectiveness of antibiotics on the isolates differed across the specimen types. The most effective antibiotics on pus swab isolates were Imipenem, Piperacillin, Piperacillin/Tazobactum and Ciprofloxacin respectively whilst in urine isolates it was Imipenem, Piperacillin/Tazobactum, Piperacillin and Ciprofloxacin respectively. This showed that there was a degree of difference in sensitivity towards Piperacillin and Piperacillin/Tazobactum amongst the isolates from the different sample types which may be due to genetic differences in the isolates. These results showed that *P. aeruginosa* in wound infections may be highly sensitive to Piperacillin as the second treatment option whilst the second treatment option in urinary tract infections may be Piperacillin/Tazobactum. Such responses may justify that the effects of *P. aeruginosa* differ according to infections.

Generally, *P. aeruginosa* isolated from both specimens responded very well to the same antibiotics (β-lactams & Ciprofloxacin) and this showed that the infections may be treated using the same antibiotics. Imipenem, Piperacillin, Piperacillin/Tazobactum and Ciprofloxacin may be considered the antibiotics of choice in treating *P. aeruginosa* infections. Regardless of high efficiency in this study, a decrease in the efficiency of Piperacillin and Piperacillin/Tazobactam was observed in Europe and the United States in a

study by Gales *et al*., (2001) and this shows that there is a possibility that the efficiency of these two antibiotics may decrease with continued use in Zimbabwe. A degree of resistance towards Piperacillin/Tazobactam was observed in the isolates and was the same as observed in a study by Rajat *et al*., (2015). Gentamicin was found to be the least effective aminoglycoside (Lister *et al.*, 2009) and this was similar to the results obtained in this study as there was high resistance of *P. aeruginosa* isolates towards Gentamicin in both sample types. On the other hand these results were different from those obtained in a study by Renuga *et al*., (2015) as Gentamicin was found to have low resistance rates. This information may support the fact that the sensitivity patterns differ according to areas. The general high resistance to Gentamicin and sensitivity patterns to Imipenem and Ciprofloxacin obtained in this study were similar to the results obtained in Egypt in a study by Mansour *et al*., (2013).

In a study carried conducted by Gales *et al*., (2001) it was found that there is no therapy that can ensure 100% effectiveness against *P. aeruginosa* worldwide thus there is a degree of resistance conferred all the time. All except for one of the isolates were resistant to at least one antibiotic in this study showing that *P. aeruginosa* has a form of resistance at any point in time. The differences in resistance frequencies in the most effective drugs may be directly related to how often they are used. Low resistance to Imipenem may show that it is not highly used by patients.

Unfortunately, the presence of low resistance frequencies may not mean that *P. aeruginosa* will always remain susceptible to the antibiotics in question as resistance may develop with time especially if the antibiotics are not used appropriately. Therefore, there is need for patients and clinicians to ensure the proper prescription and administration of antibiotics so as to try and reduce the rate of emergence of resistance. There is need for continued research on antibiotic susceptibility patterns of *P. aeruginosa* so as to clearly determine the efficacy of the antibiotics.

Selection of appropriate/ specific antibiotics and regulating their use (Mesaros *et al*., 2007) may help in the managing and treatment of infections caused by *P*. *aeruginosa*. Antibiotic resistance and consumption rates are correlated and the rotation of antibiotics can help reduce resistance as it avoids over exposure to the same antibiotic. Antibiotic dosage optimisation is essential in the treatment of Pseudomonal infections (Mesaros *et al*., 2007).

The use of combination therapy may be more effective than mono therapy as an isolate may be susceptible to at least one antibiotic amongst those used, enhancing bacterial killing thus reducing the effects of infection (Hirsch and Tam, 2010) since *P*. *aeruginosa* may be resistant to an antibiotic from a particular class and responsive to another from another class. Mono therapy using Aminoglycosides is not very successful thus combination with β-lactams is more effective (Mesaros *et al*., 2007). Studying of the extended-spectrum beta lactamase genes may play a role in the determination of antibiotic resistance in *P*. *aeruginosa* thus it can help in the selection of therapeutic choices in severe infections which may be life threatening (Strateva and Yordanov, 2009). Regardless of its high effectiveness in this study, Imipenem should be reserved as treatment for infections whereby resistance to other anti-pseudomonals has been shown so as to reduce the risk of emergence of resistance as stated by Carmeli et al. (1999).

5.6 Recommendations

Based on the findings of this study, careful and cautious prescription and use of antibiotics is recommended so as to reduce the emergence rate of antibiotic resistance in *P*. *aeruginosa* as it has shown to be resistant to all the antibiotics tested in vitro. Further studies are recommended whereby molecular characterization and screening of isolates is done so as to determine the antibiotic responses of isolates with the same genetic makeup as to determine which genes are responsible for particular resistance patterns. Other research can be carried

out using individual specific classes of antibiotics, over a longer period of time as well as considering the patient ages so as to determine the effectiveness of specific antibiotics in specific classes and which age group is more prone to infection. Since only the primary/ first line cultures were considered in this study it is recommended that subcultures may be used in further studies to try and eliminate any possible confounding factors. Since there is little to no available data on susceptibility patterns in most hospitals, it is recommended that susceptibility studies are carried out at a national level so as to create data bases which may aid clinicians in terms of treatment choices. Due to the high prevalence of *P. aeruginosa* (6.5%) it is recommended that epidemiological studies considering both private and public hospitals are carried out to monitor possible outbreaks. Overall it is recommended that health professionals and the general public cautiously prescribe and use antibiotics as treatment options are limited due to resistance in infection causing organisms.

5.7 Conclusion

This study showed that *P. aeruginosa* is prevalent in male patients than in females. *P. aeruginosa* isolates obtained from urine and pus swabs respond differently to different antibiotics regardless of being in the same classes. Isolates from the same type of infection have different antibiotypes with a few sharing similar antibiotypes. Antibiotic resistance was generally similar across all sample types. Imipenem is the drug of choice in treating *P. aeruginosa* mediated urinary tract and wound infections. Imipenem, Piperacillin, Piperacillin/Tazobactam and Ciprofloxacin may be used interchangeably as treatment options for *P. aeruginosa* mediated urinary tract and wound infections.

The latter four antibiotics may be used in combination to increase antibiotic efficacy against *P. aeruginosa*. Tetracycline may not be considered as a treatment option for *P. aeruginosa* mediated infections as resistance conferred to it by the isolates was significantly high. Beta

lactam and Quinolone classes of antibiotics had the highest efficacy against *P. aeruginosa* whilst Tetracycline, Aminoglycoside and Cephalosporin classes of antibiotics may be considered to be less effective against *P. aeruginosa*. *P. aeruginosa* related infections are difficult to treat due to limited treatment options therefore is need to continuously monitor the antibiotic resistance trends in P. aeruginosa as well as develop more antibiotics.

REFERENCES

Alo, M.N, Saidu, A. Y & Alhassan, M (2015), Prevalence and Antbiogram of Bacterial Isolates Causing Urinary Tract Infections at Federal Teaching Hospital Abakaliki I (FETHA I), British Microbiology Research Journal, **8**(2), 403–417.

Anguzu, J. R & Olila, D (2007), Drug sensitivity patterns of bacterial isolates from septic post operative wounds in a Regional Referral Hospital in Uganda, African Health Sciences, **7**(3), 148-154.

Anil, C. & Shahid, R. M (2013), Antimicrobial Susceptibility Patterns of *Pseudomonas aeruginosa* Clinical Isolates at a Tertiary Care Hospital In Kathmandu, Nepal, Asian Journal of Pharmaceutical and Clinical Research, **6**(3), 235–238.

Ansari, A, Salman, S.M & Yaqoob, S (2015), Original Research Article Antibiotic Resistance Pattern in *Pseudomonas aeruginosa* Strains Isolated at Era's Lucknow Medical College and Hospital , Lucknow , India, International Journal of Current Microbiology and Applied sciences, **1**(1), 48–58.

Asati, R. K (2013), Antimicrobial sensitivity pattern of *Escherichia coli* isolated from urine samples of UTI patients and issues related to the rational selection of selection of antimicrobials, International Journal of Pharmacology and Therapeutics, **3930**, 52-58.

Bekele, T, Tesfaye, A, Sewunet, T & Waktola, H. D (2015), *Pseudomonas aeruginosa* isolates and their antimicrobial susceptibility pattern among catheterized patients at Jimma, BMC Research Notes, **8**(488), 1–4.

Berger, S (2014), Gideon guide to antimicrobial agents, Gideon Informatics.

Carmeli, Y, Troillet, N, Eliopoulos, G. M & Samore, M. H (1999), Emergence of antibiotic-resistant *Pseudomonas aeruginosa* : comparison of risks associated with different antipseudomonal agents, American Society for Microbiology, **43**(6), 1379–1382.

Chopra, I & Roberts, M (2001), Tetracycline antibiotics: Mode of Action, Applications, Molecular biology and Epidemiology of Bacterial Resistance, Molecular Biology Reviews, **65**, 232-260.

El-hosseiny, L, El-Shenawy, M, Haroun, M & Abdullah, F (2014), Comparative Evaluation of the Inhibitory Effect of Some Essential Oils with Antibiotics against *Pseudomonas aeruginosa*. International Journal of Antibiotics, **2014**, 1–5.

Faiz, A & Basher, A (2011), Antimicrobial resistance: Bangladesh experience, Regional Health Forum, **11**(1), 1-8.

Francis, S, Smith, F & Malkinson, J (2015), Integrated Pharmacy case studies, Pharmaceutical Press, London.

Fuoco, D (2012), Classification Framework and Chemical Biology of Tetracycline-Based drugs, Antibiotics, **2012**(1), 1-13.

Frank, D.W, (2012), Research topic on *Pseudomonas aeruginosa*, biology, genetics, and host –pathogen interactions, Frontiers in Microbiology, **3**(20), 1–2.

Gales, A. C, Jones, R. N, Turnidge, J, Rennie, R & Ramphal, R (2001), Characterization of *Pseudomonas aeruginosa* isolates: Occurrence rates, Antimicrobial susceptibility patterns & Molecular typing in the Global SENTRY Antimicrobial Surveillance Program, 1997-1999 (2001), Clinical Infectious Disease, **31**(supp2), 5146-55.

Gellatly, S. L & Hancock, R. E.W (2013), *Pseudomonas aeruginosa* : new insights into pathogenesis and host defenses, Pathogens and Disease, **67**(3), 159–173.

Gelband, H, Miller-Petrie, M, Pant, S, Gandra, S, Levison, J, Barter, D, White, A and Laxminarayan (2015), The state of the World's Antibiotics 2015, Centre for Disease Dynamics, Economics and Policy, Washington DC, USA.

Hirsch, E. B & Tam, V. H (2010), Impact of multi-drug resistant *Pseudomonas aeruginosa* infection on patient outcomes, Expert Rev Pharmacoecon Outcomes Res, **10**(4), 1–18.

Igumbor, E, Gwanzura, L, Chirara, M, Obi, C & Muza, D (2000), Antibiotic sensitivity and plasmid profiles of *Pseudomonas aeruginosa*, Central African Journal of Medicine, **46**(11), 296-300.

Jalal, S, Ciofu, O. Niels, H, Gotoh, N & Wretland, B (2000), Molecular Mechanisms of Fluoroquinolone Resistance in *Pseudomonas aeruginosa* Isolates from Cystic Fibrosis Patients, Antimicrobial Agents and Chemotherapy, **44**(3), 710–712.

Jamunadevi, S, Balashanmugam, P, Muralitharan,G & Kalaichelvan, P. T (2012), Molecular Characterization of Pathogenic and Non-pathogenic *Pseudomonas aeruginosa* with Special Reference to Phenazine Gene, Journal of Modern Biotechnology, **1**(2), 70–74.

Joshi, M. C, Rashid, M. K & Joshi, H. S (2011), Study of Antibiotic Sensitivity Pattern In Urinary Tract Infection At A Tertiary Hospital, National Journal of Integrated Research in Medicine, **2**(3), 43–46.

Kimang'a, A. N (2012), A Situational Analysis of Antimicrobial Drug Resistance in Africa: Are we losing the battle, Ethiopian Journal of Health Science, **22**(2), 135-143.

Lakshmi, R, Nursin, K. S, Geory, S. A & Greelakshmi, K. S (2014), Role of Beta-lactamases on antibiotic resistance: A Review, Interanational Research Journal of Pharmacy, **5**, 2.

Lambert, P. A (2002), Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*, Journal of the Royal Society of Medicine, **95**(sup 41), 22–26.

Larson, D (2015), Clinical chemistry: Fundamentals and Laboratory techniques, Elsevier Health Sciences, UK.

Lisa, L. A, Paul D. K, Biswas, S. K, Barman, N. C & Barman, S. K (2015), Drug Resistance Profiles of Potential Gram Negative Rods Isolated from Urinary Tract Infected (UTI) Patients of Bangladesh with Four South Asian Countries – An Updated Review, International Journal of Pharmaceutical Sciences, **5**(4), 1160–1166.

Lister, P. D, Wolter, D. J & Hanson, N. D (2009), Antibacterial-Resistant *Pseudomonas aeruginosa* : Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms, Clinical Microbiology Reviews, **22**(4), 582–610.

Livermore, D. M (2002), Multiple Mechanisms of Antimicrobial Resistance in *Pseudomonas aeruginosa*,: Our worst nightmare?, Clinical Infectious Diseases, **34**(5), 634-640.

Mansour, S. A, Eldaly, O, Jiman-Fatani, M. L & Ibrahim, E. M (2013),Epidemiological characterization of *P*. *aeruginosa* isolates of intensive care units in Egypt and Saudi Arabia, Eastern Mediterranean Health Journal, **19**(1), 71–80.

Matz, C, Moreno, A. M, Morten, A, Manefield, M, Hauser, A. R, Givskov, M & Kjelleberg, S (2008), *Pseudomonas aeruginosa* uses type III secretion system to kill biofilm-associated amoebae, International Society for Microbial Ecology, **2**, 843–852.

Mesaros, N, Nordman, P, Plesiat, P, Roussel-Delvallez, M, Van Eldere, J, Glupczynski, Y, Van Lathem, Y, Jacobs, F, Lebecque, P, Malfroot, A, Tulkens, P. M & Van Bambeke, F (2007), *Pseudomonas aeruginosa*: resistance and therapeutic options at the turn of the new millennium, Clinical microbiology and infection, **13**(6), 560–78.

Mesquita, C. S, Soares-castro, P & Santos, P. M, (2013), *Pseudomonas aeruginosa* : phenotypic flexibility and antimicrobial resistance, FORMATEX, **1**, 650-665.

Milanesi, G & Ciferri, O (1966), Studies on the mechanism of action of Gentamicin on protein synthesis in cell-free extracts of *E. coli*, Biochemistry, **5**(12), 3926-3935.

Modak, B, Rahman, S, Gazi, S, Uddin, A & Rahman, T (2013), Antimicrobial potency assay of common antibiotics collected from different drug stores in Dhaka Metropolis, Stamford Journal of Microbiology, **3**(1), 26-29.

Nathwani, D, Raman, G, Sulham, K, Gavaghan, M & Menon, V (2014), Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant *Pseudomonas aeruginosa* infections : a systematic review and meta-analysis, Antimicrobial Resistance and Infection Control, **3**(32), 1–16.

Onchoa, S. A, Lopez-Montiel, F, Escalona, G, Cruz-Cordova, A, Davila, L. B, Lopez-Martinez, B, Jimenez-Tapia, Y, Giono, S, Eslava, C, Hernandez-Castro, R & Xicohtencatl-Cortes, J (2013), Pathogenic characteristics of *Pseudomonas aeruginosa* strains resistant to carbapenems associated with biofilm formation, Bol MedHosp Infant Mex, **70**(2), 133-144.

Onguru, P, Erbay, A, Bodur, H, Baran, G, Akinci, E, Balaban, N & Cevik, M. A (2008), Imipenem-Resistant *Pseudomonas aeruginosa*: Risk factors for nosocomial infections, Journal of Korean Medical Science, **23**(6), 982-987.

Parmar, H, Dholakia, A, Vasavada, D & Singhala, H (2013), The Current status of Antibiotic sensitivity of *Pseudomonas aeruginosa* isolated from various clinical samples, International Journal of Research in Medical Sciences, **2**(1), 1–6.

Papich, M. G (2015), Sanders handbook of Veterinary Drugs: Small and Large animals, Elsevier, UK.

Poole, K (2001), Multidrug Efflux Pumps and Antimicrobial Resistance in *Pseudomonas aeruginosa* and Related Organisms, Journal of Molecular Microbiology and Biotechnology, **3**(2), 255–264.

Rajat, R. M, Nimana, G. L, Mistry, K, Parmar, R, Patel, K & Vegad, M. M (2012), Antibiotic resistance pattern in *Pseudomonas aeruginosa* species isolated at a Tertiary Care Hospital, Ahmadabad, National Journal of Medical Research, **2**(2), 156-159.

Rajat, R. M, Patel, N. D, Urmila, N & Rakesh, S. K (2015), Clinical correlation of *Pseudomonas aeruginosa* isolated from clinical settings at Civil Hospital, Ahmedabad, International Archives of Integrated Medicine, **2**(5), 5-9.

Rasamiravaka, T, Labtani, Q, Duez, P & El Jaziri, M (2015), The formation of Biofilms by *Pseudomonas aeruginosa*: A Review of the Natural and Synthetic Compounds Interferring with Control Mechanisms, BioMedical Research International, 2015.

Renuga, S, Lakshmi, K, Chitralekha, S & Illamani,V (2015), Prevalence of *Pseudomonas aeruginosa* and its antibiotic susceptibility pattern in a Tertiary Care Hospital, International Journal of Research In Pharmaceutical Sciences, **6**(1), 27-30.

Rishi, M. B, Jrad, S. J, Al-Gumati, M & Aboshkiwa, M. A (2013),Nosocomial Infections in a Surgical Department , Tripoli Central Hospital,Tripoli, Libya, Ibnosina Journal of Medical and Biomedical Sciences, **5**(6), 324–329.

Rizek, C, Fu, L, Dos Santos, L, C, Leite, G, Ramos, J, Rossi, F, Guimaraes, T, Levi, A. S & Costa, S. F (2014), Characterization of carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates, carrying multiple genes coding for antibiotic resistance, Annals of Clinical Microbiology and Antimicrobials, **13**(43), 1-5.

Sadaf, M, Rao, A, Afsha & Sharma, S (2016), Antimicrobial Susceptibility Pattern in the clinical isolates of *Pseudomonas aeruginosa* at NIMS Medical College & Hospital, Jaipur, India, International Journal of Medical and Health Research, **2**(3), 104–107.

Shrestha, R. K. C. A & Sharma, V. K (2013), Bacteriological Study of Wound Infection and Antibiotic Susceptibility Pattern of the Isolates, Nepal Journal of Science and Technology, **14**(2), pp.143–150.

Stover, C. K, Pham, X. Q, Erwin, A. L, Mizoguchi, S. D, Warrener, P, Hickey, M. J, Brinkman, F. S. L. Hufnagle, W. O, Kowalik, D. J, Lagrou, M, Garber, R. L, Goltry, L, Tolentino, E, Westbrock-Wadman, S, Yuan, Y, Brody, L. L, Coulter, S. N, Folger, K. R, Kas, A, Larbig, K, Lim, R, Smith, K, Spencer, D, Wong, G. K. S, Wu, Z, Paulsen, I. T, Reizer, J, Saier, M. H, Hancock, R. E. W & Lory, S (2000), Complete genome sequence of *Pseudomonas aeruginosa* PAO1 , an opportunistic pathogen, Nature, **406**, 959–964.

Savas, L, Duran, N, Savas, N, Oulen, Y & Ocak, S (2005), The Prevalence and Resistance patterns of *Pseudomonas aeruginosa* in Intensive care units in a University Hospital, Turkish Journal of Medical Science, **35**, 317-322.

Strateva, T. & Yordanov, D (2009), *Pseudomonas aeruginosa* – a phenomenon of bacterial resistance, Journal of Medical Microbiology, **58**, 1133–1148.

Tam, V. H, Chang, K, Abdelraouf, K, Brioso, C. G, Ameka, M, McCaskey, L. A Weston, J. S, Caeiro, J & Carey, K. W (2010), Prevalence, Resistance Mechanisms and Susceptibility of

Multidrug-Resistant Bloodstream isolates of *Pseudomonas aeruginosa*, Antimicrobial Agents and Chemotherapy, **54**(3), 1160-1164.

Tangy, F, Moukkadem, M, Vindimian, E, Capman, M & Le Goffic, F (1985), Mechanisms of action of Gentamicin components, European Journal of Biochemistry, **147**, 381-386.

Todar, K (2008), Online Textbook of Bacteriology.

Vaez, H, Moghim, S, Esfahani, B. N & Safaei, H. G (2015), Clonal Relatedness among Imipenem-Resistant *Pseudomonas aeruginosa* Isolated from ICU-Hospitalized Patients, Critical Care Research and Practice,2015.

Vallerand, A. H, Sanoski, C. A (2014), Davis's Canadian guide for Nurses, F.A Davis, Canada.

www.biosite.dk (accessed 12/06/2016)

www.drugbank.ca/drugs/DB00798 (accessed 12/06/2016)

www.internetchemie.info (accessed 12/06/2016)

www.khanacademy.org (accessed 12/06/2016)

APPENDICES

APPENDIX A Media, Antibiotics and Identification tests

Table A.1: Manufacturer and Product details

Manufacture's Details	Product name
MAST GROUP	MAST Mueller Hinton agar (DM170)
	MAST Citrate Agar (IDM23)
	MAST Motility Agar (IDM28/A)
	MAST Indole (IDM34/A)
	MAST Antibiotic discs
	MAST Oxidase strips

Table A.2: Antibiotics used in susceptibility testing

Drug	Antibiotic class	Disc potency (µg)
Imipenem	β-lactam	10
Piperacillin	β-lactam	30
Norfloxacin	Quinolone	10
Tetracycline	Tetracycline	30
Gentamicin	Aminoglycoside	10
Ceftriaxone	Cephalosporin	30
Ciprofloxacin	Quinolone	5
Piperacillin/Tazobactum	β-lactam (penicillin combination)	

Table A.3: Expected identification results for Pseudomonas aeruginosa

Biochemical test/ Stain	**Expected result**
Gram stain	Gram negative rods
Oxidase	Purple colour change on strip
Indole	Negative
Motility	Positive
Citrate	Blue colour in medium

Table A4: Antibiotic zone diameters

Antibiotic	Resistant	Intermediate	Sensitive
Tetracycline	≤14		≥19
Gentamicin	≤12	13-14	≥15
Piperacillin	≤17		≥18
Ciprofloxacin	≤15	16-12	≥21
Norfloxacin	≤12	14-16	≥17
Piperacillin/Tazobactam	≤17	18-20	≥21
Ceftriaxone	≤13	14-20	≥21
Imipenem	≤13	14-15	≥16

APPENDIX B RESULTS

Table B1: urine sample results and patients' gender

Sample	Imi	Pip	Nor	Tetra	Gent	Ceft	Cip	Pip/Taz	Sex
1	S(16)	R (17)	R (11)	S (20)	R (12)	S (22)	R (15)	S (21)	F
2	S(18)	R (15)	R (10)	S (21)	R (10)	S (21)	R (12)	S (22)	M
3	S(18)	S (19)	S (18)	S (19)	S (16)	S (22)	S (21)	S (21)	M
4	S(17)	S (19)	R (11)	R (12)	S (17)	R (12)	R (11)	S (21)	F
5	S(19)	S (20)	R (9)	S (20)	S (15)	S (21)	S (21)	S (22)	M
6	S(16)	S (21)	R (12)	S (20)	S (18)	R (10)	S (22)	S (23)	F
7	S(18)	S (18)	S (18)	S (19)	S (16)	S (22)	R (10)	S (21)	M
8	S(17)	R (0)	S (19)	R (10)	S (16)	R (11)	R (0)	S (22)	M
9	S(17)	S (18)	S (20)	R (14)	R (0)	R (0)	R (13)	R (17)	M
10	S(16)	S (18)	R (10)	R (9)	R (9)	R (0)	R (15)	S (21)	F
11	S(19)	S (19)	S (19)	S (19)	S (15)	I (16)	S (21)	R (15)	M
12	S(20)	S (18)	R (12)	R (13)	R (0)	R (12)	S (23)	S (21)	M
13	S(21)	S (20)	S (20)	R (0)	R (11)	S (21)	S (21)	S (22)	M
14	S(19)	S (18)	R (11)	R (0)	R(9)	R (12)	R (9)	R (11)	F
15	R(10)	R (9)	R (9)	R (0)	R (0)	S (23)	R (0)	R (0)	M
16	R (7)	R (10)	R (10)	R (0)	R (10)	R (0)	R (11)	S (22)	M
17	S(19)	S (19)	R (12)	R (9)	R (12)	R (11)	R (14)	S (22)	M
18	S(21)	S (18)	R (8)	R (0)	R (11)	R (12)	R (12)	S (22)	M
19	S(19)	R (5)	R (9)	R (10)	R (0)	R (13)	R (15)	S (21)	F
20	S(22)	S (20)	R (12)	R (0)	S (16)	R (10)	S (22)	S (21)	F
21	R (0)	S (19)	R (0)	R (0)	S (18)	R (9)	S (22)	S (23)	M

22	S(18)	S (19)	S (17)	R (11)	S (15)	S (21)	S (21)	S (22)	M
23	R (0)	R (12)	S (18)	R (14)	S (15)	S (22)	S (22)	R (9)	F
24	S(19)	R (11)	S (18)	R (13)	R (10)	S (21)	S (23)	R (14)	M
25	S(21)	S (18)	S (17)	R (0)	R (12)	R (0)	S (21)	R (12)	M
26	S(22)	S (18)	S (19)	S (19)	R (0)	S (21)	S (21)	S (21)	F
27	R (0)	S (19)	S (20)	S (21)	R (8)	S (22)	S (22)	S (22)	M
28	R(11)	S (20)	R (10)	R (12)	R (10)	S (22)	S (23)	S (21)	M
29	R(13)	R (8)	R (0)	S (19)	R (0)	R (9)	S (21)	S (23)	M
30	S(18)	R (15)	S (19)	S (19)	R (0)	R (10)	S (21)	S (22)	F
31	S(16)	S (19)	S (20)	R (10)	S (19)	R (8)	S (22)	R (0)	M
32	S(19)	R (13)	R (9)	R (0)	S (18)	S (21)	R (15)	S (21)	F
33	S(20)	S (19)	S (19)	S (21)	R (0)	R (0)	S (22)	R (15)	F
34	S(19)	S (20)	R (7)	R (13)	R (10)	R (12)	R (13)	S (21)	F
35	S(16)	S (19)	S (20)	S (22)	R (0)	S (22)	S (22)	S (21)	M
36	S(21)	S (18)	R (0)	R (0)	R (12)	R (13)	S (21)	S (22)	M

Key- Susceptibility (zone diameter-mm), R- resistant, S- sensitive, M-male, F-female

Imi-Imipenem, Pip- Piperacillin, Nor- Norfloxacin, Tetra- Tetracycline, Gent- Gentamicin, Ceft- Ceftriaxone, Cip- Ciprofloxacin, Pip/Taz- Piperacillin/Tazobactum.

Table B2: Pus swab results and patients' gender

Sample	Imi	Pip	Nor	Tetra	Gent	Ceft	Cip	Pip/Taz	Sex
1	S (17)	S (19)	R (0)	S (19)	R (11)	R (13)	R (0)	S (21)	F
2	S (18)	S (18)	R (12)	R (9)	R (0)	R (11)	R (12)	S (22)	M
3	S (16)	S (20)	R (11)	R (0)	S (19)	R (9)	S (21)	S (21)	M
4	S (16)	S (18)	S (17)	R(0)	R (0)	S (21)	S (22)	S (21)	F
5	S (19)	S (19)	R (10)	R (12)	R (9)	R (0)	S (22)	S (23)	M
6	R (0)	S (19)	S (17)	R (10)	S (15)	S (22)	S (22)	S (21)	M
7	S (17)	R (13)	R (6)	R (14)	S (17)	R (0)	R (10)	S (21)	F
8	S (18)	S (19)	R (9)	S (20)	R (12)	R (12)	R (0)	R (0)	M
9	S (19)	S (19)	R (10)	R (0)	R (10)	S (22)	R (0)	S (22)	M
10	R (11)	R (12)	S (19)	R(11)	S (16)	S (21)	S (22)	R (12)	F
11	S (17)	R (16)	R (12)	S (19)	R (11)	S (23)	S (22)	R (16)	F
12	S (16)	S (18)	S (17)	S (20)	S (16)	R (0)	R (11)	S (22)	F
13	S (19)	S (18)	R (11)	R (0)	R (0)	R (11)	R (14)	S (22)	M
14	S (17)	S (18)	R (8)	R (0)	R (12)	I (15)	R (10)	S (22)	M
15	S (18)	S (19)	R (8)	R (9)	R (0)	S (22)	R (13)	R (0)	F
16	S (17)	S (18)	S (18)	R (11)	S (17)	R (13)	R (0)	R (9)	M
17	S (16)	S (19)	R (11)	R (13)	R (11)	R (10)	R (9)	R (10)	M
18	R (9)	R (8)	R (12)	R (0)	R (10)	R (8)	S (23)	R (15)	F
19	S (18)	S (19)	R (9)	R (0)	R (12)	R (9)	S (22)	R (13)	M
20	S (18)	S (20)	R (0)	R (12)	S (15)	R (13)	S (22)	R (10)	F
21	S (16)	R (17)	R(0)	R (0)	R (11)	R (10)	S (21)	R (12)	F
22	S (16)	R (14)	S (19)	S (19)	R (10)	R (0)	R (0)	S (21)	M

23	S (17)	R (10)	R (12)	R (13)	S (16)	R (0)	S (22)	S (21)	M
24	S (18)	R (17)	R (11)	R (12)	S (18)	R (13)	S (22)	R (0)	F
25	S (16)	R (11)	R (9)	R (14)	R (0)	S (22)	S (21)	S (21)	M
26	S (19)	R (16)	S (20)	R (10)	S (17)	S (22)	S (22)	R (0)	M
27	S (18)	S (19)	S (21)	R (12)	R (9)	R (10)	R (0)	S (22)	M
28	S (16)	S (19)	R (0)	R (0)	R (12)	R (8)	R (8)	S (23)	F
29	S (16)	S (18)	S (17)	R (9)	R (0)	R (0)	R (10)	S (22)	M
30	S (17)	S (20)	R (12)	S (19))	R (0)	R (0)	R (11)	S (21)	M
31	S (18)	S (18)	R (9)	S (19)	S (19)	S (21)	R (15)	R (0)	M
32	S (17)	S (18)	R (11)	R (0)	S (15)	R (13)	R (15)	I (20)	F
33	S (17)	S (19)	R (0)	R (0)	S (16)	S (22)	R (13)	S (21)	F
34	S (19)	S (19)	R (9)	R (0)	R (10)	R (13)	R (11)	S (22)	F
35	S (20)	S (18)	R (0)	R (11)	S (17)	S (21)	R (0)	S (22)	F
36	S (19)	S (19)	R (0)	S (19)	S (15)	S (22)	R (0)	S (22)	M
37	S (16)	R (12)	R (0)	S (20)	S (19)	S (21)	S (21)	S (22)	M
38	S (17)	R (15)	R (0)	S (19)	R (0)	S (21)	S (23)	S (23)	M
39	R (6)	R (7)	S (17)	S (21)	R (11)	R (13)	S (22)	S (21)	M
40	R(10)	S (18)	S (17)	S (20)	R (7)	R (0)	R (9)	S (21)	F
41	S (17)	S (19)	S (19)	R (0)	R (12)	S (23)	S (21)	S (22)	M
42	S (19)	S (19)	S (21)	R (0)	S (19)	S (21)	S (23)	I (19)	M

Key- Susceptibility (zone diameter), R- resistant, S- sensitive, M-male, F- female

Imi-Imipenem, Pip- Piperacillin, Nor- Norfloxacin, Tetra- Tetracycline, Gent- Gentamicin, Ceft- Ceftriaxone, Cip- Ciprofloxacin, Pip/Taz- Piperacillin/Tazobactum

Table B3: Antibiotic susceptibility pattern in pus swabs

Antibiotic	**Number (%) n= 42**		
	Sensitive	**Resistant**	**Intermediate**
Imipenem	37	5	0
Piperacillin	29	13	0
Norfloxacin	13	29	0
Tetracycline	12	30	0
Gentamicin	17	25	0
Ceftriaxone	16	25	1
Ciprofloxacin	19	23	0
Piperacillin/Tazobactum	27	13	2

Table B4: Antibiotic susceptibility pattern in urine samples.

Antibiotic	**Number (%) n= 36**		
	Sensitive	**Resistant**	**Intermediate**
Imipenem	30	6	0
Piperacillin	25	11	0
Norfloxacin	16	20	0
Tetracycline	13	23	0
Gentamicin	13	23	0
Ceftriaxone	15	20	1

Ciprofloxacin	21	15	0
Piperacillin/Tazobactum	27	9	0

Table B5: Antibiotypes of *Pseudomonas aeruginosa* in pus swab isolates.

Number	**Antibiotype**	**Number (%)**
A1 a.	$Tetra^{R}$	1 (2.38)
A2 a.	$Tetra^{R}$ $Gent^{R}$	2 (4.76)
b.	Imi^{R} $Tetra^{R}$	1 (2.38)
c.	$Ceft^{R}$ Cip^{R}	1 (2.38)
d.	Nor^{R} Cip^{R}	1 (2.38)
e.	Pip^{R} Nor^{R}	1 (2.38)
A3 a	Nor^{R} $Tetra^{R}$ $Ceft^{R}$	2 (4.76)
b.	$Ceft^{R}$ Cip^{R} Pip/Taz^{R}	1 (2.38)
c.	Pip^{R} Nor^{R} Pip/Taz^{R}	1 (2.38)
d.	Nor^{R} Cip^{R} Pip/Taz^{R}	1 (2.38)
e.	Nor^{R} $Tetra^{R}$ Cip^{R}	2 (4.76)
f.	Pip^{R} Nor^{R} $Gent^{R}$	1 (2.38)
A4 a.	Nor^{R} $Gent^{R}$ $Ceft^{R}$ Cip^{R}	2 (4.76)
b.	Nor^{R} $Tetra^{R}$ $Gent^{R}$ $Ceft^{R}$	1 (2.38)
c.	Imi^{R} Pip^{R} $Tetra^{R}$ Pip/Taz^{R}	1 (2.38)
d.	Pip^{R} Nor^{R} Gent Pip/Taz^{R}	1 (2.38)

	e.	Nor $Tetra^R$ $Ceft^R$ Pip/Taz^R	1 (2.38)
	f.	Pip^R $Gent^R$ $Ceft^R$ Cip^R	1 (2.38)
	g.	Pip^R Nor ^{R}$Tetra^R$ $Gent^R$	1 (2.38)
	h.	Imi^R Pip^R $Gent^R$ $Ceft^R$	1 (2.38)
	i.	Imi^R $Genta^R$ $Ceft^R$ Cip^R	1 (2.38)
	j.	Tet^R $Gent^R$ $Ceft^R$ Pip/Taz^R	1 (2.38)
A5	a.	Nor^R $Tetra^R$ $Genta^R$ $Ceft^R$ Cip^R	5 (11.90)
	b.	Nor^R $Tetra^R$ $Gent^R$ Cip^R Pip/Taz^R	1 (2.38)
	c.	Nor^R $Tetra^R$ $Gent^R$ $Ceft^R$ Pip/Taz^R	1 (2.38)
	d.	Pip^R Nor^R $Tetra^R$ $Ceft^R$ Pip/Taz^R	1 (2.38)
	e.	Pip^R Nor^R $Tetra^R$ $Ceft^R$ Cip^R	1 (2.38)
	f.	Nor^R $Gent^R$ $Ceft^R$ Cip^R Pip/Taz^R	1 (2.38)
	g.	Nor^R $Tetra^R$ $Genta^R$ Cip^R Pip/Taz^R	1 (2.38)
A6	a.	Nor^R $Tetra^R$ $Genta^R$ $Ceft^R$ Cip^R Pip/Taz^R	1 (2.38)
A7	a.	Imi^R Pip^R Nor^R $Tetra^R$ $Genta^R$ $Ceft^R$ Pip/Taz^R	1 (2.38)
	b.	Pip^R Nor^R $Tetra^R$ $Genta^R$ $Ceft^R$ Pip/Taz^R	1 (2.38)

Key- Imi- Imipenem, Pip- Piperacillin, Tetra- Tetracycline, Cip- Ciprofloxacin, Ceft- Ceftriaxone, Nor- Norfloxacin, Pip/Taz- Piperacillin-Tazobactum, Gent-Gentamicin.

Table B6: Antibiotypes of *Pseudomonas aeruginosa* in urine isolates

Number	Antibiotype	Number (%)
A1 a.	Nor^R	1 (2.78)
b.	Cip^R	1 (2.78)
c.	$Gent^R$	1 (2.78)
d.	Pip/Taz^R	1 (2.78)
e.	$Tetra^R$	1 (2.78)
A2 a.	$Tetra^R$ $Genta^R$	1 (2.78)
b.	Imi^R $Genta^R$	1 (2.78)
c.	Nor^R $Ceft^R$	1 (2.78)
A3 a.	$Gent^R$ $Ceft^R$ Pip/Taz^R	1 (2.78)
b.	$Tetra^R$ $Ceft^R$ Pip^R	1 (2.78)
c.	Nor^R $Tetra^R$ $Ceft^R$	1 (2.78)
d.	Pip^R $Gent^R$ $Ceft^R$	1 (2.78)
A4 a.	Pip^R Nor^R $Gent^R$ Cip^R	2 (5.56)
b.	Pip^R $Tetra^R$ $Ceft^R$ Cip^R	1 (2.78)
c.	Pip^R Nor^R $Tetra^R$ Cip^R	1 (2.78)
d.	Nor^R $Tetra^R$ $Ceft^R$ Cip^R	3 (8.34)
e.	$Tetra^R$ $Gent^R$ $Ceft^R$ Pip/Taz^R	1 (2.78)
f.	Nor^R $Tetra^R$ $Gent^R$ $Ceft^R$	2 (5.56)
g.	Imi^R Nor^R $Tetra^R$ $Ceft^R$	1 (2.78)
h.	Imi $^RPip^R$ $Tetra^R$ Pip/Taz^R	1 (2.78)

i.	Pip^R $Tetra^R$ $Gent^R$ Pip/Taz^R	1 (2.78)
j.	Imi^R Nor^R $Tetra^R$ $Gent^R$	1 (2.78)
A5 a.	Imi^R Pip^R Nor^R $Gent^R$ $Ceft^R$	1 (2.78)
b.	$Tetra^R$ $Gent^R$ $Ceft^R$ Cip^R Pip/Taz^R	1 (2.78)
c.	Nor^R $Tetra^R$ $Gent^R$ $Ceft^R$ Cip^R	2 (5.56)
A6 a.	Nor^R $Tetra^R$ $Gent^R$ $Ceft^R$ Cip^R Pip/Taz^R	1 (2.78)
b.	Pip^R Nor^R $Tetra^R$ $Gent^R$ $Ceft^R$ Cip^R	1 (2.78)
A7 a.	Imi^R Pip^R Nor^R $Tetra^R$ $Gent^R$ Cip^R Pip/Taz	1 (2.78)
b.	Imi^R Pip^R Nor^R $Tetra^R$ $Gent^R$ $Ceft^R$ Cip^R	1 (2.78)
*	1 isolate had 100% sensitivity to all drugs	1 (2.78)

Key- Imi- Imipenem, Pip- Piperacillin, Tetra- Tetracycline, Cip- Ciprofloxacin, Ceft- Ceftriaxone, Nor- Norfloxacin, Pip/Taz- Piperacillin-Tazobactam, Gent-Gentamicin.

APPENDIX C SPSS OUTPUTS 95% confidence interval

C1. Chi-Square Heterogeneity of proportions in pus swab samples

H_O –the proportion of sensitive isolates is equal across all drugs.

H_1 –the proportion of sensitive isolates was different for atleast 2 drugs

Table C1.1: Heterogeneity of proportions of all drugs

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	52.942[a]	7	.000
Likelihood Ratio	56.801	7	.000
Linear-by-Linear Association	5.439	1	.020
N of Valid Cases	333		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 19.58.

Table C1.2: Heterogeneity of proportions of 6 drugs (Nor, Tetra, Genta, Ceft, Cip, Pip/Taz)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	16.280[a]	5	.006
Likelihood Ratio	16.352	5	.006
Linear-by-Linear Association	12.685	1	.000
N of Valid Cases	249		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.71.

NB- the 2 most performing antibiotics (Imi & Pip) were removed.

Table C1.3: Heterogeneity of proportions of the 4 least performing drugs (Nor, Tetra, Gent, Ceft)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.912[a]	3	.591
Likelihood Ratio	1.920	3	.589
Linear-by-Linear Association	1.194	1	.275
N of Valid Cases	167		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 14.24.

Table C1.4: Heterogeneity of proportions of the 4 most performing drugs (Imi, Pip, Cip, Pip/Taz)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	17.646[a]	3	.001
Likelihood Ratio	18.502	3	.000
Linear-by-Linear Association	9.049	1	.003
N of Valid Cases	166		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 13.01.

Table C1.5: Heterogeneity of proportions of best 2 drugs (Imi & Pip)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.525[a]	1	.033		
Continuity Correction[b]	3.465	1	.063		
Likelihood Ratio	4.655	1	.031		
Fisher's Exact Test				.061	.030
Linear-by-Linear Association	4.471	1	.034		
N of Valid Cases	84				

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 9.00.

b. Computed only for a 2x2 table

Table C 1.5.1: Percentage proportion in best 2 drugs

Antibiotic susceptibility in pus swabs * Antibiotic Crosstabulation

			Antibiotic		Total
			Imi	Pip	
Antibiotic susceptibility in pus swabs	sensitive	Count	37	29	66
		Expected Count	33.0	33.0	66.0
		% within Antibiotic susceptibility in pus swabs	56.1%	43.9%	100.0%
		% within Antibiotic	88.1%	69.0%	78.6%
	resistant	Count	5	13	18
		Expected Count	9.0	9.0	18.0
		% within Antibiotic susceptibility in pus swabs	27.8%	72.2%	100.0%
		% within Antibiotic	11.9%	31.0%	21.4%
Total		Count	42	42	84
		Expected Count	42.0	42.0	84.0
		% within Antibiotic susceptibility in pus swabs	50.0%	50.0%	100.0%
		% within Antibiotic	100.0%	100.0%	100.0%

C 2 Heterogeneity of proportions in urine samples.

H_O –the proportion of sensitive isolates is equal across all drugs.

H_1 –the proportion of sensitive isolates was different for at least 2 drugs

Table C 2.1: Heterogeneity of proportions of all drugs

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	34.825[a]	7	.000
Likelihood Ratio	36.469	7	.000
Linear-by-Linear Association	1.198	1	.274
N of Valid Cases	287		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 15.49.

Table C 2.2: Heterogeneity of proportions of 6 drugs (Pip, Nor, Tetra, Gent, Ceft, Cip)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.804[a]	5	.025
Likelihood Ratio	13.010	5	.023
Linear-by-Linear Association	.739	1	.390
N of Valid Cases	215		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 16.77.

Table C 2.3 : Heterogeneity of proportions of 4 least performing drugs (Nor, Tetra, Gent, Ceft)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.869[a]	3	.833
Likelihood Ratio	.869	3	.833
Linear-by-Linear Association	.019	1	.890
N of Valid Cases	143		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 13.95.

Table C 2.4: Heterogeneity of proportions of 4 best performing drugs (Imi, Pip, Cip, Pip/Taz)

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.831[a]	3	.120
Likelihood Ratio	5.893	3	.117
Linear-by-Linear Association	1.538	1	.215
N of Valid Cases	144		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 10.25.

Table C2.4.1: percentage proportions in best 4 antibiotics.

Antibiotic susceptibility * Antibiotic Crosstabulation

			Antibiotic				Total
			Imi	Pip	Cip	Pip/Taz	
Antibiotic susceptibility	sensitive	Count	30	25	21	27	103
		Expected Count	25.8	25.8	25.8	25.8	103.0
		% within Antibiotic susceptibility	29.1%	24.3%	20.4%	26.2%	100.0%
		% within Antibiotic	83.3%	69.4%	58.3%	75.0%	71.5%
	resistant	Count	6	11	15	9	41
		Expected Count	10.3	10.3	10.3	10.3	41.0
		% within Antibiotic susceptibility	14.6%	26.8%	36.6%	22.0%	100.0%
		% within Antibiotic	16.7%	30.6%	41.7%	25.0%	28.5%
Total		Count	36	36	36	36	144
		Expected Count	36.0	36.0	36.0	36.0	144.0
		% within Antibiotic susceptibility	25.0%	25.0%	25.0%	25.0%	100.0%
		% within Antibiotic	100.0%	100.0%	100.0%	100.0%	100.0%