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**Deanship of Graduate Studies**

# Evaluation of antibiotics resistance including fosfomycin in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients in Palestinian Hospital over 5 years

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**M.Sc. Thesis.**

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# Evaluation of antibiotics resistance including fosfomycin in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients in Palestinian Hospital over 5 years

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**This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Pharmaceutical science in Pharmacy Department.**

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## Thesis Approval

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**Jerusalem–Palestine**

**1442/2021**

**Dedication**

**This thesis is proudly dedicated to all my beloved family (my mother, my late father, my husband, my brothers and all my friends), to my teachers and future students who can use this thesis as guide in their studies**

**Thanks for your endless of love, sacrifices, supports and advices**

**Declaration.**

**I Certify that this thesis is submitted to the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this study (or any part of the same) has not been submitted for a higher degree to any other university or institution.**

**Signed……..**

**Bayan Asad Subb Laban**

**Date:**

# Acknowledgements

**I’m writing the last part of my thesis after working hard for one year, I will not forget all the bittersweet moments of this journey which ended with the completion of my research “Evaluation of antibiotics resistance including fosfomycin in *Pseudomonas aeruginosa* isolates from cystic fibrosis patients in Palestinian Hospital over 5 years”. First of all, I would like to thank Allah to enabling me to complete this research, and then I would like to thank all the greatest people who were always there for helping and supporting me during this time.**

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# Abstract.

**Background:** Antipseudomonal agents, like other antibiotics, will develop a resistance when taken continuously for many years. In diseases like cystic fibrosis (CF),the most dangerous pathogen colonizing the lungs is *Pseudomonas aeruginosa* and antibiotic resistance has a high chance to occur. In Palestine, there is no accurate data about resistance in these bacteria among CF patients.

The increased prevalence of multi-drug resistant strains, allergic reactions, nephrotoxicity and other side effects of antibiotic used among patients with CF limits the number of antibiotics available to treat pulmonary exacerbations. Fosfomycin, a unique broad spectrum bactericidal antibiotic, might offer an alternative therapeutic option in such cases.

**Objectives:** The aim of this study was to determine the level and change in the rates of resistance over the years for mostly commonly used antibiotics in Pseudomonal infection in CF patients. In addition, the study evaluated the effect of fosfomycin on this bacteria.

**Methods:** To study antibiotics resistance tested in sputum samples over the past 5 years, a retrospective study was carried out in the Department of Microbiology at Caritas Baby Hospital. Clinical and microbiological data were extracted from medical database for pediatric CF patients. The results were analyzed using Microsoft Excel Software 2010 and Statistical Package for Social Sciences (SPSS) program version 20.

In addition, 129*Pseudomonas aeruginosa* sputum samples from CF patients were tested for fosfomycin sensitivity by using two different methods minimum inhibitor concentration (MIC) and disc diffusion(DD).

**Results:** The sensitivity to commonly used antibiotics in treatment *P.aeruginosa* infection in CF pediatric patients in Palestine was92.3%, 91.9%, 89.1%, 87.7%, 76.5% and 73.5% for ciprofloxacin, ceftazidime, piperacillin-tazobactam, meropenem, amikacin and gentamicin; respectively. Over the past 5 years, the sensitivity was oscillating slightly with no deterioration in sensitivity toward these antibiotics.

The vast majority of patients are from the south of the west bank (70.1%), and also the resistance in this area is higher for all antibiotics. Males are slightly more sensitive than females. Regarding ages, as the age increased, resistance to antibiotics increased.

In vitro, due to the lack of susceptibility breakpoint of fosfomycin for *P.aeruginosa* in CLSI and EUCAST, sensitivity to fosfomycin according to MIC results was determined based on the existing CLSI breakpoints for the Enterobacteriaceae; ≤64 is sensitive. MIC method showed good activity for fosfomycin toward *P.aeruginosa* isolates from CF patients; nearly 40% sensitive, the most sensitivity was in mucoid type (57.5%). DD method showed a strong correlation with MIC method, Pearson Correlation between MIC and DD 200 μg was -0.889, between MIC and DD 50 μg was -0.768, and also 0.932 between DD 200μg and DD 50μg.

**Conclusions:** In Palestine, the sensitivity to commonly used antibiotics in treatment *P.aeruginosa* infection in CF pediatric patients was stable, and there was no deterioration in sensitivity toward these antibiotics over the past 5 years. Fosfomycin showed a good sensitivity towards Palestinian *P.aeruginosa* isolates in CF patients, so it can be considered as a choice in the antibiotics used in treating *P.aeruginosa* infection (particularly the mucoid type) in CF patients in the future, but it still needs more research.

**Keywords: Cystic Fibrosis, *Pseudomonas Aeruginosa*, Fosfomycin, Bacterial Resistance.**

# List of Abbreviations

ASL: Airway Surface Liquid

ATCC: American Type Culture Collection

ATP: Adenosine triphosphate

CBH: Caritas Baby Hospital

CF: Cystic Fibrosis

CFTR: Cystic Fibrosis Transmembrane conductance Regulator

CLSI: Clinical & Laboratory standards institute

CRPA: Carbapenem resistant pseudomonas aeruginosa

CLSI: Clinical and Laboratory Standard Institute

DD: Disc Diffusion

ENAC: Epithelial Na+ Channel

EUCAST: European Committee on Antibiotic Susceptibility Testing

G-6-P: Glucose-6-phosphate

*H. influanzae: Haemophilus influenzae*

MDR: Multidrug Resistant Bacteria

MIC: Minimum Inhibitory Concentration

M: Mucoid

NM: Non-Mucoid

*P. aeruginosa: Pseudomonas aeruginosa*

RTI: Respiratory Tract Infection

*S. aereus: Staphylococcus aureus*

TSI: Triple Sugar Iron

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# Chapter One

## 1. Introduction.

### 1.1 Background

#### 1.1.1 CF disease:

CF is an inherited disease, it was first described by Anderson in the late 1930s as a gastrointestinal disease, but after that it well recognized as a multisystem disorder with lung involvement as the major cause of morbidity and mortality. It characterized by buildup of thick, sticky mucus that can damage these organs in the body(Ramsey et al., 1993).

In addition to affecting the lungs, CF also affects the upper respiratory tract, hepato-biliary system, pancreas, gastrointestinal tract, endocrine system, sweat glands and reproductive tract; such as obstructive aspermia in men and thick cervical mucus in women(Ramsey et al., 1993).

For the airway disease, commonly accepted explanation in CF is the “low volume” hypothesis, a reduced volume of airway surface liquid (ASL) which causes failure of mucociliary clearance, the lungs’ innate defense mechanism. The mucociliary dysfunction means that a patient with CF cannot effectively clear inhaled bacteria and there is an excessive inflammatory response to pathogens. For a given bacterial load, a person with CF will have up to 10 times more inflammation than a person with a lower respiratory tract infection but without the disease. Also, CF patients suffer from repeated acute exacerbation and scar formation in cysts in the lung which

finally lead to respiratory failure(Davies, Alton, & Bush, 2007). For other organs, the mucus prevents release of digestive enzymes that allow the body to break down food and absorb nutrients(Davies et al., 2007).

Symptoms in patients with CF include accumulation of thick mucus, recurrent lung infections, persistent cough at times associated with phlegm with or without hemoptysis, wheezing, poor growth/weight loss, frequent greasy, bulky stools or difficulty in bowel movements, and electrolyte imbalance particularly in countries with warm weather(Banjar & Angyalosi, 2015). There are three common tests used to diagnose CF: the newborn screening test, the sweat test, and the genetic test. The sweat test is convenient and fast, with no needles involved. It can be done in less than an hour, and results can be obtained on the same day the test is performed. A genetic test could be further performed to confirm individuals with a positive sweat test result(Farrell et al., 2008).

### 1.1.2 Causes and inheritance:

CF is a multisystem disorder caused by an autosomal recessive inheritance of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. A mutation (defect) in this gene changes a membrane protein that regulates the movement of salt in and out of cells(Stoltz, Meyerholz, & Welsh, 2015)Children need to inherit two copies of the gene, one copy from each parent, in order to have the disease. If children inherit only one copy, they won't develop CF. However, they will be carriers the gene, and possibly pass it to their own children.

### 1.1.3 CFTR and lung disease:

CFTR protein is a membrane protein encoded by CFTR gene on chromosome 7, it belongs to a group of proteins which bind ATP and are implicated in ion transportation across membranes(Chinet, 1995).

In a healthy state, CFTR protein on the apical membrane secretes chloride ions CL− onto the airway surface liquid and inhibits sodium absorption by blocking the Epithelial Na+ Channel (ENAC), allowing water movement via osmosis to hydrate the airway surface liquid (ASL). This function is deleted in CF epithelial cells, resulting in the retention of chloride and sodium within the cell, causing water to move from the ASL into the cell which resulting in dehydrated mucus, which leads to a decrease in ASL volume and impairment in mucociliary clearance, as shown in figure (1.1)(Chinet, 1995).Obstruction of the airways with viscous secretions limits pathogen clearance and permits a cycle of inflammation, infections and mucus impaction.

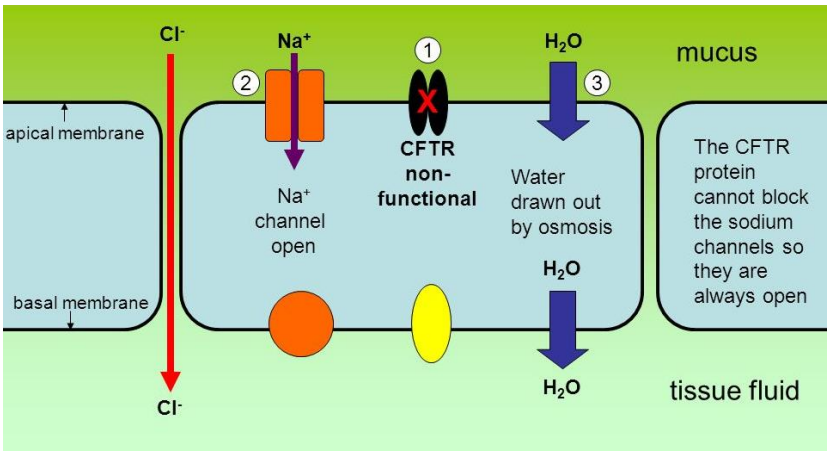



**Figure 1.1:** CFTR and lung disease

### 1.1.4 Pulmonary infection in CF patients:

Lung infections accounts for most of the complications and deaths related to CF (Cohn, 1998). The etiologic diagnosis of respiratory infections in CF patients is established through culture of respiratory tract samples, such as sputum and oropharyngeal swabs, the latter method generally

being used in infants and children who are unable to expectorate sputum(Gibson, Burns, & Ramsey, 2003).

Lungs of CF patients are often colonized or infected in infancy and early childhood with organisms, such as *S. aureus* and *H. influenzae,* that may damage the epithelial surfaces, leading to increased attachment of, and eventual replacement by gram negative bacilli *P. aeruginosa*(Silva Filho et al., 2013).

Chronic infection with *P.aeruginosa* is associated with a worse prognosis in children, adolescents and adults(Eber & Zach, 2010). In children, the 8-year risk of death was 2.6 times higher in children who had respiratory cultures positive for *P. aeruginosa* than in children without *P. aeruginosa* in their respiratory cultures(Emerson, Rosenfeld, McNamara, Ramsey, & Gibson, 2002).

In the early stage of colonization, the organism can be eradicated, while during chronic colonization or exacerbations, reduction of bacterial density is desirable(Cantón et al., 2005). After chronic colonization is established, the eradication of this pathogen is almost impossible because of the nature of CF itself and the appearance of mucoid phenotypes of *P. aeruginosa* which forming micro-colonies on the mucosal surfaces which are surrounded by an abundant mass of the microbial exopolysaccharide alginate. This unique bacterial product may present a poly-anionic barrier to antimicrobial agents and preventing its action(Govan & Deretic, 1996; Pedersen, Høiby, Espersen, & Koch, 1992). Patients with mucoid *P.aeruginosa* strains do worse than those with non-mucoid strains(Eber & Zach, 2010).

Also, there is another reason for eradication failure which is increasing in *P. aeruginosa* due to resistance to antibiotics by several mechanisms, including porin loss, overexpression of efflux pumps and production of inactivating enzymes, such as β-lactamases. Also, the generation of

alginate polysaccharide biofilms is another key mechanism of resistance; these are complex structures, which have a barrier protection and diffusion limitation mechanisms to induce resistance(Lambert, 2002).

Regarding MDR *P. aeruginosa*, there is disagreement within the medical community as to the definition of multidrug resistance. Multidrug resistance is a heterogeneous phenotype, which may result from combination of different resistance mechanisms. A review of studies reporting on MDR P. *aeruginosa* infections revealed huge different definitions used in the literature, ranging from resistance to a single antibiotic agent or class to resistance to all tested antibiotics(Falagas, Koletsi, & Bliziotis, 2006). In the majority of the published studies, multidrug resistance was defined as resistance to at least three drugs from a variety of antibiotic classes, mainly aminoglycosides, β-lactam (antipseudomonal penicillin's and cephalosporins), carbapenems and fluoroquinolones antibiotics(Falagas et al., 2006).

## 1.2 Antibiotic treatment for *P. aeruginosa* infection in CF:

### 1.2.1 Commonly used antibiotics:

Without antibiotic treatment, the infant with CF is at risk of early infection and inflammation and ultimately progressing to fatal respiratory failure.

Antibiotic therapy strategies aimed to preventing or delaying progression from initial acquisition of *P. aeruginosa* to chronic infection consider the central to the management of patients with CF, which is the major reason for increased patient survival.

Colistin (polymixin E) was the first antibacterial agent used for *P. aeruginosa* infection in CF patients. Unfortunately, clinical efficacy didn't match in vitro susceptibility and adverse effects such as neurotoxicity and nephrotoxicity were reported.

After that, extended-spectrum penicillins have been developed for use, these include ticarcillin, azlocillin, and piperacillin in conjugation with β -lactamase inhibitor. But in patients with CF, they have common adverse effects include rash, urticaria and pruritis (up to 30%).

Ceftazidime, a cephalosporin, is a commonly used cell wall synthesis inhibitor antibiotic, it is highly stable to β-lactamases produced by *p. aeruginosa*. also, it has generally good activity in vitro and advantages include ease of parental administration, no requirement for monitoring drug concentrations, twice daily administration is preferred and low toxicity. However recently, new strain of *p. aeruginosa* has arrived Palestine which is resistant to Ceftazidime.

Ciprofloxacin, a fluoroquinolone, has the advantage in that is available asoral preparation. Oral bioavailability is (70-80) % and a $t_{1/2}$ of 5 hours allows twice daily administration regimens. Elimination is partly via hepatic metabolism by cytochrome P450 enzymes, this mean there is potential for drug interaction by cyt-P450 inhibitors or reducers. Also, constant prophylactic therapy is undesirable because of a steady increase over time in the minimum inhibitory concentration (MIC) to *P. aeruginosa*. this will eventually lead to resistance. So, this agent should be used sparingly and for special circumstances.

Other β-lactam antibiotics used are carbapenems (imipenem and meropenem). They were developed to deal with multi drug resistant *P. aeruginosa*. Imipenem is given intravenously and is broken down at the proximal tubule by a dehydropeptidase. Meropenem has advantage on imipenem in that it isn't broken down by dehydropeptidase.

Aminoglycosides include gentamicin, tobramycin and amikacin are used; they act by inhibiting bacterial protein synthesis. They are not absorbed in the gastrointestinal tract and the serum $t_{1/2}$ is 2 to 3 hours. Elimination is renal, so care with the dosage should be taken in patients with renal impairment. Serum concentrations are measured just prior intravenous administration and 1 hour

after. It is also likely that frequency of aminoglycoside courses will be found to have an important role in toxicity such as, ototoxicity and nephrotoxicity(Banerjee & Stableforth, 2000).

A combination of two antibiotics with a different mechanism of action should be used for intravenous treatment in CF patients. Ceftazidime and tobramycin are commonly used, also meropenem and colistin is a suitable alternative combination.

As a result of the repeated use of antimicrobial agents in the management of CF, there may be a subsequent emergence of resistant *P. aeruginosa*(Spencker, Staber, Lietz, Schille, & Rodloff, 2003). Furthermore, repeated use of the same antibiotics results in patient intolerance and increased side effects(Mirakhur, Gallagher, Ledson, Hart, & Walshaw, 2003).

Therefore, we have looked for other antibiotics that possess anti-pseudomonal activity, one such antibiotic is fosfomycin.

1.2.2 Fosfomycin for *P. aeruginosa* in CF:

Fosfomycin is a unique broad-spectrum bactericidal antibiotic, that interferes with cell wall synthesis in both Gram-positive and Gram-negative bacteria by inhibiting the initial step involving phosphoenolpyruvate synthetase, it enters the cells of fosfomycin-susceptible bacteria by two different transport uptake systems: a constitutively functional l-α-glycerophosphate transport system (GlpT) and the hexose–phosphate uptake system (UhpT). It inhibits the synthesis of peptidoglycan by blocking the formation of N-acetylmuramic acid(Frederick, M. K. et al., 1974).Chemically, it is unrelated to any other antimicrobial agent. It is available in intravenous formulation as fosfomycin disodium and in oral formulations as fosfomycin calcium or fosfomycin trometamol. Following intravenous administration of the disodium salt, 80–95% of the drug is excreted unchanged in the urine within 24 h, the t½ is 1.5–2 h and it is not bound to serum proteins so its volume of distribution is large. It's concentration in lung tissue can be up

to 50% of serum levels 1–2 hr. after administration. It is taken up actively into bacterial cells through two nutrient transport systems present in various bacteria including *P. aeruginosa*, and inhibits the initial step in cell wall synthesis. So, it may be a good choice for treatment *P. aeruginosa* infections in CF patients with less side effect which caused by commonly used antibiotics(Mirakhur et al., 2003).

Fosfomycin has an excellent side-effect profile, the main side effects are gastrointestinal (nausea, vomiting, diarrhea and a transient increase in serum transaminase levels) thought to be most common with the oral preparations occurring in 2–8% of cases. There are no specific reports in the literature of side effects with the IV administration(Jardin, 1990).

Fosfomycin can be used for CF patients alone or in combination with other standard antibiotics(Mirakhur et al., 2003).

Because fosfomycin acts on a different synthetic pathway, synergistic effect against *P. aeruginosa* has been demonstrated when fosfomycin used with other antibiotics including β-lactams, aminoglycosides, macrolides and tetracyclines. Also, the potential for the development of cross-resistance with other classes of antibiotics is reduced(Mirakhur et al., 2003).

## 1.3 Statement of problem and study rational:

According to CF foundation, the major cause of hospitalization for CF patients is respiratory tract infections, more than one-third of the CF patients are hospitalized each year and 20% are hospitalized more than twice a year, and of all these hospitalizations more than 75% is for treatment of RTIs. *P.aeruginosa* is the organism most commonly known to cause an exacerbations in pulmonary infection(Pressler, 2008).

Chronic infection and frequent exacerbation require treatment with antibiotics and as we know, the repeated treatment any infection with the same antibiotics for a long time will result in the

emerging a new strain which is more resistant to these antibiotics. In many countries there were many studies illustrating the decreased sensitivity of pseudomonas aeruginosa in CF patients to mostly used antibiotics over years. These studies can help the doctors in choosing the right antibiotic, which leads to good managements of the infection in a safe way and saving the patient's live.

For the same reason, there is an effort to find new antibiotics that are chemically and pharmacologically different from others and possess anti-pseudomonal activity in order to bypassing this resistance and side effects associated with the traditional pseudomonal aeruginosa antibiotics, one such antibiotic is fosfomycin, which also studied for its efficacy in *P. aeruginosa* strain in CF patients worldwide.

In Palestine, we have a quiet a few patients with CF disease and also have reported death related to lung infection in these patients, in one report there was two patients that died at 8 and 17 years of age due to lung infections.(Siryani et al., 2015)

Therefore, the sensitivity and resistance pattern over the years for each antibiotic used in treatment pseudomonal pulmonary infection in CF patients is an important issue in Palestine in order to maintain control over the efficacy of treatment along with prevention increasing in resistance over time as much as possible.

## 1.4 Aims of the study:

### 1.4.1 Main objectives

1. To determine the level of resistance to mostly used anti-pseudomonal antibiotics for treatment infections in CF pediatric patients attending Caritas Baby Hospital in Palestine where the vast majority of CF patients in the West Bank is treated in Caritas.

2. To predict the change in the rates of antibiotic resistance over 5 years (2016-2020) for different species of *P. aeruginosa* for each antibiotic.
3. To investigate the activity of fosfomycin antibiotic on *P. aeruginosa* species in CF patients, and differentiate its activity between mucoid, non-mucoid and CRPA species.

### 1.4.2 Specific objectives

1.To differentiate the sensitivity pattern for all antibiotics between mucoid and non-mucoid *P. aeruginosa.*

2.To predict the influence of sex, age, geographic distribution on sensitivity for all antibiotics.

3. To find MIC and zone diameter breakpoints for fosfomycin antibiotic on *P. aeruginosa* bacteria which is not available yet in CLSI (Clinical and Laboratory Standard Institute) and EUCAST (European Committee on Antibiotic Susceptibility Testing) clinical breakpoints tables and also recommend these breakpoints to the responsible committee.

Chapter Two

## 2.Literature Review:

For *P. aeruginosa* in CF patients the literature review included:

**1- *P. aeruginosa sensitivity to different antibiotics around the world*.**

In Italy, a study conducted at CF department in Treviso Hospital for 6 antibiotics on *P. aeruginosa* isolates from 57 CF patients showed that during 4 years sensitivity decreased for Ciprofloxacin from 47.11% in 2010 to 30.87% in 2013, for Levofloxacin from 41.66% to 30.10% and for Ceftazidime from 82.61% to 79.34%, while it increased for Colistin from 93.12% to 98.47% and Amikacin from 0% to 48.72%, whereas, Meropenem susceptibility was constant during the study period (72.08% in 2010, 71.17 in 2013). Also, Sensitivity was greater in the younger group in 2010 for all the antibiotic agents and in 2013 similar sensitivity prevalence with non-significantly difference detectable among the two populations were reported(Lucca et al., 2018).

In the CF Centre of Gaslini Children's Hospital in Italy, the susceptibility patterns of 1315 mucoid and non-mucoid *P. aeruginosa* strains from 224 patients were determined along with antibiotic utilization in a CF Centre from 1993 to1997. Ceftazidime was the most active agent (86.0% sensitive isolates),followed by piperacillin-tazobactam (81.7%), aztreonam (80.3%),

imipenem (80%), piperacillin (76.8%), tobramycin (76.5%), ciprofloxacin(73.7%), ticarcillin (72.4%), ticarcillin-clavulanic acid (70.2%), amikacin (69.5%), netilmicin (56.5%), meropenem (79%) and imipenem(75.5%).Trend testing from 1993 to 1997 showed a significant decline of susceptibility to aminoglycosides, imipenem and ciprofloxacin, while the susceptibility to piperacillin and ceftazidime was stable(Manno et al., 2005).

In Germany, from April 1994 to April 1996, 399 isolates of *P. aeruginosa* collected from 34 children in CF center at the Department of Pediatrics of the Leipzig University. This study showed that regarding to aminoglycosides, the *P. aeruginosa* isolates were most susceptible totobramycin, followed by gentamicin and amikacin. Among the quinolones ciprofloxacin showed the highest activity, followed by trovafloxacin, levofloxacin and moxifloxacin. Also, over 2 years there was a moderate increase of MICs for most antibiotics. Ceftazidime, imipenem and meropenem are the least affected.

Regarding mucoid and non-mucoid phenotypes, the mucoid isolates were, except for the aminoglycosides, less susceptible to the anti-pseudomonal agents, but significance was only achieved by imipenem, for aminoglycosides, the most susceptibility was to gentamicin, tobramycin, and amikacin(Spencker et al., 2003).

In Germany 2002, Tanja Schulin collected 385 mucoid and non-mucoid strains of *P. aeruginosa* isolated from 192 sputa from 57 adult CF patients. Susceptibility testing using an agar dilution technique was performed for 7 antibiotics. For all antibiotics tested, except tobramycin, the mucoid isolates were more susceptible than the non-mucoid strains. Meropenem, ceftazidime and piperacillin were the most potent agents (susceptibility 86.2%, 84.2% and 84%, respectively). Tobramycin and colistin susceptibility rates were lower, but in light of higher intra-bronchial concentrations of these drugs a greater percentage of strains might still be

clinically susceptible. Only 46.2% and 41.8% of isolates were susceptible to ciprofloxacin and fosfomycin, respectively(Schülin, 2002).

## 2- P. aeruginosa Sensitivity to fosfomycin.

There was no reference guideline for determining sensitivity for fosfomycin on *P.aeruginosa* bacteria in CLSI and EUCAST.

In 2011, Ching-Lan Lu and others studied the susceptibility of different MDR bacteria to fosfomycin, regarding to *P. aeruginosa*, the modal MICs was high at 64 µg/ml. They concluded that a significant proportion of wild-type *P. aeruginosa* isolates have MICs below the susceptible breakpoint. However further work, including clinical studies, is needed to determine if wild type *P. aeruginosa* strains are truly susceptible to fosfomycin(Lu et al., 2011).

In Greece, 30 sample of *P.aeruginosa* isolates were collected randomly from patients in a general tertiary care hospital in Athens, to evaluate the antimicrobial activity of fosfomycin against this bacteria, the result showed that the fosfomycin MICs of the *P. aeruginosa* strains had a distribution across a range of 4 to over 512 µg/ml; $MIC_{50}$ was 32 µg/ml and $MIC_{90}$ 128 µg/ml. 12 isolates from 30 had MIC level of 32 µg/ml, which considered sensitive according to CASFM(Falagas et al., 2008).

In 2015, Clare C. Walsh and his colleagues investigated the in vitro activity of fosfomycin on 64 *P. aeruginosa* isolates, 59 isolates were from CF patients, they concluded that 61% of isolates considered fosfomycin susceptible (MIC < 64 mg/L). The MIC distributions for MDR and non-MDR isolates were similar. Also, they found that Most concentrations resulted in complete replacement of fosfomycin-susceptible colonies by fosfomycin-resistant colonies. These data suggest mono-therapy with fosfomycin may be problematic for the treatment of infections

caused by *P. aeruginosa*, so further investigation of fosfomycin combination therapy is warranted(Walsh, McIntosh, Peleg, Kirkpatrick, & Bergen, 2015).

In 2003, IV fosfomycin was used in combination with other antibiotics to treat 30 pulmonary exacerbations in 15 adult CF patients colonized by *P. aeruginosa*, mainly MDR strains. The study showed that IV fosfomycin is well tolerated by adult patients with CF (just one patient experienced nausea during combination treatment with fosfomycin, and the drug was withdrawn. And no other side effects were founded) and can be useful in the treatment of those colonized with multi-resistant *P. aeruginosa*(Mirakhur et al., 2003).

In vitro, a synergistic effect has been demonstrated for fosfomycin in combination with ofloxacin against *P. aeruginosa* growing in a biofilm(Kumon, Ono, Iida, & Nickel, 1995).And also with ciprofloxacin against *P. aeruginosa* isolates from CF patients(Figueredo & Neu, 1988)

Regarding to carbapenems, $MIC_{90}$ of fosfomycin alone, fosfomycin in combination with carbapenem, carbapenems alone and carbapenems in combination with fosfomycin were 1,024, 1,024, 32 and 32 mg/ml, for multidrug resistant (MDR)-PA and 512, 128, 8 and 3 mg/ml respectively, for non-MDR *P. aeruginosa*. So, prolonged infusion of fosfomycin combined with extended carbapenem infusion could be used in non-MDR *P. aeruginosa* treatment(Asuphon, Montakantikul, Houngsaitong, Kiratisin, & Sonthisombat, 2016).

In the presence of mucin, fosfomycin enhances the active uptake of tobramycin into *P. aeruginosa* resulting in greater inhibition of protein synthesis, enhanced bacterial killing, and ultimately a lower frequency of resistance development(MacLeod et al., 2012). It was observed that (fosfomycin-tobramycin) combination and tobramycin alone demonstrated comparable activity on biofilm formation and disruption. So, this may lead to a decrease in negative side effects of aminoglycosides(Anderson, Kenney, Macleod, Henig, & O'Toole, 2013).

For 15 MDR P. aeruginosa isolates, synergy of fosfomycin with imipenem, meropenem, doripenem, colistin, netilmicin, and tigecycline was observed for 46.7%, 53.3%, 73.3%, 13.3%, 13.3%, and 13.3% of the isolates, respectively(Samonis, Maraki, Karageorgopoulos, Vouloumanou, & Falagas, 2012).

Study carried out before 2 years concluded that treatment of pulmonary exacerbation of CF with antibiotic regimens including fosfomycin appear to be safe and clinically effective. Fosfomycin should, therefore, be considered as an add-on therapy in patients who failed to respond to initial treatment and with multiple drug side effects(Spoletini et al., 2018).

The recent study was carried out in early 2020, in Italy, the results concluded that the percentage of sensitivity measured by the agar dilution method was 77% and 78% for mucoid and non-mucoid strains, respectively. MIC50 and MIC90 were equal to 32 mg/ml and 64mg/ml, respectively(Bressan et al., 2020).

## 3- Antimicrobial sensitivity in P. aeruginosa studies in Arab world.

In the state of Qatar, a total of 61 *P. aeruginosa* samples were collected from 30 CF patients, since October 2014 to September 2015 at Hamad Medical Corporation. The study showed that *P. aeruginosa* in lower respiratory samples of 30 CF patients showed the highest sensitivity to piperacillin/tazobactam (90.2%) followed by meropenem (88.5%), ciprofloxacin (77%), cefepime (70.5%), amikacin (67.2%), and gentamicin (59%) and all the isolates were susceptible to colistin during the study period.

Among 30 samples, there were 12 samples were positive for MDR-*P. aeruginosa* (7 non-mucoid and 5 mucoid isolates), the antimicrobial susceptibility pattern of these MDR-*P. aeruginosa* isolates showed the highest rate of resistance (100%) toward each gentamycin, amikacin, and

cefepime, followed by 91.7% to ciprofloxacin, 75% to tobramycin, 58.3% to meropenem, and 50% to piperacillin-tazobactam(AbdulWahab et al., 2017).

In Palestine, no previous studies have been carried out regarding evolution of resistance in *P. aeruginosa* in CF patients and no one studied the efficacy of Fosfomycin in this topic.

# Chapter Three

## 3.Methodology:

### 3.1 Study setting:

This research was done in Caritas Baby Hospital, the only children's hospital in West Bankthat offers medical and social services to every child in need, irrespective of origin or religion. Treatments are offered to Palestinian children from birth up to 18 years of age in the outpatient department and the inpatient wards of the hospital.

Caritas Baby Hospital focuses on three pediatric subspecialties; pulmonology, neurology, and neonatal and pediatric intensive care. It consider the only hospital that treat CF patients in the west bank. For this reason we chose it to do this research which related to CF disease.

All microbiological data in this study was taken from Caritas Baby Hospital loboratory, it is considered a reliable source for microbiological data because they follow the CLSI guidelines for working in this area. Ethical approval was taken from Caritas Baby Hospital to sharing us this data, the form in Appendix 10.

### 3.2 Materials:

- Strains: One hundred and twenty nine samples of *P. aeruginosa* were extracted from bacterial saving boxes;that were kept frozen at -60ºC, the samples included three categories (non-mucoid , mucoid and carbapenem-resistant *P.aeruginosa* CRPA). All samples were from CF patients.

- Volumetric flasks, graduated cylinder, electronic balance, magnetic heater stirrer,distilled water, Sterile petri dishes and Muller Hinton agar powder (Oxoid Ltd, Hamshire, UK) for media preparation.
- Fosfomycin Disodium powder 100mg (Sigma- Aldrich, Buchs, Switzerland)
- Glucose-6-phosphate powder (G -6- P) ( Roche, Germany)
- Glass test tubes, sterile volumetric tubes, D/W (B. Braun, Melsungen, Germany), sterile pipettes and Micropipette for antibiotic dillution.
- Normal saline 0.9% (B. Braun, Melsungen, Germany)

## 3.3 *P. aeruginosa* strains identification:

Once CF sputum sample enter the laboratory, it is cultured on 5 types of plates (Sheep blood agar, Chocolate agar, Maconkey agar, *Burkhulderia capacia* medium and Mannitol agar), on Macconkey agar looking for any non-fermenter colonies. On Gram stain; Gram negative rods. By oxidase test; positive. Then, the bacteria will be inoculated on other two tube medias; TSI and nutrient broth at 42ºC. In the same time sensitivity test will be done, next day after incubation overnight, the tubes will be checked; TSI must give K \ K ie red color on slant and bottom, nutrient broth must give growth (the broth becomes turbid).

From sensitivity test, chloramphenicol disc must be resistant, always *P.aeruginosa* resistant to this antibiotic. In some cases, when there was a confusion about if its *P.aeruginosa* or not, API non E strip can be done to give a definitive diagnosis.

## 3.4 Commonly used antibiotics sensitivity testing (disc diffusion method):

From 2016 to 2020, sensitivity test was performed for all isolates using Kirby–Bauerdisk diffusion method on Muller-Hinton agar plates (Becton, Dickinson and company, Sparks, MD, USA) according to the Clinical and Laboratory Standard Institute guidelines (CLSI 2016) for the following antibiotics: ceftazidime (30µg), meropenem (10 µg), amikacin (30µg), gentamycin (10 µg), piperacillin-tazobactam (10:1 110µg), and ciprofloxacin (5 µg). (Oxoid Ltd, Hamshire, UK).

## 3.5 Antibiotics sensitivity over 5 years data analysis:

Clinical and microbiological data were extracted from medical database for pediatric CF patients in Caritas Baby Hospital from January 2016 to December 2020.The results were analyzed using Microsoft Excel Software 2010 and Statistical Package for Social Sciences (SPSS) program version 20.

## 3.6 Fosfomycin Susceptibility Testing:

129 sputum samples from CF patients had been rolled in the fosfomycin sensitivity tests, these samples include the three types of p. aeruginosa as following: 79 non-mucoid, 40 mucoid and 10 CRPA.

Fosfomycin susceptibility testing was done in two different methods, disc diffusion method (using 200µg fosfomycin/ 50µg G-6-p and 50 µg fosfomycin discs) and agar dilution MIC method.

1- **MIC agar dilution method:**

MICs for fosfomycin was determined by agar dilution method according to CLSI using 0.5 McFarland inoculum and the Steers-Replicator apparatus (Figure 2.1). This allowed the testing of a total of 37 microorganisms simultaneously on a single agar plate.

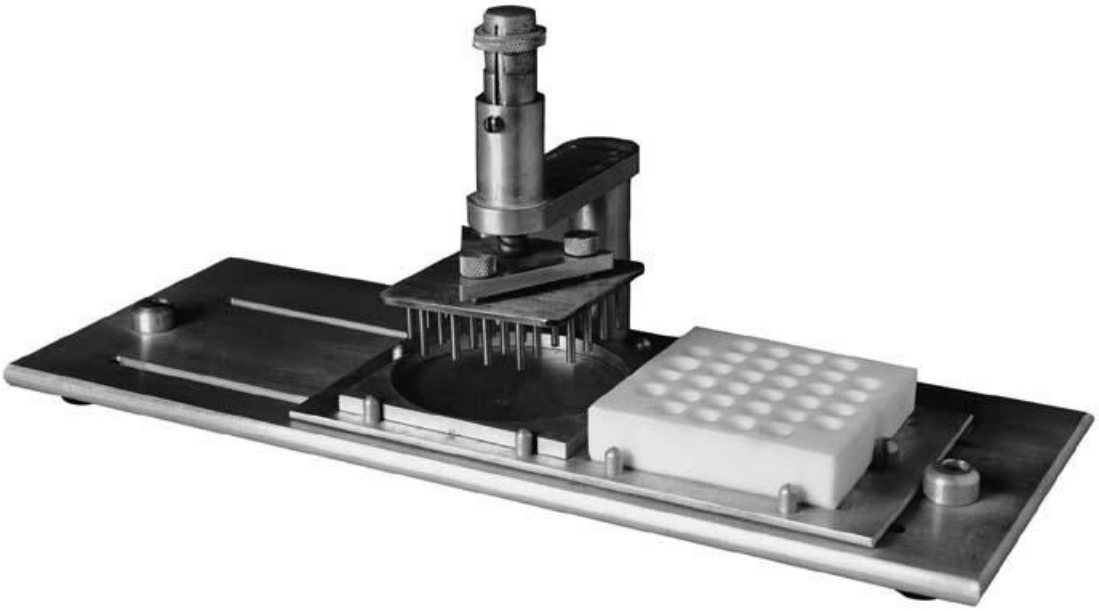


**Figure 2.1:** Steer's Replicator

Method validation for agar dilution method was done by using Ceftriaxone 1000 mg antibiotic (Panpharma UK Ltd, Liverpool, England) on *Escherichia coli* ATCC 25922, *pseudomonas aeruginosa* ATCC27853 and *Staphylococcus aureus* ATCC 29213 to ensure that the method is reliable. Ceftriaxone powder was diluted in D/W (B. Braun, Melsungen, Germany)according to Clinical Laboratory Standard Institute (CLSI) standards. The concentrations which tested are: 0.03, 0.06, 0.125, 0.25, 0.5. 1, 2, 4, 8, 16, 32, 64, 128 and 256 μg/ml. This done in triplicate times for each isolate.

For fosfomycin powder, D/W was also used for dilution and the concentrations which tested are 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256 µg/ml. and also this done in triplicate times for each sample.

The bacteria were taken from saving tube by sterile pipette or sterile loop and cultured directly on sheep blood agar, and then the cultured bacteria stayed in incubator for 24 hrs. After that, the bacteria were re-cultured on Macconkey agar as a step in refusing any contaminated samples, and incubated for 24 hrs. After eliminating any contaminated samples, the bacteria were re-cultured from Macconkey plates onto sheep blood agar and incubated it for another 24 hrs in order to use it for sensitivity test. I was preparing Muller Hinton agar with different concentrations of fosfomycin antibiotic in these steps:

- Stock solution (10 ml) of fosfomycin was prepared by adding 9.765 ml of distilled water, this stock solution has a concentration of 10240 mg/L.
- Three other stock solutions were prepared with these concentrations respectively: 2560, 320 and 40 mg/L by following dilution equation (M1*V1 = M2*V2).
- From theses stock solutions, all concentrations for fosfomycin sensitivity were prepared as shown in the following table (2.1):

  **Table (2.1):**Preparation of dilution of agents for use in agar dilution susceptibility tests.

| Antimicrobial concentration (mg/L) in stock solution | Volume stock solution (mL) | Volume distilled water (mL) | Antimicrobial concentration obtained (mg/L) | Final concentration in medium after addition of 19 mL of agar |
|---|---|---|---|---|
| 10240 | 1 | 0 | 10240 | 512 |
| 10240 | 1 | 1 | 5120 | 256 |
| 10240 | 1 | 3 | 2560 | 128 |
| 2560 | 1 | 1 | 1280 | 64 |
| 2560 | 1 | 3 | 640 | 32 |
| 2560 | 1 | 7 | 320 | 16 |
| 320 | 1 | 1 | 160 | 8 |
| 320 | 1 | 3 | 80 | 4 |
| 320 | 1 | 7 | 40 | 2 |
| 40 | 1 | 1 | 20 | 1 |
| 40 | 1 | 3 | 10 | 0.5 |
| 40 | 1 | 7 | 5 | 0.25 |
| 5 | 1 | 1 | 2.5 | 0.125 |
| 5 | 1 | 3 | 1.25 | 0.06 |
| 5 | 1 | 7 | 0.625 | 0.03 |
| 0.625 | 1 | 1 | 0.3125 | 0.015 |
| 0.625 | 1 | 3 | 0.1562 | 0.008 |
| 0.625 | 1 | 7 | 0.0781 | 0.004 |

G-6-P powder was added to Muller Hinton media (25 mg/L) in the flask after autoclaving when the temperature reaches 42-50 ºC.

Finally, the Muller Hinton and fosfomycin were poured in the Petri dish and left to dry.

On the next day, the media had dried, I had prepared 0.5 McFarland inoculums for each sample and also for controls (*Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922). And by using sterile pipette I had took a small volume of bacteria's solution and put it in its place in steers replicator apparatus and the last step was cultured 35 sample, *P. aeruginosa* ATCC 27853 and normal saline as positive and negative controls, respectively. And finally, put the plates after culturing in incubator. After 20 hr., I read the result for sensitivity, MIC here is defined as the lowest concentration of fosfomycin that prevents visible growth of a microorganism.

**2- Disc diffusion method:**

Fosfomycin sensitivity test was performed for all isolates using Kirby–Bauerdisk diffusion method on Muller-Hinton agar plates (Becton, Dickinson, Sparks, MD, USA) according to the

Clinical and Laboratory Standard Institute guidelines (CLSI 2016) for two different discs: fosfomycin 50 µg(Oxoid Ltd, Hamshire, UK) and fosfomycin 200 µg with glucose-6-phosphate 50 µg(Becton, Dickinson, Sparks, MD, USA). Each isolate was done in duplicate.

# Chapter Four

## 4.Results:

## 4.1 Sensitivity analysis from Jan.2016 till Dec. 2020:

### 4.1.1 Study population:

Clinical isolates of *P. aeruginosa* bacteria were isolated from sputum samples of 435 CF patients presenting with pulmonary infection at Caritas Baby Hospital in the period between 2016 and 2020. Geographical distribution of patients includes all areas of the West Bank in Palestine from the north to the center and the south. Patients ages are distributed from newborn to less than 18 years according to the international classification of pediatric ages, patients are distributed as described the table (3.1):

**Table (3.1):**Geographical area, sex and age distribution of the patients enrolled in the analysis.

| | | Geographical Area | | | |
|---|---|---|---|---|---|
| | | North N(%) | Central N(%) | South N(%) | Total N(%) |
| Gender | Male | 35(51.5%) | 37(59.7%) | 154(50.5%) | 226(52%) |
| | Female | 33(48.5%) | 25(40.3%) | 151(49.5%) | 209(48%) |
| | Total | 68(100%) | 62(100%) | 305(100%) | 435(100%) |
| Age (year) | <1 | 12(17.6%) | 10(16.1%) | 93(30.5%) | 115(26.4%) |
| | 1 - <2 | 6(8.8%) | 4(6.5%) | 19(6.2%) | 29(6.7%) |
| | 2 - <5 | 4(5.9%) | 11(17.7%) | 46(15.1%) | 61(14%) |
| | 5 - <10 | 24(35.3%) | 19(30.6%) | 76(24.9%) | 119(27.4%) |
| | 10 - <15 | 18(26.5%) | 16(25.8%) | 56(18.4%) | 90(20.7%) |
| | 15 - <18 | 4(5.9%) | 2(3.2%) | 15(4.9%) | 21(4.8%) |
| | Total | 68(100%) | 62(100%) | 305(100%) | 435(100%) |

**3.1.2 Bacterial types in CF patients:** Sputum samples of CF patients are colonized by different types of bacteria. *S. aureus, H. influenza* and *P. aeruginosa* are the three major types which affected the lung, from 2016 to 2020 the bacteria which affected CF patients are as the following figure (3.1):

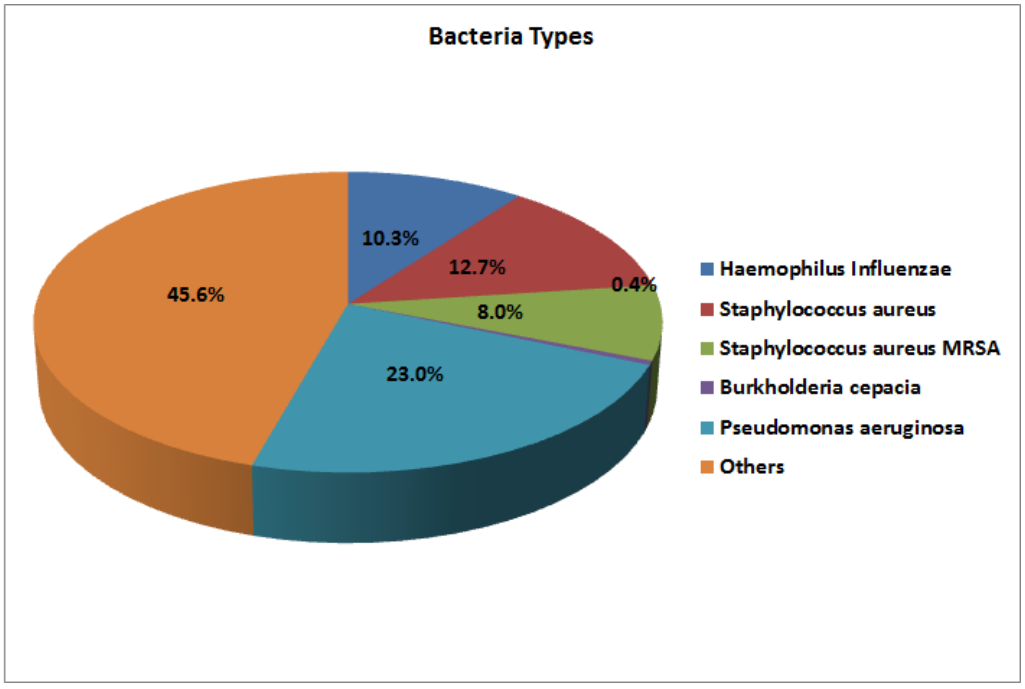



**Figure 3.1:** Types of bacteria in CF samples

In addition, *P. aeruginosa* was distributed between 3 categories, mucoid, non-mucoid and carbapenems-resistant as illustrated in the figure (3.2):

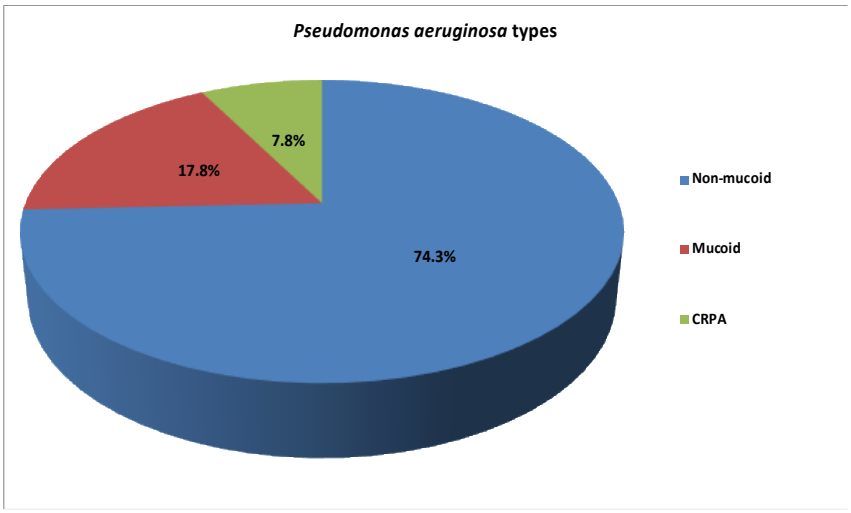



**Figure 3.2:** *P.aeruginosa* types in CF samples

The majority of *P. aeruginosa* was from non-mucoid type 74.3 %, following by mucoid type 17.8% and the lowest one is CRPA only 7.8%.

These *P. aeruginosa* isolates were collected from 348 samples of 94 CF patients. So, there were patients with multiple samples, this multiplicity in samples is characterized as the following table (3.2):

**Table (3.2):**Multiplicity in *P.aeruginosa* samples

| Number of samples | Number of patients with this number of samples |
|---|---|
| 1 | 46 |
| 2 | 17 |
| 3 | 5 |
| 4 | 3 |
| 5 | 3 |
| 6 | 2 |
| 7 | 3 |
| 8 | 3 |
| 10 | 3 |
| 11 | 3 |
| 14 | 2 |
| 17 | 1 |
| 18 | 1 |
| 20 | 1 |

| | |
|---|---|
| 23 | 1 |
| Grand Total | 94 |

### 3.1.3 Difference in sensitivity between antibiotics and the 3 types of P. aeruginosa among 5 years:

regardless of years, from 2016 to 2020 the sensitivity for each antibiotic was illustrated in the following figure (3.3):

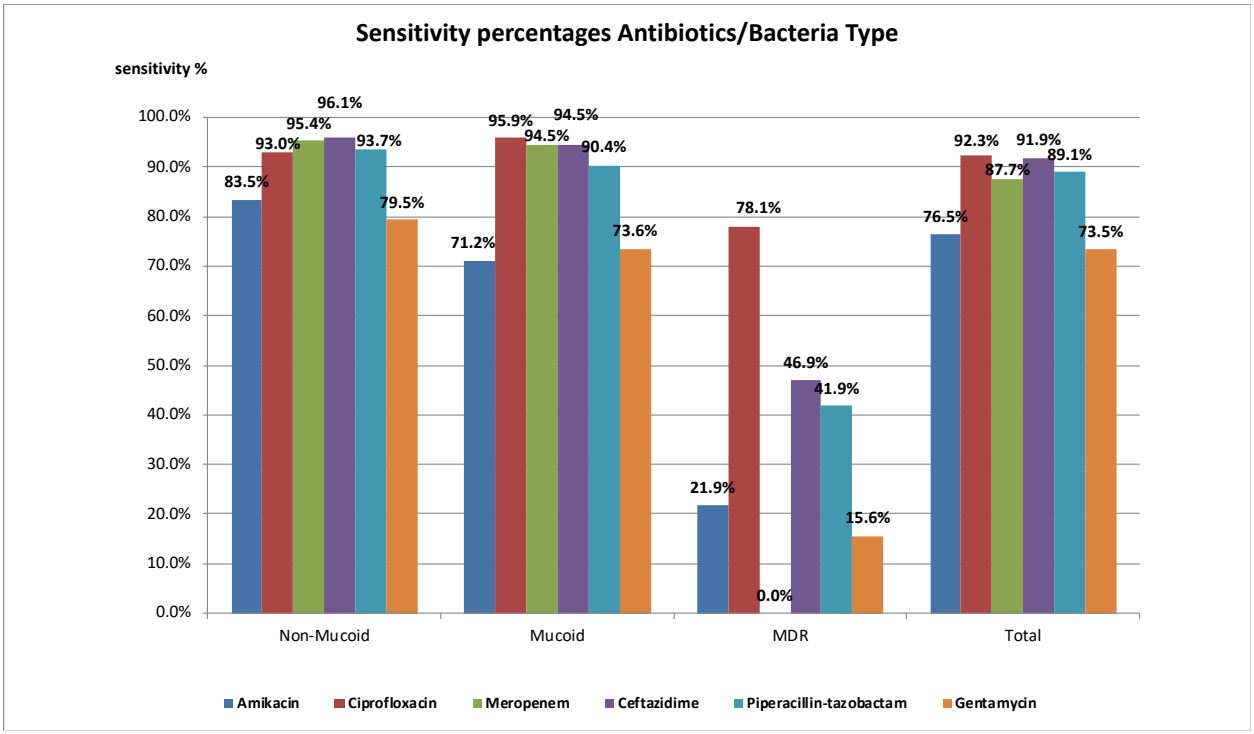



**Figure 3.3:***P. aeruginosa* antimicrobial sensitivity in general

A general view of the results show that the lowest sensitivity always exists corresponding to CRPA bacteria for all antibiotics, where the best sensitivity was for ciprofloxacin which is only 78.1% and lower sensitivity for other antibiotics; all are less than 50% and for meropenem is 0%.

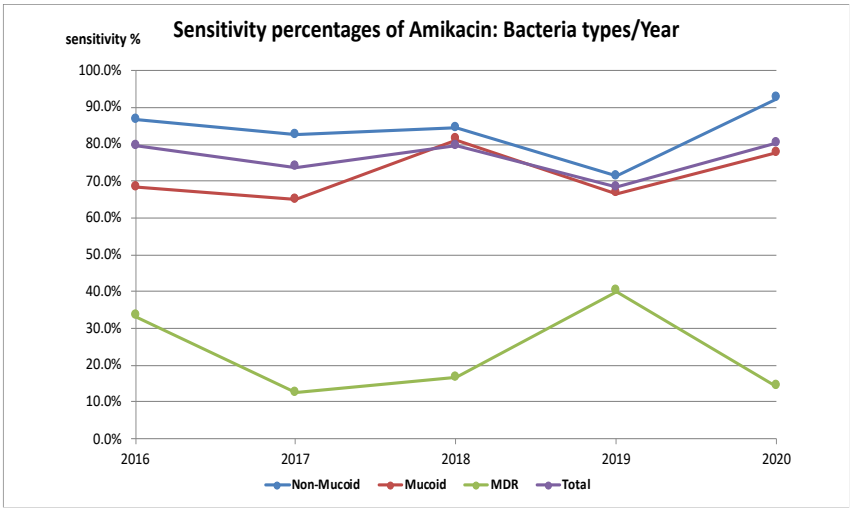
For non-mucoid bacteria the best sensitivity was for ceftazidime (96.1%), and the lowest was for gentamicin (79.5%).

For mucoid bacteria the best sensitivity was for ciprofloxacin (95.9%) and the lowest was for amikacin (71.2%).

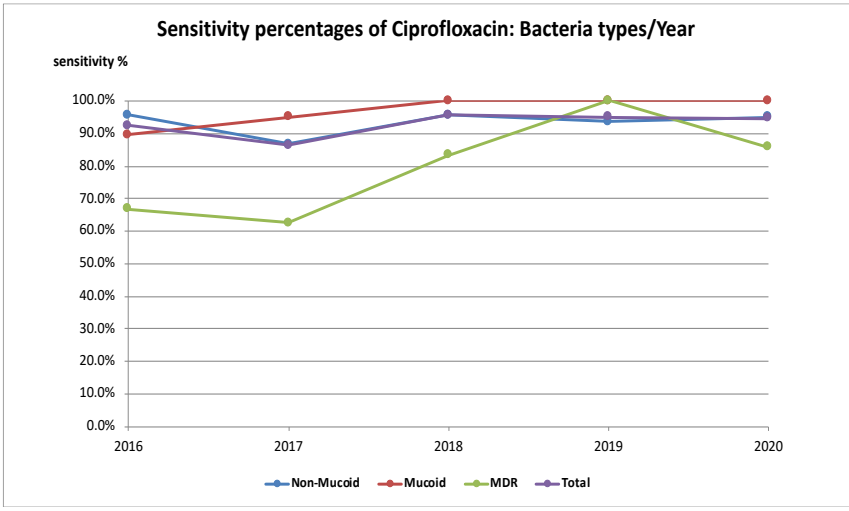
Statistical analysis (shown in appendix 3), indicates that there are significant differences at the level 0.05 in sensitivity between antibiotics over 5 years of usage for each bacteria type since all P-values of Chi-Square Tests for antibiotics are less than 0.05.

### 3.1.4 Differences in sensitivity between years for each type of P. aeruginosa regarding antibiotics:

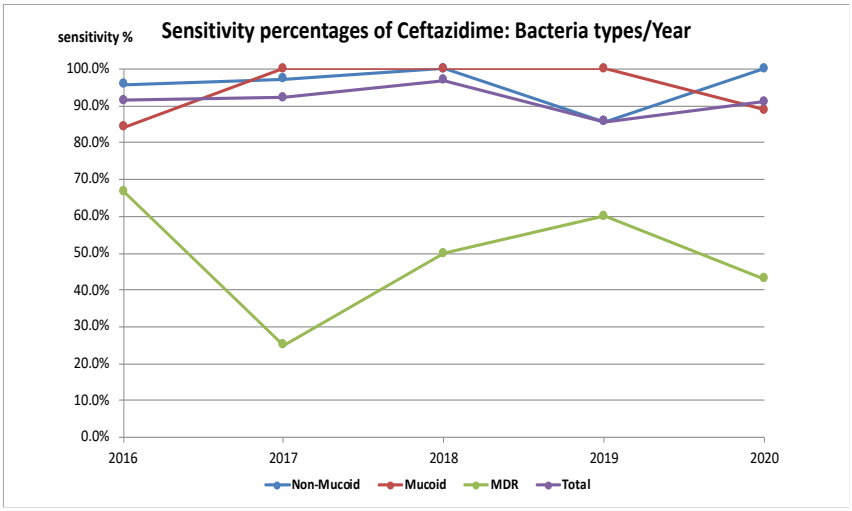
To understand how the sensitivity for each antibiotic was changed with the passage of time, the data was summarized in the charts below for each antibiotic as the following figure (3.4) and statistical analysis is shown in appendix (4,5):



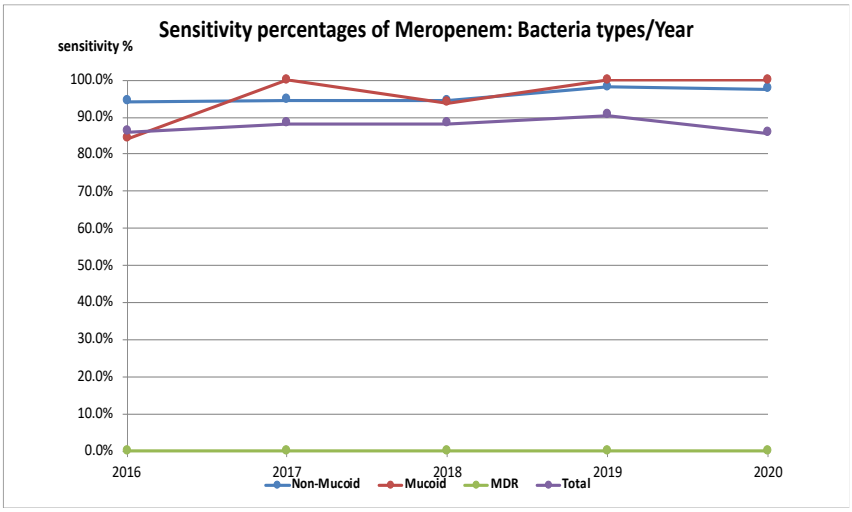

(a)



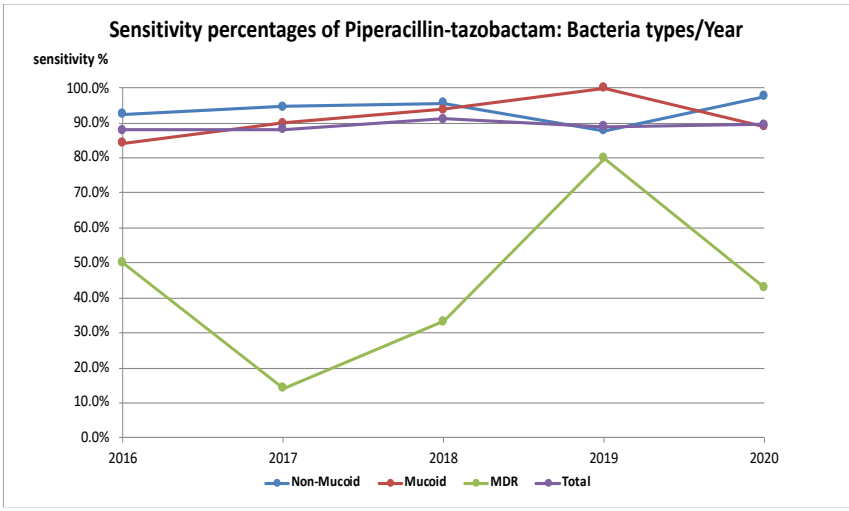

(b)


Sensitivity percentages of Ceftazidime: Bacteria types/Year
sensitivity %
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
2016
2017
2018
2019
2020
Non-Mucoid
Mucoid
MDR
Total

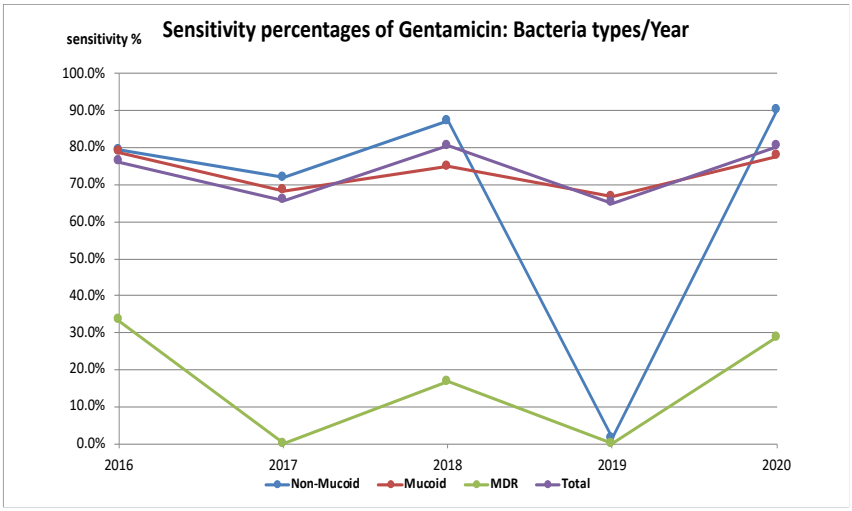
(c)


Sensitivity percentages of Meropenem: Bacteria types/Year
sensitivity %
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
2016
2017
2018
2019
2020
Non-Mucoid
Mucoid
MDR
Total

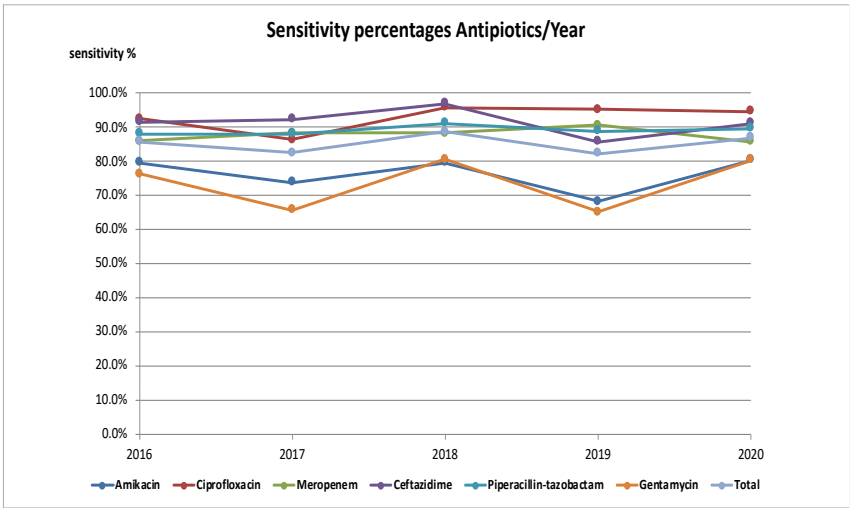
(d)


Sensitivity percentages of Piperacillin-tazobactam: Bacteria types/Year
sensitivity %
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
2016
2017
2018
2019
2020
Non-Mucoid
Mucoid
MDR
Total

(e)




(f)




(g)

**Figure 3.4:** Antibiotics sensitivity from 2016 until 2020

Generally, the results show that the sensitivity for all antibiotics corresponding to all bacteria types are excellent and are oscillating slightly with time without a big change in its percentages over the past 5 years. Figure (3.4g).

The only type that had poor sensitivity is CRPA, which had 0% sensitivity toward meropenem, and for other antibiotics less than 40% except ciprofloxacin which has sensitivity more than 60% over 5 years.

For amikacin, the results in appendix 3 show that there are significant differences at the level 0.05 in sensitivity between the five years corresponding to Non-Mucoid bacteria type only, since its P-value of Chi-Square Test for Years is less than 0.05.

Regarding non-mucoid bacteria type, the best sensitivity was (92.5%) in the year (2020) which is significantly higher than the worst sensitivity (71.4%) in the year (2019). Regarding Mucoid Bacteria type, the best sensitivity was (81.3%) in the year (2018) and the worst sensitivity was (65%) in the year (2017). Regarding the CRPA type, the best sensitivity was (40%) in the year (2019) and the worst sensitivity was (12.5%) in the year (2017). Figure (3.4a)

For ciprofloxacin, there are no significant differences at the level 0.05 in sensitivity between the five years for each bacteria type, since all P-values of Chi-Square Tests for Years are more than 0.05. Regarding non-mucoid bacteria type, the best sensitivity was (95.7%) and (95.6%) in the years (2018) and (2016) respectively, while the worst sensitivity was (86.7%) in the year (2017). Regarding mucoid bacteria type, it is clear that the sensitivity increasing over time, the best sensitivity was in the last three years by (100%) sensitivity percentage, while the worst sensitivity was (89.5%) in the year (2016). Regarding the CRPA type, the best sensitivity was (100%) in the year (2019) and the worst sensitivity was (62.5%) in the year (2017). Figure (3.4b)

For meropenem, there are no significant differences at the level 0.05 in sensitivity between the five years for each bacteria type, since all P-values of Chi-Square Tests for Years are more than 0.05. Also, the results show that there is no sensitivity (0%) corresponding to CRPA bacteria for all the five years. Regarding non-mucoid bacteria type, the best sensitivity was (98%) and (97.5%) in the years (2019) and (2020) respectively, while the worst sensitivity was in the first three years (94.1%, 94.6, 94.4). Regarding mucoid bacteria type, the best sensitivity was in the

years 2017, 2019 and 2020 by (100%) sensitivity percentage, while the worst sensitivity was (84.2%) in the year (2016). Figure (3.4c)

For ceftazidime, there are no significant differences at the level 0.05 in sensitivity between the five years for each bacteria type, since all P-values of Chi-Square Tests for Years are more than 0.05. Regarding Non-Mucoid Bacteria type, the best sensitivity was (100%) in the years (2018) and (2020), while the worst sensitivity was (85.7%) in the year (2019). Regarding Mucoid Bacteria type, the best sensitivity was in the three middle years by (100%) sensitivity percentage, while the worst sensitivity was (84.2%) in the year (2016). Regarding the CRPA type, the best sensitivity was (66.7%) in the year (2016) while the worst sensitivity was (25%) in the year (2017). Figure (3.4d)

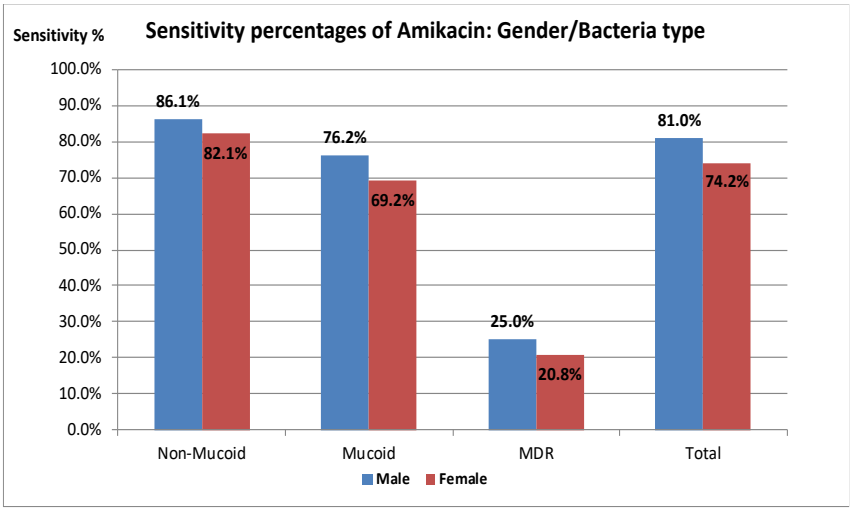
For Piperacillin-tazobactam, there are no significant differences at the level 0.05 in sensitivity between the five years for each bacteria type, since all P-values of Chi-Square Tests for Years are more than 0.05. Regarding non-mucoid bacteria type, the best sensitivity was (97.6%) in the year (2020), while the worst sensitivity was (87.8%) in the year (2019). Regarding mucoid bacteria type, the best sensitivity was in the year (2019) by (100%) sensitivity percentage, while the worst sensitivity was (84.2%) in the year (2016). Regarding the CRPA type, the best sensitivity was (80%) in the year (2019) while the worst sensitivity was (14.3%) in the year (2017). Figure (3.4e)

For gentamicin, there are significant differences at the level 0.05 in sensitivity between the five years only for the Non-Mucoid bacteria type regarding Gentamicin, since its P-value of Chi-Square Tests for Years is less than 0.05. Regarding non-mucoid bacteria type, the best sensitivity was (90%) in the year (2020) which is significantly higher than the worst sensitivity (71.4%) in the year (2019). Regarding mucoid bacteria type, the best sensitivity was in the year (2016) by

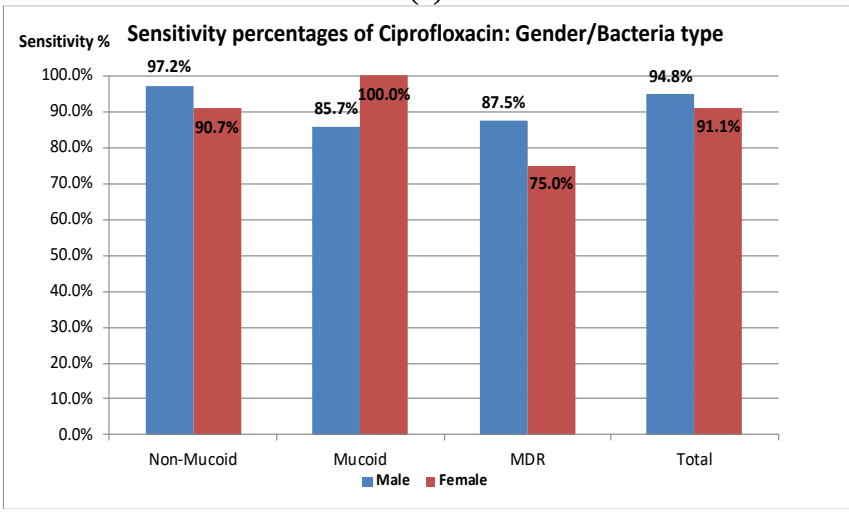
(78.9%), while the worst sensitivity was (66.7%) in the year (2019). Regarding the CRPA type, the best sensitivity was (33.3%) in the year (2016) while the worst sensitivity was (0%) in the years (2017) and (2019). Figure (3.4f)

### 3.1.5 Difference in sensitivity between three types of P. aeruginosa types for each antibiotic regarding sex:

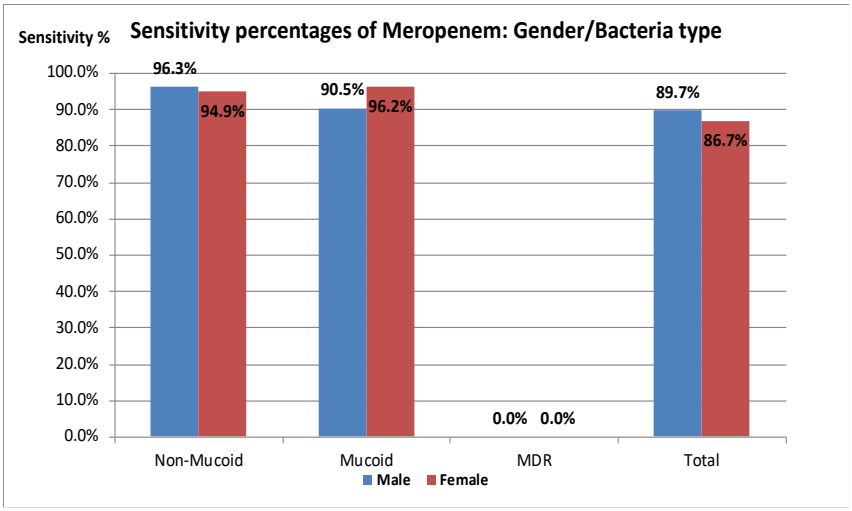
the data was analyzed among 5 years related to sex to predict if this parameter has an influence on the sensitivity to different types of antibiotic, and the results was as the following figure (3.5) and statistical analysis is shown in appendix (6):



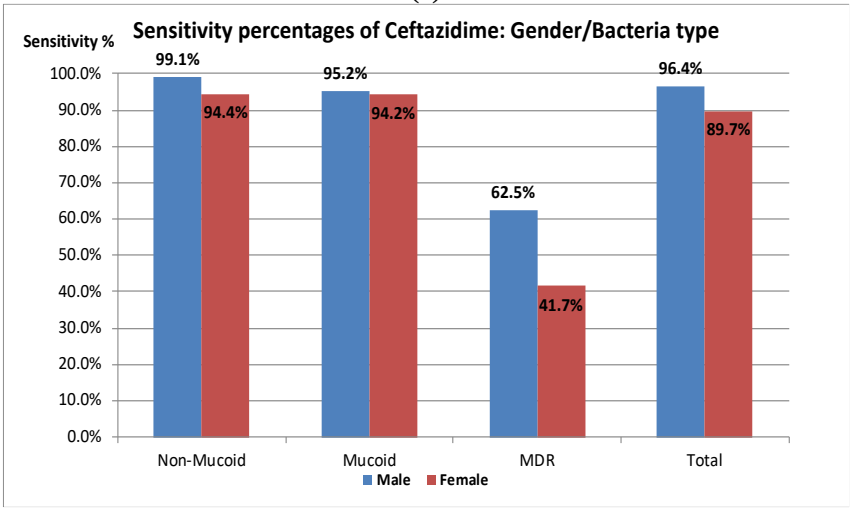

(a)



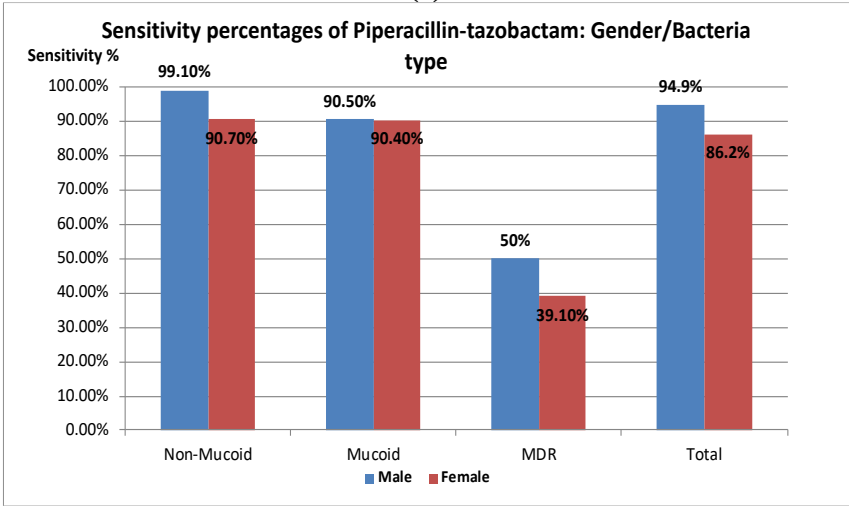

(b)


Sensitivity %
Sensitivity percentages of Meropenem: Gender/Bacteria type
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
96.3%
94.9%
90.5%
96.2%
0.0%
0.0%
89.7%
86.7%
Non-Mucoid
Mucoid
MDR
Total
Male
Female

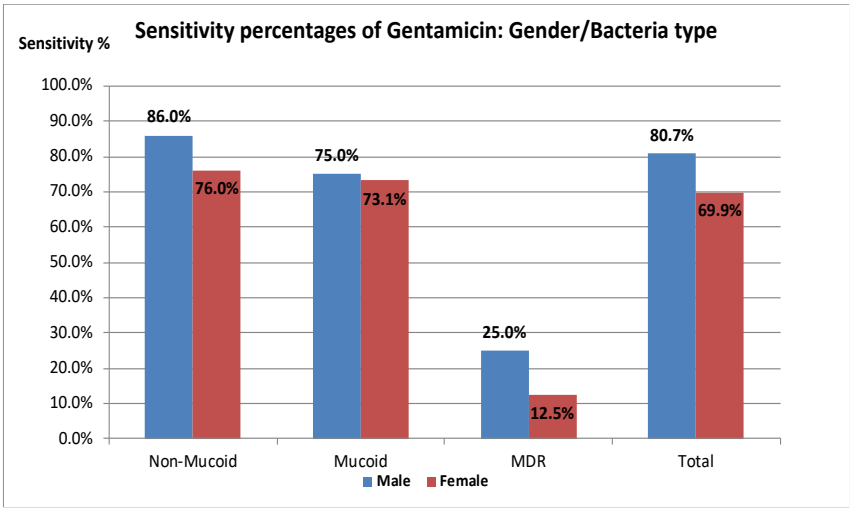
(c)


Sensitivity %
Sensitivity percentages of Ceftazidime: Gender/Bacteria type
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
99.1%
94.4%
95.2%
94.2%
62.5%
41.7%
96.4%
89.7%
Non-Mucoid
Mucoid
MDR
Total
Male
Female

(d)


Sensitivity %
Sensitivity percentages of Piperacillin-tazobactam: Gender/Bacteria type
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
99.10%
90.70%
90.50%
90.40%
50%
39.10%
94.9%
86.2%
Non-Mucoid
Mucoid
MDR
Total
Male
Female

(e)




(f)

**Figure3.5:** Gender influence on antibiotics sensitivity

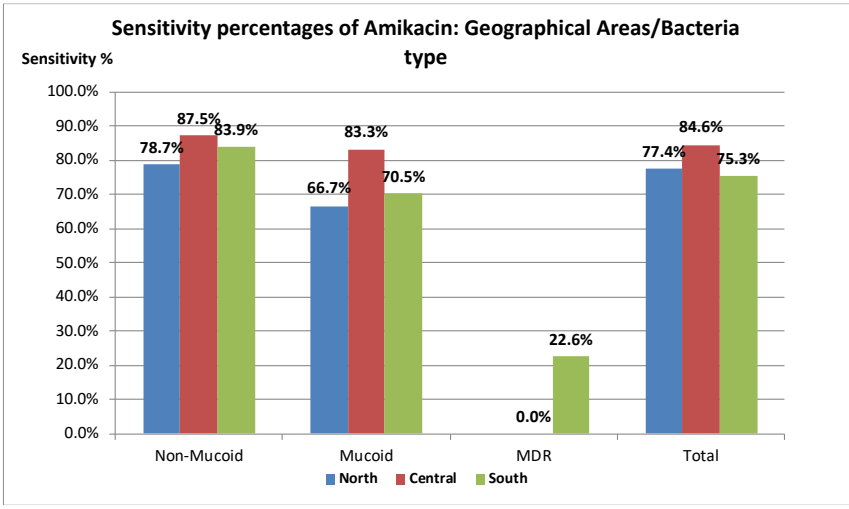
In general, all antibiotics have an excellent sensitivity toward both male and female, and in male is slightly higher than female with no big difference in percentages.

For non-mucoid type, all antibiotics have higher sensitivity in male than female. For mucoid, sensitivity for piperacillin-tazobactam is almost equal (90.5% and 90.4%) for male and female, respectively, amikacin, ceftazidime and gentamycin have higher sensitivity in male than female, and only meropenem and ciprofloxacin have higher sensitivity in female than male. Regarding CRPA, all antibiotics have higher sensitivity in male than female.

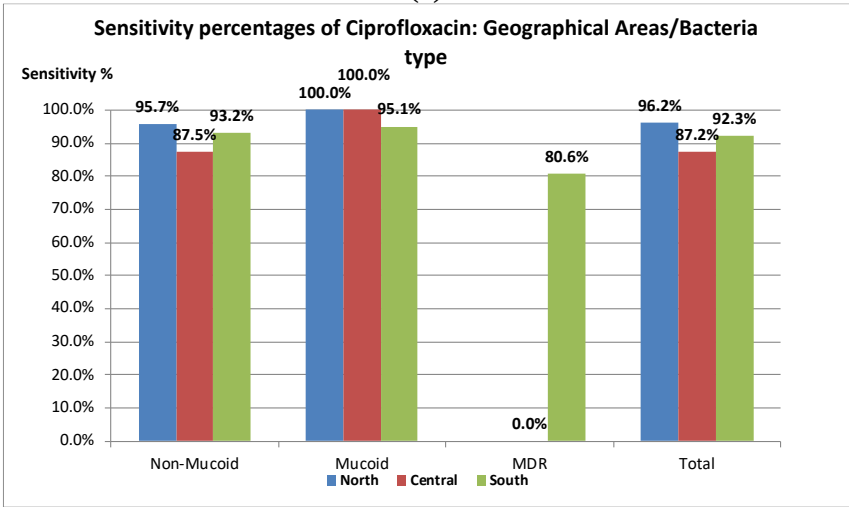
Statistically, regarding non-mucoid type; there are significant differences at the level 0.05 in sensitivity between male and female corresponding to all antibiotics except meropenem. For mucoid type, there are significant differences at the level 0.05 in sensitivity between male and female corresponding to amikacin, ciprofloxacin and piperacillin-tazobactam. Moreover, for CRPA, there are significant differences at the level 0.05 in sensitivity between male and female corresponding to amikacin and piperacillin-tazobactam only.

### 3.1.6 Difference in sensitivity between three types of *P. aeruginosa* types for each antibiotic regarding the area of geographical distribution:

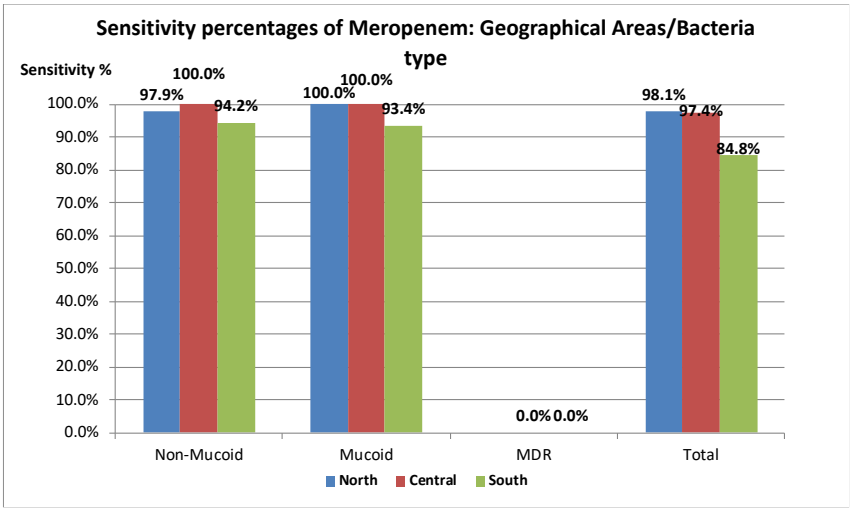
The data was analyzed among 5 years related to geographical distribution to predict if this parameter has an influence on the sensitivity to different types of antibiotic, and the results was as the following figure (3.6) and statistical analysis is shown in appendix (7):



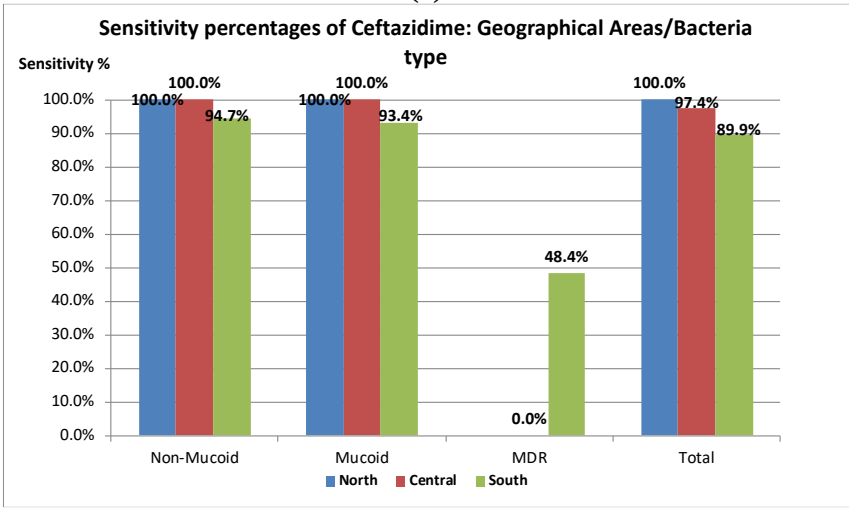

(a)



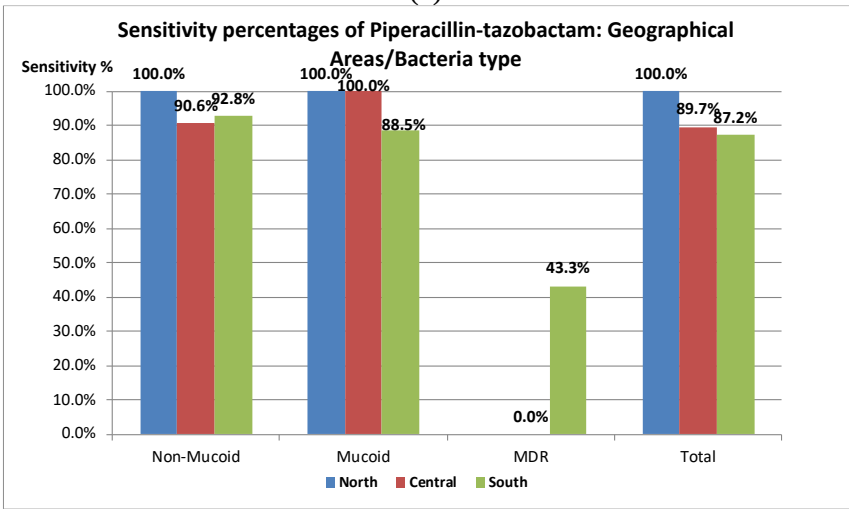

(b)


Sensitivity percentages of Meropenem: Geographical Areas/Bacteria type
Sensitivity %
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
97.9%
100.0%
94.2%
100.0%
100.0%
93.4%
0.0%
0.0%
98.1%
97.4%
84.8%
Non-Mucoid
Mucoid
MDR
Total
North
Central
South

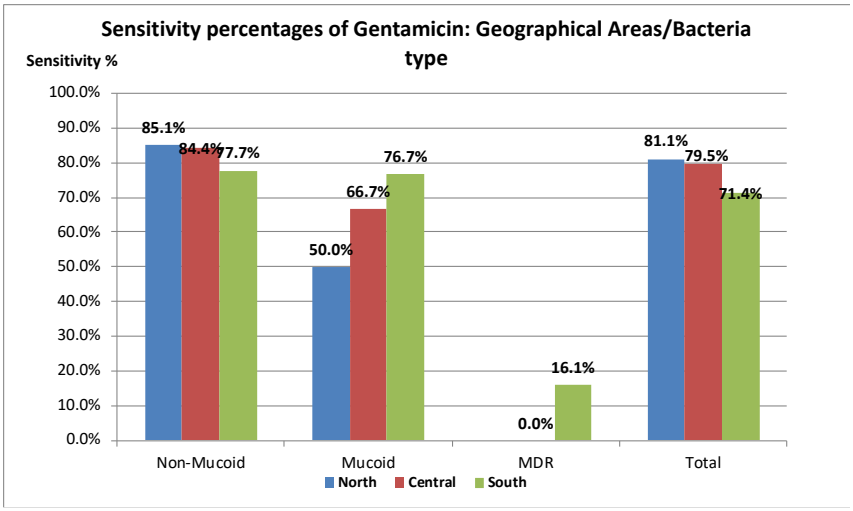
(c)


Sensitivity percentages of Ceftazidime: Geographical Areas/Bacteria type
Sensitivity %
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
100.0%
100.0%
94.7%
100.0%
100.0%
93.4%
0.0%
48.4%
100.0%
97.4%
89.9%
Non-Mucoid
Mucoid
MDR
Total
North
Central
South

(d)


Sensitivity percentages of Piperacillin-tazobactam: Geographical Areas/Bacteria type
Sensitivity %
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
100.0%
90.6%
92.8%
100.0%
100.0%
88.5%
0.0%
43.3%
100.0%
89.7%
87.2%
Non-Mucoid
Mucoid
MDR
Total
North
Central
South

(e)

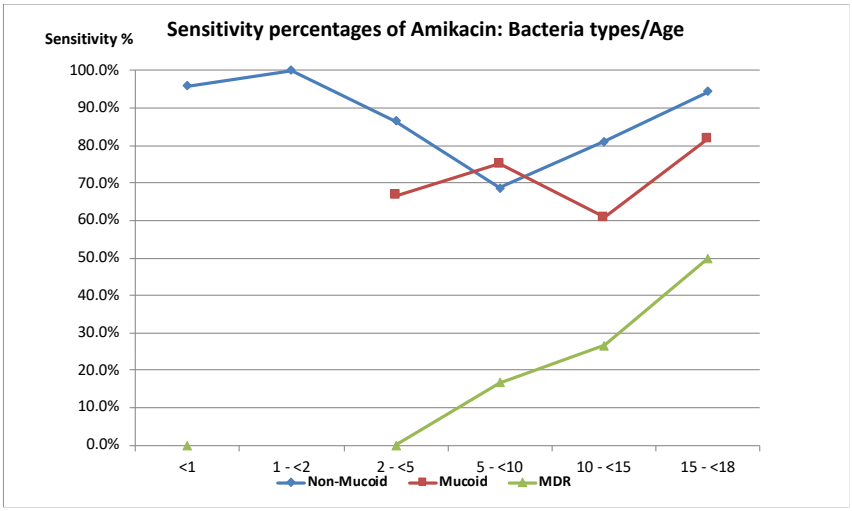



(f)

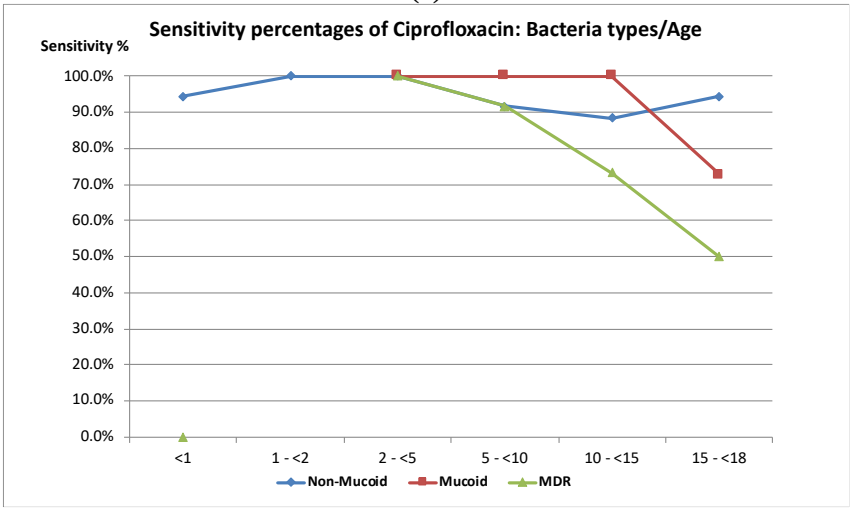
**Figure 3.6:** Geographical distribution influence on antibiotics sensitivity

In general, all antibiotics have an excellent sensitivity towards all types of *P. aeruginosa* bacteria among different geographical area, the south origin has the lowest sensitivity among three origins for all antibiotics except for ciprofloxacin where it has least sensitivity in central area. Regarding subtypes of *P. aeruginosa*, the results in appendix (6) show that there are no significant differences at the level 0.05 in sensitivity between Geographical Areas for all antibiotics corresponding to each bacteria type, since all P-values of Chi-Square Test for Geographical Areas are more than 0.05.

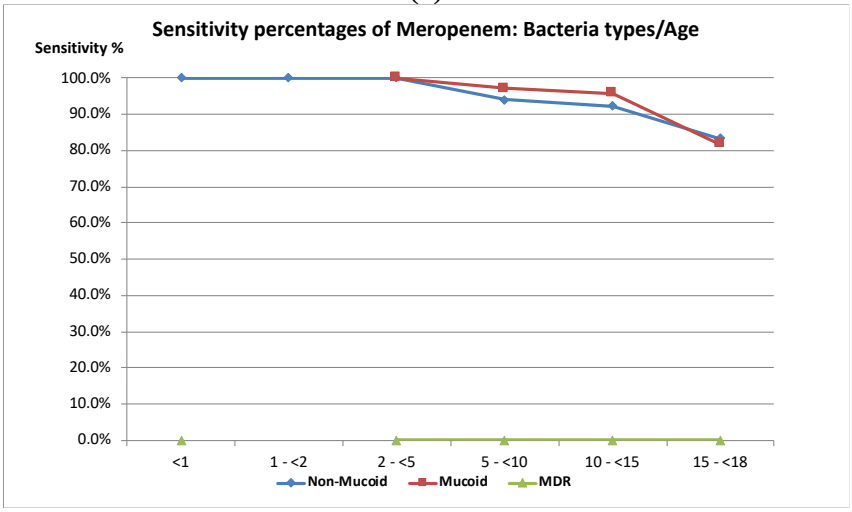
**3.1.7 Difference in sensitivity between three types of *P. aeruginosa* types for each antibiotic regarding the age:** we have 6 age categories in pediatric population, and the next figure (3.7) illustrated the difference in sensitivity between these 6 categories. Statistical analysis is shown in appendix (8):


Sensitivity %
Sensitivity percentages of Amikacin: Bacteria types/Age
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
<1
1 - <2
2 - <5
5 - <10
10 - <15
15 - <18
Non-Mucoid
Mucoid
MDR

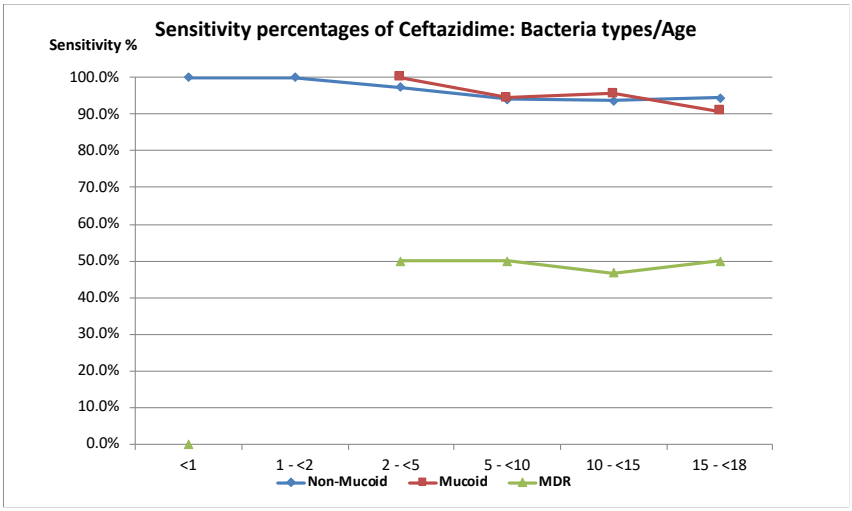
(a)


Sensitivity %
Sensitivity percentages of Ciprofloxacin: Bacteria types/Age
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
<1
1 - <2
2 - <5
5 - <10
10 - <15
15 - <18
Non-Mucoid
Mucoid
MDR

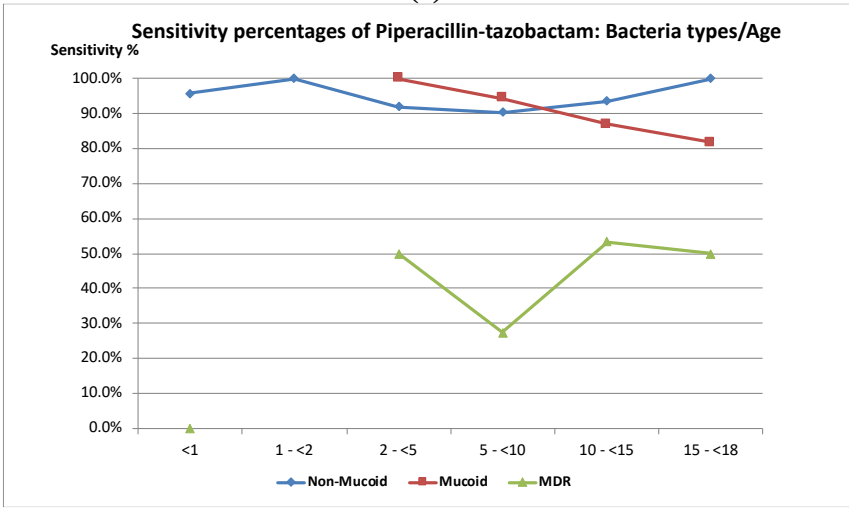
(b)


Sensitivity %
Sensitivity percentages of Meropenem: Bacteria types/Age
100.0%
90.0%
80.0%
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
<1
1 - <2
2 - <5
5 - <10
10 - <15
15 - <18
Non-Mucoid
Mucoid
MDR

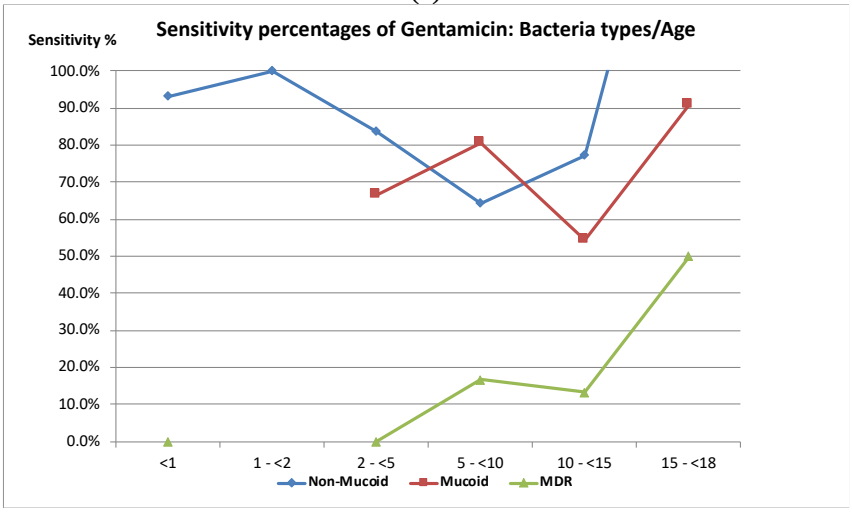
(c)




(d)




(e)




(f)

**Figure 3.7:** Age influence on antibiotics sensitivity

In general, the results show that as the age increased, the sensitivity to all antibiotics decreased.

At all categories, mucoid and non-mucoid types have an excellent sensitivity toward all antibiotics, and there is no big difference in sensitivity between mucoid and non-mucoid types. While CRPA has bad sensitivity toward all antibiotics at all age categories, except ciprofloxacin which show an excellent sensitivity in middle age categories, 100% and 91.7% for 2-<5 and 5-<10 age categories, respectively, and then going worse at higher age categories, 73.3% and 50% for 10-<15 and 15-<18 age categories, respectively.

On the other hand, the figures show that at first two categories there was no mucoid bacteria found and only one isolate CRPA founded in the first age, so we can say that mucoid and CRPA started to infect CF patients from age 2 years and above.

Statistically, regarding non-mucoid type; there are significant differences at the level 0.05 in sensitivity between ages corresponding to amikacin, meropenem and gentamicin only. For mucoid type, there are significant differences at the level 0.05 in sensitivity between ages corresponding to ciprofloxacin and gentamicin only.

In addition, for CRPA, there are significant differences at the level 0.05 in sensitivity between ages corresponding to amikacin, ciprofloxacin, ceftazidime and piperacillin-tazobactam.

**3.2 Fosfomycin data analysis:**

**3.2.1 Agar dilution method validation results:** method validation for agar dilution method was done on the Steer's Replicator apparatus three times for each bacteria and the results were as the following table (3.3):

**Table (3.3):** MIC validation results

| MIC / Bacteria tested | MIC 1 (µg/ml) | MIC 2 (µg/ml) | MIC 3 (µg/ml) | MIC QC range* (µg/ml) |
|---|---|---|---|---|
| *Pseudomonas aeruginosa* ATCC 27853 | 8 | 8 | 8 | 8-64 |

| | | | | |
|---|---|---|---|---|
| *Escherichia coli.* ATCC 25922 | 0.06 | 0.06 | 0.06 | 0.03-0.12 |
| *Staphylococcus aureus* ATCC 29213 | 2 | 2 | 2 | 1-8 |

*CLSI standards.

ATCC: American Type Culture Collection.

MIC: minimum inhibitory concentration

QC: Quality control

This result shows us that this method is reliable and we can use it on this apparatus comfortably.

### 3.2.2 Fosfomycin MIC and DD results:

MIC and DD methods were performed in triplicate on all isolates (129 *P. aeruginosa* samples) for fosfomycin, the results were illustrated in appendix (9).

MIC methods was done by using steer's replicator apparatus and the results was read from plates as shown in figure (3.8).

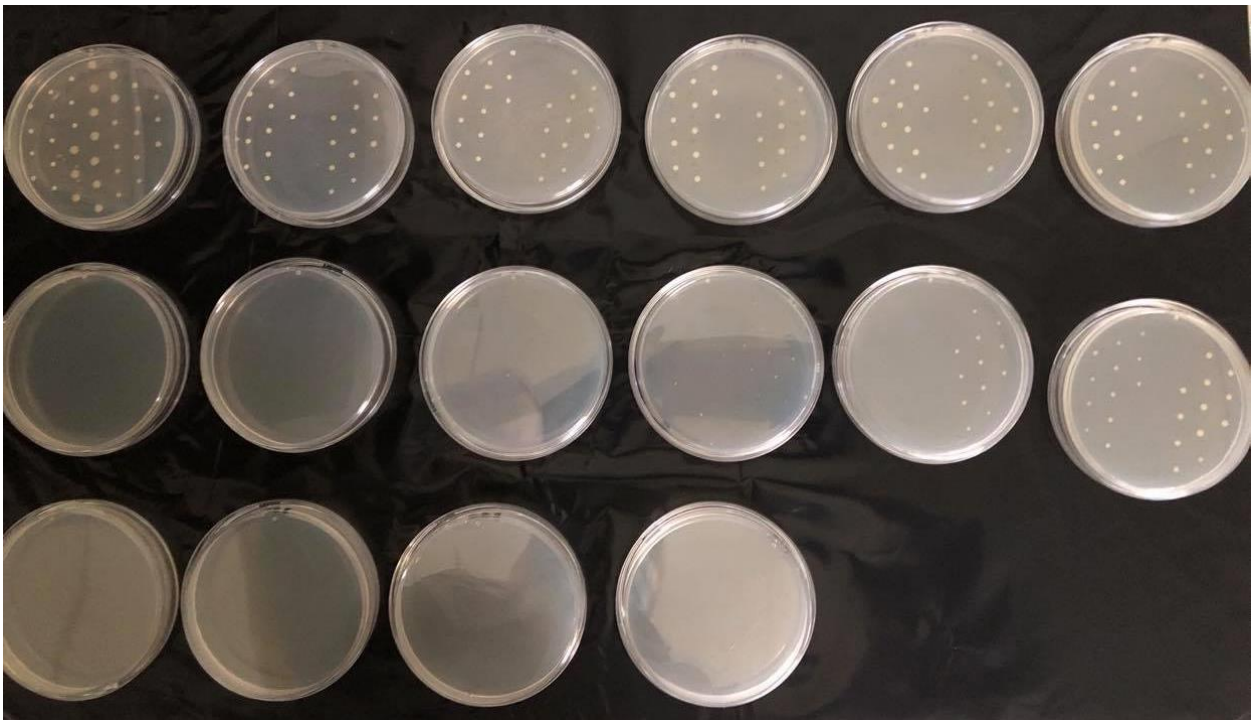


**Figure 3.8:** *P. aeruginosa* fosfomycin MIC method by agar dilution

At present, due to the lack of susceptibility breakpoint of fosfomycin for *P.aeruginosa* in CLSI and EUCAST, sensitivity to fosfomycin according to MIC results was determined based on the existing CLSI breakpoints for the Enterobacteriaceae≤64, 128 and ≥256 mg/L for susceptibility, intermediacy and resistance, respectively(Clinical and Laboratory Standards Institute.,. 2020).For the purposes of this research, we considered all isolates with an MIC ≤64 mg/L to be susceptible and >64 mg/L to be resistant. Concentrations of ≤64 mg/L are readily achievable in plasma and other sites such as subcutaneous, interstitial and bone tissue at normal therapeutic doses(Roussos N et al., 2009).

Depending on MIC results, the sensitivity for all isolates in general to fosfomycin was 39.53%. The sensitivity for non-mucoid, mucoid, and CRPA were 34.18%, 57.5%, 10% respectively.

Table (3.4)

**Table (3.4):** Three types of *P. aeruginosa* bacteria and their MIC numbers.

| **Type of isolate** | **Number of isolates** | **Number of isolates with indicated MIC (µg/mL)** | | | | | | | | **Sensitivity %** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 4 | 8 | 16 | 32 | 64 | 128 | 256 | More than 256 | |
| Non-mucoid | 79 | 2 | 1 | - | 1 | 23 | 26 | 15 | 11 | **34.18%** |
| Mucoid | 40 | - | 2 | - | 1 | 20 | 11 | 4 | 2 | **57.5%** |
| CRPA | 10 | - | - | - | - | 1 | 3 | 2 | 4 | **10%** |
| Total | 129 | 2 | 3 | - | 2 | 44 | 40 | 21 | 17 | **39.53%** |

*MIC: Minimum Inhibitor Concentration
*CRPA: Carbapenem Resistant *Pseudomonas aeruginosa*

So, the mucoid type was the most type sensitive to fosfomycin, followed by non-mucoid type, and the lowest sensitivity was CRPA.

The correlation between two methods, MIC and DD were calculated and the results were illustrated in table (3.4)

**Table (3.5):** MIC and DD Pearson Correlation Matrix

| Pearson Correlation Matrix | | | | |
|---|---|---|---|---|
| | | MIC | Zone200* | Zone50** |
| MIC | Pearson Correlation | 1 | -0.889-*** | -0.768-*** |
| | Sig. (2-tailed) | | 0.000 | 0.000 |
| | N | 129 | 129 | 129 |
| Zone200 | Pearson Correlation | -0.889-*** | 1 | 0.932*** |
| | Sig. (2-tailed) | 0.000 | | 0.000 |
| | N | 129 | 129 | 129 |
| Zone50 | Pearson Correlation | -0.768-*** | 0.932*** | 1 |
| | Sig. (2-tailed) | 0.000 | 0.000 | |
| | N | 129 | 129 | 129 |
| * disc 200µg fosfomycin\50 µg g-6-p<br>** disc 50 µg fosfomycin<br>*** Correlation is significant at the 0.05 level.<br>MIC: Minimum Inhibitor Concentration<br>Zone: Inhibition Zone | | | | |

This result shows us that there was a strong correlation between MIC, zone 50 and zone 200, and the data was distributed between MIC and DD values as the following figure (3.9):

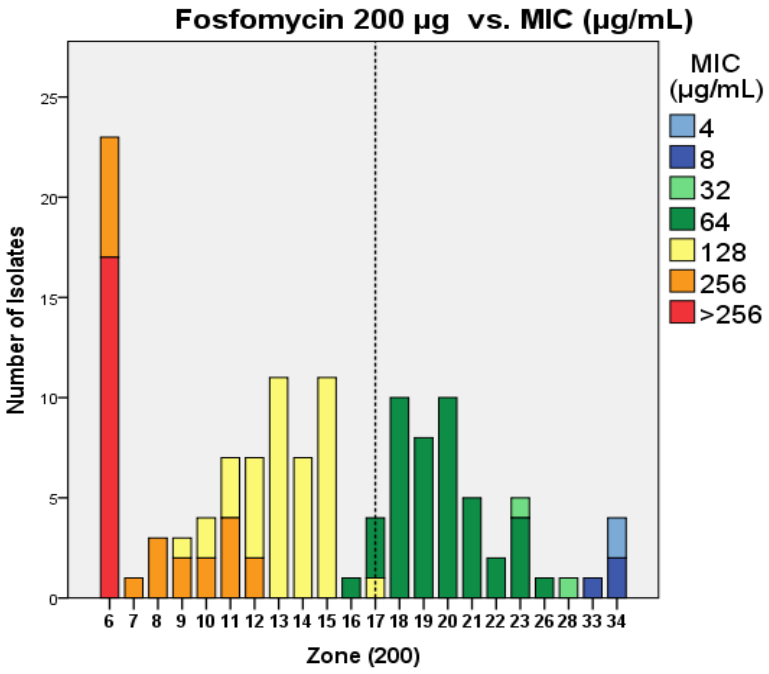



**Figure 3.9:** MIC (µg/mL) and inhibition zone diameter (mm).

# Chapter Five

## 5.Discussion.

*P. aeruginosa*, *S. aureus* and *H. influenzae* were detected in a half of the samples; this is in line with what had been shown in related studies. But *P. aeruginosa* was found in approximately a quarter of the samples (23%); the higher percent of bacteria detected, this is inconsistent with what publication concluded; the lungs of CF patients are often colonized or infected in infancy and early childhood with organisms, such as *S. aureus* and *H.influenzae*(Silva Filho et al., 2013).This highly colonization with *P. aeruginosa* is due to poor infection control in our society, such as; there is no separation of clinics in hospitals between CF clinic and other chest diseases clinics so there is mixing of patients and transmitting infection between them, in addition to the lack of separation between clinic appointments, because most of patients come to the hospital suddenly without a prior appointment, moreover; inpatients are placed in common rooms and there are no isolation units for them, on the family level, among the reasons is the multiplicity of CF patients in the same family and poor hygiene. All these reasons increase the incidence of transition P. *aeruginosa* microorganism between patients. This illustrated how much *P. aeruginosa* is important and danger in this disease.

*Burkholderiacepacia* complex; the most dangerous bacteria which can pose serious risk to the health of a person with CF because they are often resistant to many antibiotics and difficult to treat once they infect the lungs; were detected in our samples. This is not good result for CF patient's situation in Palestine.

The majority of *P. aeruginosa* detected was from non-mucoid type, but also from 100 *P. aeruginosa* samples detected, there was 18 samples from mucoid type, mucoid type as we notice

before is associated with a significant increase in morbidity and mortality. This finding about the type of *P. aeruginosa* found in CF patients is not so good.

A recurrent use of the same antibiotics can decrease its efficacy over time and leading to bacterial resistance. This development in antibiotic resistance is a serious public health concern.

In our study, we focused on the *pseudomonas aeruginosa* strains which affecting CF pediatric patients and their sensitivity patterns to different groups of antibiotics commonly administered to treat the infections over the period of 5 years.

From the result, we saw that ciprofloxacin has the highest sensitivity in mucoid and CRPA, so we can say that it would be the best choice when other safer antibiotic failed in treatment, especially that is the only antibiotic can be given orally, for that reason it is used only for outpatients. But we must be attentive when giving it because it's possible side effect on cartilage damage.

The second-best sensitivity was for ceftazidime, first choice in non-mucoid and second choice in mucoid and CRPA, it is an excellent antibiotic which currently used as the first choice in CRPA in treatment.

Meropenem has good activity against *P. aeruginosa* strains but is be used only for highly resistant samples.

Gentamicin had the lowest sensitivity compared with other antibiotics, this can be explained by the fact that it was the most commonly used antibiotics in this disease, it can be given to inpatients as IV solution and also to outpatients as inhalation solution, for patients whom colonized with *P. aeruginosa*, gentamicin had been given to them as inhalation in cycles; one month and one month off for a long of life.

From 5 years ago till now, there was no a big change in sensitivity toward all antibiotics used corresponding to all types of *P.aeruginosa* bacteria, this is a good sign that there was a good control on using antibiotic in Palestine, the CF patients were still sensitive to commonly used antibiotics.

Regarding gender, female showed lower sensitivity compared with male, so the same antibiotic may be affected differently depending on gender particularly for non-mucoid type.

Most of patients are from the south (70%), this high percent reflects the extent of the disease in the south, and one of the reasons for this is the spread of the phenomenon of consanguineous marriage. Moreover, most of patients whom had multiple samples are from the south, this can explain why the resistance to antibiotics in the south was more than north and central.

In patients less than 2 years, only non-mucoid type was detected in their samples, this is in line with what had been mentioned by researchers; the first type of *P. aeruginosa* can infect patients is non-mucoid after that mucoid type can be appear. Also, we notice that as the age increased, the sensitivity decreased, this can be explained by as the age increased, the patient will have an infection more times and *P. aeruginosa* be colonized in the lungs and will have transformation to become more resistant.

For fosfomycin MIC results, our isolates of *P.aeruginosa* showed a good sensitivity towards fosfomycin, nearly 40% of all isolates are susceptible, especially in mucoid type where its sensitivity was nearly 50%. So, we can say that fosfomycin could be an option in treating *P. aeruginosa* infection in CF patients, but we still need another research to study its efficacy in vivo.

When we match the results between MIC and DD methods, we notice a big correlation between the results, we can say that in DD method all samples that have a zone diameter ≥ 16 mm are susceptible to fosfomycin, by comparing it with ≥ 64 susceptibility in MIC method.\

# Chapter Six

## 6. Conclusions .

The current study has demonstrated that in Palestine the sensitivity to commonly used antibiotics in treatment *P.aeruginosa* infection in CF pediatric patients was good, and there was no deterioration in sensitivity toward these antibiotics over the past 5 years. We hope that this situation still in that manner and the control on using antibiotic still good.

Regarding the geographical distribution of the patients, we saw that the vast majority of patients are from the south, and also the resistance in this area is more than others to all antibiotics, so we need more monitoring on patient's situation in the south.

On the other hand, age had contributed on sensitivity, as we saw, as the age increased the resistance increased.

Fosfomycin showed a good sensitivity towards Palestinian *P.aeruginosa* isolates in CF patients, so it can be considered as a choice in the antibiotics used in treating *P.aeruginosa* infection in CF patients especially that it has a good safety profile with no annoying side effects, but it still needs more studies.

# Chapter Seven

## 7.Study limitations and future direction

**Materials insufficiency and their import from abroad:** materials like fosfomycin powder and disc and glucose-6-phosphate are not available in Palestine, so we are forced to recommend it from outside and wait months for it arrives.

**Limited access to data:** although I can access to the laboratory information with the help of Dr. Musa Hindiyeh, a big thanks to his person, but also there was a difficulty in access to other medical and clinical history for patients, especially because of time constraints.

**Unavailability of reference data:** unavailability of reference breakpoints for sensitivity for fosfomycin on *p. aeruginosa* in CLSI and EUCAST in different methods.

**The data was collected from one hospital**: so, the results may not represent status in all the West Bank.

**The data was collected from historic hospital records:** this thing may introduce some errors.

Despite the existence of these challenges, we were able to carry out our project and we were able to get good results, especially because it is the first study of its kind in the Arab world for fosfomycin sensitivity and in Palestine for two objects.

In the future we can do more researches related to this study such as:

- Study the efficacy of fosfomycin in combination with other antibiotics used in treating pseudomonal infection in CF patients; like gentamycin or ceftazidime and comparing the efficacy of each antibiotic alone and in combination.
- Conducting scientific research to find out the extent to which CF patients are affected by the side effects of the antibiotics used such as ototoxicity from gentamicin and amikacin, cartilage damage due to ciprofloxacin and other possible side effects.
- Study and evaluation resistance to other bacteria that affect CF patients such as *H. influanzae* and *S. aereus* and the change of sensitivity for years.

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## Appendix 1: Bacterial types in CF patients

| Bacteria types | Frequency | Percent |
|---|---|---|
| *Haemophilus Influenzae* | 183 | 10.3% |
| *Staphylococcus aureus* | 226 | 12.7% |
| *Staphylococcus aureus* MRSA | 143 | 8.0% |
| *Burkholderiacepacia* | 8 | 0.4% |
| *Pseudomonas aeruginosa* | 409 | 23.0% |
| Others | 813 | 45.6% |
| Total | 1782 | 100.0% |

## Appendix 2: *P. aeruginosa* types in CF samples

| **Pseudomonas type** | **count** | **percentage%** |
|---|---|---|
| *Pseudomonas aeruginosa* | 304 | 74.3% |
| *Pseudomonas aeruginosa* / Mucoid | 73 | 17.8% |
| CRPA | 32 | 7.8% |
| Total | 409 | 100.0% |

## Appendix 3: Difference in sensitivity between antibiotics and the 3 types of *P. aeruginosa* among 5 years

| | | Antibiotic | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Bacteria type | | Amikacin | Ciprofloxacin | Meropenem | Ceftazidime | Piperacillin-tazobactam | Gentamicin | Chi-Square Test for Antibiotics | |
| | | | | | | | | Chi-Square | P-value |
| Non-Mucoid | | 253(83.5%) | 279(93%) | 288(95.4%) | 292(96.1%) | 281(93.7%) | 241(79.5%) | 81.817 | 0.000 |
| Mucoid | | 52(71.2%) | 70(95.9%) | 69(94.5%) | 69(94.5%) | 66(90.4%) | 53(73.6%) | 39.875 | 0.000 |
| CRPA | | 7(21.9%) | 25(78.1%) | 0(0%) | 15(46.9%) | 13(41.9%) | 5(15.6%) | 54.370 | 0.000 |
| Total | | 312(76.5%) | 374(92.3%) | 357(87.7%) | 376(91.9%) | 360(89.1%) | 299(73.5%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 62.440 | 10.635 | 248.016 | 95.201 | 77.632 | 60.649 | | |
| | P-value | 0.000 | 0.005 | 0.000 | 0.000 | 0.000 | 0.000 | | |

## Appendix 4: Differences in sensitivity between years for each type of *P. aeruginosa* regarding antibiotics

| Antibiotic | Bacteria type | Year | | | | | Chi-Square Test for Years | |
|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | 2018 | 2019 | 2020 | Chi-Square | P-value |
| Amikacin | Non-Mucoid | 59(86.8%) | 62(82.7%) | 60(84.5%) | 35(71.4%) | 37(92.5%) | 14.174 | 0.007 |
| | Mucoid | 13(68.4%) | 13(65%) | 13(81.3%) | 6(66.7%) | 7(77.8%) | 1.516 | 0.824 |
| | CRPA | 2(33.3%) | 1(12.5%) | 1(16.7%) | 2(40%) | 1(14.3%) | 2.165 | 0.706 |
| | Total | 74(79.6%) | 76(73.8%) | 74(79.6%) | 43(68.3%) | 45(80.4%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 11.509 | 19.391 | 15.697 | 45.256 | 23.134 | | |
| | P-value | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | | |

| Antibiotic | Bacteria type | Year | | | | | Chi-Square Test for Years | |
|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | 2018 | 2019 | 2020 | Chi-Square | P-value |
| Ciprofloxacin | Non-Mucoid | 65(95.6%) | 65(86.7%) | 67(95.7%) | 44(93.6%) | 38(95%) | 6.386 | 0.172 |
| | Mucoid | 17(89.5%) | 19(95%) | 16(100%) | 9(100%) | 9(100%) | 3.483 | 0.481 |
| | CRPA | 4(66.7%) | 5(62.5%) | 5(83.3%) | 5(100%) | 6(85.7%) | 3.335 | 0.503 |
| | Total | 86(92.5%) | 89(86.4%) | 88(95.7%) | 58(95.1%) | 53(94.6%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 6.934 | 66.427 | 2.917 | 0.940 | 1.620 | | |
| | P-value | 0.031 | 0.000 | 0.233 | 0.625 | 0.445 | | |

| Antibiotic | Bacteria type | Year | | | | | Chi-Square Test for Years | |
|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | 2018 | 2019 | 2020 | Chi-Square | P-value |
| Meropenem | Non-Mucoid | 64(94.1%) | 70(94.6%) | 67(94.4%) | 48(98%) | 39(97.5%) | 1.657 | 0.798 |
| | Mucoid | 16(84.2%) | 20(100%) | 15(93.8%) | 9(100%) | 9(100%) | 6.121 | 0.190 |
| | CRPA | 0(0%) | 0(0%) | 0(0%) | 0(0%) | 0(0%) | --- | --- |
| | Total | 80(86%) | 90(88.2%) | 82(88.2%) | 57(90.5%) | 48(85.7%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 40.682 | 65.550 | 47.817 | 51.632 | 48.038 | | |
| | P-value | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | | |

| Antibiotic | Bacteria type | Year | | | | | Chi-Square Test for Years | |
|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | 2018 | 2019 | 2020 | Chi-Square | P-value |
| Ceftazidime | Non-Mucoid | 66(95.7%) | 73(97.3%) | 71(100%) | 42(85.7%) | 40(100%) | 0.263 | 0.608 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mucoid | 16(84.2%) | 20(100%) | 16(100%) | 9(100%) | 8(88.9%) | 0.789 | 0.374 |
| | CRPA | 4(66.7%) | 2(25%) | 3(50%) | 3(60%) | 3(42.9%) | 0.017 | 0.897 |
| | Total | 86(91.5%) | 95(92.2%) | 90(96.8%) | 54(85.7%) | 51(91.1%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 7.577 | 54.887 | 44.950 | 49.000 | 23.986 | | |
| | P-value | 0.023 | 0.000 | 0.000 | 0.000 | 0.000 | | |

| Antibiotic | Bacteria type | Year | | | | | Chi-Square Test for Years | |
|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | 2018 | 2019 | 2020 | Chi-Square | P-value |
| Piperacillin-tazobactam | Non-Mucoid | 62(92.5%) | 70(94.6%) | 66(95.7%) | 43(87.8%) | 40(97.6%) | 0.040 | 0.842 |
| | Mucoid | 16(84.2%) | 18(90%) | 15(93.8%) | 9(100%) | 8(88.9%) | 0.816 | 0.366 |
| | CRPA | 3(50%) | 1(14.3%) | 2(33.3%) | 4(80%) | 3(42.9%) | 0.559 | 0.455 |
| | Total | 81(88%) | 89(88.1%) | 83(91.2%) | 56(88.9%) | 51(89.5%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 9.800 | 39.480 | 26.892 | 48.250 | 19.002 | | |
| | P-value | 0.007 | 0.000 | 0.000 | 0.000 | 0.000 | | |

| Antibiotic | Bacteria type | Year | | | | | Chi-Square Test for Years | |
|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | 2018 | 2019 | 2020 | Chi-Square | P-value |
| Gentamycin | Non-Mucoid | 54(79.4%) | 54(72%) | 62(87.3%) | 35(71.4%) | 36(90%) | 12.050 | 0.017 |
| | Mucoid | 15(78.9%) | 13(68.4%) | 12(75%) | 6(66.7%) | 7(77.8%) | 0.033 | 0.855 |
| | CRPA | 2(33.3%) | 0(0%) | 1(16.7%) | 0(0%) | 2(28.6%) | 0.003 | 0.958 |
| | Total | 71(76.3%) | 67(65.7%) | 75(80.6%) | 41(65.1%) | 45(80.4%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 6.572 | 16.704 | 18.090 | 10.197 | 14.287 | | |
| | P-value | 0.037 | 0.000 | 0.000 | 0.006 | 0.001 | | |

## Appendix 5: Differences in sensitivity between years for each antibiotic

| | | Year | | | | | Chi-Square Test for Years | |
|---|---|---|---|---|---|---|---|---|
| Antibiotic | | 2016 | 2017 | 2018 | 2019 | 2020 | Chi-Square | P-value |
| Amikacin | | 74(79.6%) | 76(73.8%) | 74(79.6%) | 43(68.3%) | 45(80.4%) | 4.239 | 0.375 |
| Ciprofloxacin | | 86(92.5%) | 89(86.4%) | 88(95.7%) | 58(95.1%) | 53(94.6%) | 7.627 | 0.106 |
| Meropenem | | 80(86%) | 90(88.2%) | 82(88.2%) | 57(90.5%) | 48(85.7%) | 0.945 | 0.918 |
| Ceftazidime | | 86(91.5%) | 95(92.2%) | 90(96.8%) | 54(85.7%) | 51(91.1%) | 6.317 | 0.177 |
| Piperacillin-tazobactam | | 81(88%) | 89(88.1%) | 83(91.2%) | 56(88.9%) | 51(89.5%) | 0.634 | 0.959 |
| Gentamicin | | 71(76.3%) | 67(65.7%) | 75(80.6%) | 41(65.1%) | 45(80.4%) | 9.658 | 0.047 |
| Total | | 478(85.7%) | 506(82.4%) | 492(88.6%) | 309(82.2%) | 293(86.9%) | | |
| Chi-Square Test for Antibiotics | Chi-Square | 15.932 | 37.615 | 24.738 | 33.293 | 8.441 | | |
| | P-value | 0.007 | 0.000 | 0.000 | 0.000 | 0.134 | | |

## Appendix 6: Differences in sensitivity between Males and Females for each antibiotic for the three types of bacteria over 5 years

| | | Gender | | Chi-Square Test for Gender | |
|---|---|---|---|---|---|
| Antibiotic | Bacteria type | Male | Female | Chi-Square | P-value |
| Amikacin | Non-Mucoid | 93(86.1%) | 160(82.1%) | 0.831 | 0.362 |
| | Mucoid | 16(76.2%) | 36(69.2%) | 0.354 | 0.552 |
| | CRPA | 2(25%) | 5(20.8%) | 0.061 | 0.805 |
| | Total | 111(81%) | 201(74.2%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 18.467 | 42.622 | | |
| | P-value | 0.000 | 0.000 | | |
| Ciprofloxacin | Non-Mucoid | 103(97.2%) | 176(90.7%) | 4.378 | 0.036 |
| | Mucoid | 18(85.7%) | 52(100%) | 7.747 | 0.005 |
| | CRPA | 7(87.5%) | 18(75%) | 0.549 | 0.459 |
| | Total | 128(94.8%) | 246(91.1%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 5.604 | 12.802 | | |
| | P-value | 0.061 | 0.002 | | |
| Meropenem | Non-Mucoid | 103(96.3%) | 185(94.9%) | 0.302 | 0.583 |
| | Mucoid | 19(90.5%) | 50(96.2%) | 0.931 | 0.335 |

| | | | | | |
|---|---|---|---|---|---|
| | CRPA | 0(0%) | 0(0%) | ---- | ---- |
| | Total | 122(89.7%) | 235(86.7%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 74.708 | 171.948 | | |
| | P-value | 0.000 | 0.000 | | |
| Ceftazidime | Non-Mucoid | 107(99.1%) | 185(94.4%) | 4.033 | 0.045 |
| | Mucoid | 20(95.2%) | 49(94.2%) | 0.029 | 0.864 |
| | CRPA | 5(62.5%) | 10(41.7%) | 1.046 | 0.306 |
| | Total | 132(96.4%) | 244(89.7%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 28.421 | 65.784 | | |
| | P-value | 0.000 | 0.000 | | |
| Piperacillin-tazobactam | Non-Mucoid | 106(99.1%) | 175(90.7%) | 8.172 | 0.004 |
| | Mucoid | 19(90.5%) | 47(90.4%) | 0.000 | 0.990 |
| | CRPA | 4(50%) | 9(39.1%) | 0.288 | 0.592 |
| | Total | 129(94.9%) | 231(86.2%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 37.679 | 46.833 | | |
| | P-value | 0.000 | 0.000 | | |
| Gentamycin | Non-Mucoid | 92(86%) | 149(76%) | 4.220 | 0.040 |
| | Mucoid | 15(75%) | 38(73.1%) | 0.027 | 0.868 |
| | CRPA | 2(25%) | 3(12.5%) | 0.711 | 0.399 |
| | Total | 109(80.7%) | 190(69.9%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 18.298 | 41.285 | | |
| | P-value | 0.000 | 0.000 | | |

## Appendix 7: Differences in sensitivity between Geographical Areas for each antibiotic for the three types of bacteria over 5 years

| | | Geographical Area | | | Chi-Square Test for Geographical Area | |
|---|---|---|---|---|---|---|
| Antibiotic | Bacteria type | North | Central | South | Chi-Square | P-value |
| Amikacin | Non-Mucoid | 37(78.7%) | 28(87.5%) | 188(83.9%) | 1.180 | 0.554 |
| | Mucoid | 4(66.7%) | 5(83.3%) | 43(70.5%) | 0.506 | 0.776 |
| | CRPA | *** | 0(0%) | 7(22.6%) | 0.289 | 0.591 |
| | Total | 41(77.4%) | 33(84.6%) | 238(75.3%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 0.442 | 16.030 | 56.074 | | |
| | P-value | 0.506 | 0.000 | 0.000 | | |
| Ciprofloxacin | Non-Mucoid | 45(95.7%) | 28(87.5%) | 206(93.2%) | 2.046 | 0.359 |
| | Mucoid | 6(100%) | 6(100%) | 58(95.1%) | 0.615 | 0.735 |
| | CRPA | *** | 0(0%) | 25(80.6%) | 3.687 | 0.055 |
| | Total | 51(96.2%) | 34(87.2%) | 289(92.3%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 0.265 | 7.685 | 6.874 | | |
| | P-value | 0.606 | 0.021 | 0.032 | | |
| Meropenem | Non-Mucoid | 46(97.9%) | 31(100%) | 211(94.2%) | 2.867 | 0.239 |
| | Mucoid | 6(100%) | 6(100%) | 57(93.4%) | 0.833 | 0.660 |
| | CRPA | *** | 0(0%) | 0(0%) | ---- | ---- |
| | Total | 52(98.1%) | 37(97.4%) | 268(84.8%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 0.130 | 38.000 | 191.931 | | |
| | P-value | 0.718 | 0.000 | 0.000 | | |
| Ceftazidime | Non-Mucoid | 47(100%) | 32(100%) | 213(94.7%) | 4.386 | 0.112 |
| | Mucoid | 6(100%) | 6(100%) | 57(93.4%) | 0.833 | 0.660 |
| | CRPA | *** | 0(0%) | 15(48.4%) | 0.911 | 0.340 |
| | Total | 53(100%) | 38(97.4%) | 285(89.9%) | | |
| Chi-Square Test for Bacteria | Chi-Square | ---- | 39.000 | 65.341 | | |
| | P-value | ---- | 0.000 | 0.000 | | |
| Piperacillin-tazobactam | Non-Mucoid | 47(100%) | 29(90.6%) | 205(92.8%) | 3.983 | 0.136 |
| | Mucoid | 6(100%) | 6(100%) | 54(88.5%) | 1.523 | 0.467 |
| | CRPA | *** | 0(0%) | 13(43.3%) | 0.746 | 0.388 |
| | Total | 53(100%) | 35(89.7%) | 272(87.2%) | | |
| Chi-Square Test for Bacteria | Chi-Square | ---- | 9.463 | 57.859 | | |
| | P-value | ---- | 0.009 | 0.000 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Gentamycin | Non-Mucoid | 40(85.1%) | 27(84.4%) | 174(77.7%) | 1.831 | 0.400 |
| | Mucoid | 3(50%) | 4(66.7%) | 46(76.7%) | 2.159 | 0.340 |
| | CRPA | *** | 0(0%) | 5(16.1%) | 0.191 | 0.662 |
| | Total | 43(81.1%) | 31(79.5%) | 225(71.4%) | | |
| Chi-Square Test for Bacteria | Chi-Square | 4.284 | 17.065 | 51.546 | | |
| | P-value | 0.038 | 0.000 | 0.000 | | |

## Appendix 8: Differences in sensitivity between Age categories for each antibiotic for the three types of bacteria over 5 years

| | | Age | | | | | | Chi-Square Test for Ages | |
|---|---|---|---|---|---|---|---|---|---|
| Antibiotic | Bacteria type | <1 | 1 - <2 | 2 - <5 | 5 - <10 | 10 - <15 | 15 - <18 | Chi-Square | P-value |
| Amikacin | Non-Mucoid | 70(95.9%) | 13(100%) | 32(86.5%) | 57(68.7%) | 64(81%) | 17(94.4%) | 26.101 | 0.000 |
| | Mucoid | *** | *** | 2(66.7%) | 27(75%) | 14(60.9%) | 9(81.8%) | 2.087 | 0.555 |
| | CRPA | 0(0%) | *** | 0(0%) | 2(16.7%) | 4(26.7%) | 1(50%) | 10.875 | 0.028 |
| | Total | 70(94.6%) | 13(100%) | 34(81%) | 86(65.6%) | 82(70.1%) | 27(87.1%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 17.740 | *** | 9.632 | 14.500 | 18.919 | 14.222 | | |
| | P-value | 0.000 | *** | 0.008 | 0.001 | 0.000 | 0.001 | | |
| Ciprofloxacin | Non-Mucoid | 68(94.4%) | 13(100%) | 37(100%) | 76(91.6%) | 68(88.3%) | 17(94.4%) | 6.914 | 0.227 |
| | Mucoid | *** | *** | 3(100%) | 36(100%) | 23(100%) | 8(72.7%) | 17.634 | 0.001 |
| | CRPA | 0(0%) | *** | 2(100%) | 11(91.7%) | 11(73.3%) | 1(50%) | 14.520 | 0.002 |
| | Total | 68(93.2%) | 13(100%) | 42(100%) | 123(93.9%) | 102(88.7%) | 26(83.9%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 13.789 | *** | *** | 3.229 | 6.473 | 14.846 | | |
| | P-value | 0.000 | *** | *** | 0.199 | 0.039 | 0.001 | | |
| Meropenem | Non-Mucoid | 72(100%) | 13(100%) | 37(100%) | 79(94%) | 72(92.3%) | 15(83.3%) | 13.802 | 0.017 |
| | Mucoid | *** | *** | 3(100%) | 35(97.2%) | 22(95.7%) | 9(81.8%) | 4.165 | 0.244 |
| | CRPA | 0(0%) | *** | 0(0%) | 0(0%) | 0(0%) | 0(0%) | *** | *** |
| | Total | 72(98.6%) | 13(100%) | 40(95.2%) | 114(86.4%) | 94(81%) | 24(77.4%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 73.000 | *** | 42.000 | 83.816 | 73.739 | 7.339 | | |
| | P-value | 0.000 | *** | 0.000 | 0.000 | 0.000 | 0.025 | | |
| Ceftazidime | Non-Mucoid | 73(100%) | 13(100%) | 36(97.3%) | 79(94%) | 74(93.7%) | 17(94.4%) | 5.881 | 0.318 |
| | Mucoid | *** | *** | 3(100%) | 34(94.4%) | 22(95.7%) | 10(90.9%) | 0.508 | 0.917 |
| | CRPA | 0(0%) | *** | 1(50%) | 6(50%) | 7(46.7%) | 1(50%) | 8.200 | 0.042 |
| | Total | 73(98.6%) | 13(100%) | 40(95.2%) | 119(90.2%) | 103(88%) | 28(90.3%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 74.000 | *** | 9.521 | 23.973 | 28.018 | 13.786 | | |
| | P-value | 0.000 | *** | 0.009 | 0.000 | 0.000 | 0.001 | | |
| Piperacillin-tazobactam | Non-Mucoid | 70(95.9%) | 13(100%) | 34(91.9%) | 75(90.4%) | 72(93.5%) | 17(100%) | 4.365 | 0.498 |
| | Mucoid | *** | *** | 3(100%) | 34(94.4%) | 20(87%) | 9(81.8%) | 2.247 | 0.523 |
| | CRPA | 0(0%) | *** | 1(50%) | 3(27.3%) | 8(53.3%) | 1(50%) | 10.077 | 0.018 |
| | Total | 70(94.6%) | 13(100%) | 38(90.5%) | 112(86.2%) | 100(87%) | 27(90%) | | |
| Chi-Square Test for | Chi-Square | 17.740 | *** | 54.053 | 35.276 | 17.864 | 6.263 | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Bacteria types | P-value | 0.000 | *** | 0.000 | 0.000 | 0.000 | 0.044 | | |
| Gentamycin | Non-Mucoid | 68(93.2%) | 13(100%) | 31(83.8%) | 54(64.3%) | 61(77.2%) | 14(82.4%) | 24.417 | 0.000 |
| | Mucoid | *** | *** | 2(66.7%) | 29(80.6%) | 12(54.5%) | 10(90.9%) | 29.189 | 0.000 |
| | CRPA | 0(0%) | *** | 0(0%) | 2(16.7%) | 2(13.3%) | 1(50%) | 2.418 | 0.659 |
| | Total | 68(91.9%) | 13(100%) | 33(78.6%) | 85(64.4%) | 75(64.7%) | 25(83.3%) | | |
| Chi-Square Test for Bacteria types | Chi-Square | 11.489 | *** | 8.183 | 16.023 | 23.726 | 10.640 | | |
| | P-value | 0.001 | *** | 0.017 | 0.000 | 0.000 | 0.005 | | |

***** Out of comparisons because No cases have sensitivity or Non-sensitivity results.**

## Appendix 9: Fosfomycin MIC and DD results

| Number | Phenotype | Morphology | MIC (µg/mL) | Zone 200 µg (mm) | Zone 50 µg (mm) |
|---|---|---|---|---|---|
| | *P. aeruginosa* ATCC 27853 | | 4 | 30 | 30 |
| | *E. coli* ATCC 25922 | | 1 | 29 | 27 |
| 1 | CRPA | Tiny | >256 | 6 | 6 |
| 2 | NM | Large wide-spread | 256 | 6 | 6 |
| 3 | NM | Medium | 128 | 12 | 10 |
| 4 | NM | Tiny | 64 | 21 | 18 |
| 5 | M | Tiny transparent | 256 | 6 | 6 |
| 6 | NM | Tiny transparent | >256 | 6 | 6 |
| 7 | CRPA | Tiny transparent | >256 | 6 | 6 |
| 8 | NM | Large wide-spread | 64 | 19 | 18 |
| 9 | NM | Tiny | 128 | 15 | 11 |
| 10 | NM | Tiny | >256 | 6 | 6 |
| 11 | NM | Medium | 64 | 19 | 14 |
| 12 | M | Large wide-spread | 4 | 34 | 32 |
| 13 | NM | Large | 256 | 7 | 6 |
| 14 | NM | Large | 128 | 10 | 6 |
| 15 | NM | Large | 256 | 6 | 6 |
| 16 | NM | Large | 128 | 13 | 6 |
| 17 | NM | Large wide-spread | 128 | 14 | 8 |
| 18 | M | Large wide-spread | 64 | 20 | 18 |
| 19 | NM | Large | 256 | 11 | 6 |
| 20 | M | | 128 | 14 | 8 |
| 21 | NM | Large | 128 | 11 | 6 |
| 22 | M | Large shiny | 128 | 11 | 6 |
| 23 | CRPA | Large | 64 | 18 | 13 |
| 24 | NM | Large irregular size | 64 | 19 | 15 |
| 25 | NM | Tiny | 256 | 11 | 6 |
| 26 | M | | 64 | 18 | 10 |
| 27 | M | | 64 | 19 | 19 |

| | | | | | |
|---|---|---|---|---|---|
| 28 | CRPA | Tiny | 128 | 12 | 10 |
| 29 | M | White | 256 | 8 | 6 |
| 30 | M | | 8 | 34 | 30 |
| 31 | M | | 64 | 19 | 10 |
| 32 | M | | 8 | 34 | 30 |
| 33 | M | | 128 | 14 | 10 |
| 34 | NM | Medium | 128 | 15 | 13 |
| 35 | NM | Medium | 64 | 26 | 22 |
| 36 | M | | 128 | 15 | 12 |
| 37 | NM | Large | 64 | 18 | 12 |
| 38 | NM | | 128 | 9 | 6 |
| 39 | NM | Tiny transparent | >256 | 6 | 6 |
| 40 | M | Tiny | 64 | 23 | 23 |
| 41 | NM | Irregular | >256 | 6 | 6 |
| 42 | NM | Large | 8 | 33 | 32 |
| 43 | NM | Tiny transparent | >256 | 6 | 6 |
| 44 | M | | 64 | 19 | 13 |
| 45 | NM | Large | 64 | 22 | 18 |
| 46 | NM | Large | 256 | 8 | 6 |
| 47 | M | | 64 | 20 | 18 |
| 48 | CRPA | Tiny | 256 | 11 | 9 |
| 49 | M | | 64 | 23 | 18 |
| 50 | CRPA | Tiny transparent | >256 | 6 | 6 |
| 51 | M | Large | 64 | 21 | 17 |
| 52 | M | | 128 | 13 | 10 |
| 53 | M | | 64 | 16 | 14 |
| 54 | M | | 128 | 13 | 9 |
| 55 | M | | 128 | 11 | 6 |
| 56 | NM | Large | 64 | 20 | 10 |
| 57 | NM | | 64 | 18 | 14 |
| 58 | CRPA | Tiny | 128 | 15 | 6 |
| 59 | M | White | 256 | 6 | 6 |
| 60 | M | Large | 64 | 23 | 22 |
| 61 | CRPA | | >256 | 6 | 6 |
| 62 | M | | 64 | 18 | 14 |
| 63 | M | | >256 | 6 | 6 |

| | | | | | |
|---|---|---|---|---|---|
| 64 | M | | 64 | 20 | 12 |
| 65 | M | | 64 | 19 | 16 |
| 66 | M | | 128 | 12 | 6 |
| 67 | M | | 256 | 12 | 6 |
| 68 | NM | Large | 256 | 11 | 6 |
| 69 | NM | Medium | 128 | 14 | 10 |
| 70 | NM | Tiny | 64 | 21 | 22 |
| 71 | NM | Small | 128 | 15 | 12 |
| 72 | NM | Medium | 64 | 23 | 19 |
| 73 | CRPA | Medium | 128 | 17 | 16 |
| 74 | NM | Large | 128 | 15 | 10 |
| 75 | NM | Tiny | 64 | 21 | 17 |
| 76 | NM | Tiny | >256 | 6 | 6 |
| 77 | NM | Medium | 128 | 15 | 10 |
| 78 | NM | Tiny | 64 | 19 | 16 |
| 79 | M | Tiny | 128 | 15 | 12 |
| 80 | M | | 64 | 20 | 16 |
| 81 | NM | Small | >256 | 6 | 6 |
| 82 | NM | Medium | 128 | 13 | 8 |
| 83 | NM | Tiny | >256 | 6 | 6 |
| 84 | NM | Medium | 64 | 20 | 16 |
| 85 | NM | Medium | 256 | 8 | 6 |
| 86 | NM | Medium | 64 | 21 | 16 |
| 87 | NM | Tiny | >256 | 6 | 6 |
| 88 | NM | Large | 128 | 14 | 10 |
| 89 | M | | 64 | 20 | 16 |
| 90 | NM | Medium | 64 | 18 | 14 |
| 91 | M | | >256 | 6 | 6 |
| 92 | NM | Tiny | 128 | 13 | 9 |
| 93 | NM | Tiny | 64 | 20 | 17 |
| 94 | NM | Medium | 128 | 14 | 10 |
| 95 | NM | Medium | 256 | 12 | 7 |
| 96 | M | | 128 | 10 | 8 |
| 97 | NM | Large | 64 | 18 | 12 |
| 98 | NM | Large | 64 | 18 | 14 |
| 99 | M | Small | 64 | 17 | 12 |

| | | | | | |
|---|---|---|---|---|---|
| 100 | NM | Small | 256 | 6 | 6 |
| 101 | NM | Small | 128 | 13 | 8 |
| 102 | M | Large | 64 | 22 | 18 |
| 103 | M | | 128 | 13 | 8 |
| 104 | NM | Medium | 4 | 34 | 31 |
| 105 | M | | 32 | 28 | 25 |
| 106 | NM | | 128 | 13 | 11 |
| 107 | NM | Medium | 128 | 12 | 6 |
| 108 | M | Tiny | 64 | 20 | 17 |
| 109 | NM | Large | 128 | 12 | 6 |
| 110 | NM | Large | 32 | 23 | 20 |
| 111 | NM | Tiny | >256 | 6 | 6 |
| 112 | NM | Small | 64 | 18 | 13 |
| 113 | NM | Medium | 64 | 17 | 12 |
| 114 | NM | Medium | 128 | 15 | 9 |
| 115 | NM | Medium | 256 | 9 | 6 |
| 116 | NM | | 256 | 10 | 6 |
| 117 | M | Medium | 64 | 20 | 16 |
| 118 | NM | Tiny | >256 | 6 | 6 |
| 119 | NM | Tiny | 256 | 9 | 6 |
| 120 | NM | Large | 128 | 13 | 6 |
| 121 | NM | Small | 128 | 15 | 9 |
| 122 | NM | Medium | 256 | 6 | 6 |
| 123 | NM | Medium | 256 | 10 | 6 |
| 124 | NM | Large | 128 | 15 | 9 |
| 125 | NM | Small | 64 | 18 | 12 |
| 126 | NM | Small | 128 | 13 | 7 |
| 127 | NM | Small | 64 | 17 | 10 |
| 128 | CRPA | Medium | 128 | 14 | 6 |
| 129 | NM | Medium | 128 | 13 | 6 |

# Appendix 10: Ethical approval from Caritas Baby Hospital

Children's Relief Bethlehem

Caritas Baby Hospital
P.O.Box 84, Bethlehem
Tel. +972 2 275 85 00, Fax +972 2 275 85 01
info@crb-mail.org, www.childrens-relief-bethlehem.org
Arab Bank, Bethlehem, Acct. 702200

**Medical Research Agreement of Principles**

Date: 12-10-2019

This is to certify that Caritas Baby Hospital represented by: __**Dr. Musa Hindiyeh**__ will be collaborating with ____**Alquds University**_____ represented by: _ **Dr. Hussein Halaq** ___ to conduct a Medical Research Project entitled: _ **Evaluation of antibiotics resistance including fosfomycin in Pseudomonas aeruginosa isolates from cystic fibrosis patients in Palestinian Hospital over 5 years**___. The research project will be conducted by: _____**Bayan Subb Laban** _____.

The above research project was reviewed by memebrs of Caritas Medical Research Committee and was approved on____**1-1-2019**_____ and given MRC-Project Number _____**MRC-43**_____.

After the fruitful accomplishment of the project both parties agree to publsih the work in peer reviewed journal and the authorship location in the manuscript will be as follows:

First author: **Bayan Subb Laban**
Second author:
Third author:
Before last author: **Hussein Halaq**
Last author: **Musa Hindiyeh**

Principle Invesigator

**Hussein Halaq**

Caritas Baby Hospital
Medical Rearch Committe Representative

**Musa Hindiyeh**

**تقييم حساسية المضادات الحيوية من ضمنها الفوسفومايسين في خلايا الزائفة الزنجارية البكتيرية لمرضى التليف الكيسي في مستشفى فلسطيني خلال خمس سنوات**

**اعداد الطالبة :بيان صب لبن.**
**المشرف : الدكتور حسين الحلاق**
**الملخص.**

**المقدمة:**

مضادات الزائفة الزنجارية، مثلها مثل كل المضادات الحيوية، ممكن ان يحدث لها مقاومة إذا استخدمت بشكل متواصل لعدة سنوات. في مرض التليف الكيسي، أخطر بكتيريا ممكن أن تستعمر الرئتين هي الزائفة الزنجارية ونسبة مقاومة المضادات الحيوية لها تعتبر كبيرة. في فلسطين، لا يوجد هناك دراسات تلخص مقاومة هذه البكتيريا للمضادات الحيوية عند مرضى التليف الكيسي.

زيادة انتشار البكتيريا المقاومة للمضادات الحيوية، ردود الفعل التحسسية، التسمم الكلوي وغيرهم من الآثار الجانبية للمضادات الحيوية عند مرضى التليف الكيسي يمكن ان يحد من العدد المتوفر من المضادات الحيوية المستخدمة في علاج الالتهابات الرئوية. الفوسفومايسين، مضاد حيوي مختلف وذو تغطية بكتيرية واسعة، يمكن ان يقدم علاج بديل في مثل هذه الحالات.

**الأهداف:**

هدف هذه الدراسة تحديد مستوى التغير في مقاومة الزائفة الزنجارية للمضادات الحيوية المستخدمة عادة في علاج الالتهابات الرئوية الناتجة عن تلك البكتيريا عند مرضى التليف الكيسي، بالإضافة إلى تقييم فعالية الفوسفومايسين ضد هذه البكتيريا.

**الطريقة:**

لدراسة مقاومة المضادات الحيوية خلال خمس سنوات، تم القيام بدراسة رجعية في قسم المختبر في مستشفى كاريتاس الطبي للأطفال. تم تجميع المعلومات الطبية والمخبرية المتعلقة بالمضادات الحيوية المستخدمة لمرضى التليف الكيسي من فئة الأطفال. تم تحليل النتائج باستخدام برنامج ميكروسوفت اكسل ٢٠١٠ وبرنامج SPSS.

بالإضافة إلى ذلك، تم اخذ ١٢٩ عينة تحتوي الزائفة الزنجارية مأخوذة من مرضى التليف الكيسي وتم دراسة فعالية الفوسفومايسين عليها باستخدام طريقتين مختلفتين.

**النتائج:**

فعالية المضادات الحيوية المستخدمة في علاج الالتهابات الناتجة عن البكتيريا الزائفة الزنجارية عند مرضى التليف الكيسي في فلسطين كانت كالتالي ٩٢،٣٪، ٩١،٩٪، ٨٩،١٪، ٨٧،٧٪، ٧٦،٥٪ و٧٣،٥٪ للمضادات الحيوية التالية سيبروفلوكساسين، سيفتازيديم، بايبراسيلين-تازوباكتام، ميروبينيم، اميكاسينوجنتامايسين بالتتالي. خلال الخمس سنوات الماضية، حساسية البكتيريا لهذه المضادات الحيوية كانت تتأرجح بشكل بسيط من دون حدوث تدهور في حساسيتها تجاه هذه المضادات.

الغالبية العظمى من المرضى كانت من منطقة الجنوب (٧٠،١٪)، وأيضا المقاومة لجميع المضادات الحيوية في هذه المنطقة كانت أكبر ما يمكن. حساسية المضادات الحيوية عند الرجال كانت أفضل منها عند النساء. بالنسبة للعمر، تم ملاحظة أنه كلما زاد العمر كلما زادت مقاومة البكتيريا للمضادات الحيوية.

مخبريا، لعدم وجود susceptibility breakpoint للفوسفومايسين على الزائفة الزنجارية في CLSI وEUCAST،الحساسية للفوسفومايسين تم تحديدها بناء على breakpoint الموجودة لفصيلة الامعائيات،≤ ٦٤ تعتبر حساس. MICأظهرت فعالية جيدة للفوسفومايسين على بكتيريا الزائفة الزنجارية المأخوذة من مرضى التليف الكيسي؛ تقريبا نسبة الفعالية ٤٠ ٪، والأكثر حساسية للمضادات الحيوية كانت نوع mucoidبنسبة (٥٧،٥٪). أيضا طريقة DDأظهرت علاقة قوية مع طريقةMIC . حيث كان معامل ارتباط بيرسون بين MICوDD 200 µg -٠،٨٨٩، بينMIC وDD 50 µg -٠،٧٦٨ وأيضا بين DD 200 µg وDD50 µg ٠،٩٣٢.

**الاستنتاج:**

في فلسطين، حساسية الزائفة الزنجارية للمضادات الحيوية الأكثر استخداما في مقاومتها عند مرضى التليف الكيسي الأطفال تعتبر جيدة، ولا يوجد هناك تدهور في هذه الحساسية خلال الخمس سنوات الماضية. الفوسفومايسين اظهر فعالية جيدة تجاه خلايا الزائفة الزنجارية المأخوذة من مرضى التليف الكيسي في فلسطين، لذلك يمكن القول انه في المستقبل ممكن أن يتم استخدامه في علاج الالتهابات الناتجة عن الزائفة الزنجارية عند مرضى التليف الكيسي ولكنه بحاجه إلى دراسات أخرى.