ALI MIFTAH ALSALAMI

THE PROBLEM OF AMINOGLYCOSIDES RESISTANCE TO NON-ENTERIC GRAM-NEGATIVE

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NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

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M.Sc. THESIS

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Approval

We certify that we have read the thesis submitted by **Ali Miftah Alsalami** titled **"The Problem of Aminoglycosides Resistance to NE-GNB in Clinical Samples"** and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Educational Sciences.

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Declaration

I hereby declare that all information in this document has been obtained and presented in accordance with the academic rules and ethical guidelines of the Graduate School of Educational Sciences, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this study.

Ali Miftah Alsalami

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Day/Month/Year

Acknowledgement

I would like to thank Allah for blessing virtues to implant the soul of endurance and faith in me for completing this study. Also, I have no appropriate words that fully express the immense indebtedness and deep gratitude I owe to my worthy learned research Advisor Prof. Dr. NEDIM ÇAKIR for his keen interest, admirable guidance, constructive suggestions, affectionate supervision, inspiring behavior and valuable knowledge which it contributed in this work multitude ways. Whenever I needed him was near and assisted me.

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Dedication

This thesis is dedicated to my parents especially to soul of my father who passed away last year December, 2022, my family as well as my supervisor Prof. Dr. Nedim Çakir for his tireless support.

Abstract

The Problem of Aminoglycosides Antibiotics

Resistance to Non-enteric Gram-negative bacilli in Clinical Samples

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Objective: Aminoglycosides are a crucial part of the modern anti-bacterial therapy. They are a therapeutically beneficial family of medications across a wide range of infection types eexcept for anaerobic microorganisms because of their broad spectrum of activity, quick bactericidal action and good chemical and pharmacokinetic characteristics, we studied on four Aminoglycoside antibiotics including Gentamycin Tobramycin, Amikacin, Netilmicin resistance to **Non-enteric Gram-negative bacilli** (NE-GNB), majorly *Pseudomonas aeruginosa* and *Acinetobacter baumanii.* **Material and methods:** we used the material and methods for gram staining and culture and isolation according to the Microbiological standards. The data were obtained from patient files at the archive and electronic system retrospectively. Totally 659 patient's records were admitted to study in Near East University Hospital from August 2020 to June, 2022. In this study the processes of isolation and identification of NE-GNB and susceptibility to aminoglycosides including Amikacin, Gentamicin, Tobramycin, and Netilmicin had done according to the Microbiological Standards, Antibiotic resistance results for aminoglycosides and demographic and clinical characteristics of the patients were evaluated. **Results:** The results show the aminoglycosides percentage of resistance and sensitive as follow :Amikacin resistance was found in 36.1% of bacteria, whereas 63.9% were sensitive. Tobramycin resistance was found in 38.8% of bacteria, whereas 61.2% were sensitive. Gentamicin resistance was found in 46.3% of bacteria, whereas 53.7% were sensitive. Netilmicin resistance was found in 56.5% of bacteria, whereas 43.5% were sensitive. But it less than some group of B-Lactams for example Aztreonam resistance was found in 85.9% of bacteria resistance also for Trimethoprim/Sulfamethoxazole resistance was 85% high resistance .

KEYWODS: aminoglycosides, resistance, non-enteric gram-negative rods.

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List of Abbreviations

AMR	Antimicrobial Resistance
ESBL	Extended-Spectrum Beta-lactamase
GRE	Enterobacteriaceae
GNB	Gram-Negative Bacilli
ICU	Intensive Care Unit
MDROs	Multi-drug Resistant Organisms
MDR	Multidrug resistance
MRSDA	Methicillin Resistance Staphylococcus Aureus
NGB	Neurogenic Bladder
NEUH	Near East University Hospital
NE-GNB	Non-enteric Gram-negative bacilli
SD	Standard Deviation
SPSS	Statistical Package of Social Science
VRE	Vancomycin-resistant Enterococcus
WHO	World Health Organization

CHAPTER 1

Introduction

1.1 Background

A worldwide health issue has emerged due to the growing emergency in antimicrobial resistance (AMR) (Ahmed et al., 2022). It is challenging to design regional and national policies and strategies for reducing AMR as well as to determine the financial Effect of AMR. The difficulties in closely monitoring the dynamics of AMR have not yet been adequately addressed. Higher rates of resistance are closely correlated with increased antibiotic use. Antimicrobial drugs are commonly used to treat various infections worldwide. Consumption of antibiotics, particularly broad-spectrum antibiotics, may indicate that doctors are concerned and that severely ill patients require effective therapy. Antimicrobial abuse and overuse are common place, not just in developing nations but also in developed. The major cause of these problems is the prevalence of uneducated physicians. According to studies, hospitals use up to 50% more antibiotics than necessary, and in the previous 30 years, no regulations addressing antibiotic usage and resistance have been formed.

Aminoglycosides have a significant class of antibiotics utilized for treating infections caused by bacteria in latest filed of medicine. Aminoglycosides are efficient against many gram-negative bacteria as well as certain gram-positive bacteria. They are a therapeutically beneficial family of medications across a wide range of infection types, including certain protozoal diseases, because of their broad spectrum of activity, quick bactericidal action, and good chemical and pharmacokinetic characteristics. Because newer types of broad-spectrum pharmaceuticals with better safety profiles came into existence, the usage of aminoglycosides began to decline. However, the rise of multidrug resistance (MDR),such as “Methicillin Resistant *Staphylococcus aureu*s (, MRSA) Extended-spectrum beta-lactamase (ESBl) positive baclli,Carbapenem-resistant Enterobacteriaceae (CRE), Vancomycin-resistant Enterococcus (VRE)” (De Lencastre, 2007)have increased during the the time. Infections have rekindled interest in this family of medications. MDR The capacity of bacteria to tolerate the effects of numerous antibiotics, leaving them resistant to a wide variety of medications, is referred to as multidrug resistance (Sulaiman, et al., 2022). Adopting tailored dosage regimens that

increase safety while preserving efficacy results from an improved understanding of the determinants of toxicity and efficacy. The synthesis and development of new medicines, particularly created to avoid resistance while maintaining efficacy against fully susceptible isolates, have been made possible by advances in medicine chemistry and an increased understanding of essential aminoglycoside resistance pathways. Further modification of the aminoglycoside scaffold is necessary to produce new drugs with greater efficacy against MDR bacteria and an enhanced safety profile. This is necessary given the lack of novel antibiotic medicines in the pipeline and the growing threat of resistance (Krause, et al., 2016).

1.2 Statement of the Problem and Rationale of the Study

Antibiotic resistance mechanisms are continually changing in microorganisms, making microorganisms more virulent and difficult to eliminate, creating a difficulty in treating sick people. Aminoglycosides are widely utilized medications in hospitalized patients, as previously stated in the literature study (Bailly, et al., 2008). However, these drugs have largely bacteriostatic effects and become bactericidal only at greater dosages. Furthermore, bacteria have developed a variety of resistance tactics to these antibiotics, increasing their sensitivity to drug resistance. As a result, research into medication resistance in hospitalized patients are critical, allowing for preventative measures and preparation in treating patients afflicted with such resistant microorganisms. The mechanisms of resist of these antibiotics in varied maneuvers, which makes the resistance to these drugs highly susceptible. Hence, it is critical to investigate drug resistance among hospitalized patients in order to take action and be prepared to treat patients infected with such resistant bacteria.

1.3 Research Question

- What are the types of NE-GNB *(Pseudomonas aeruginosa* as well as *Acinetobacter baumannii*) that are resistant to aminoglycosides?.

1.4 Significance of the Study

The findings of this study lay the foundation for the creation of practical measures that may be used to instruct patients and guide clinicians in Cyprus on the proper use of

antibiotics. The current study, the first of its kind in Cyprus, highlighted

the paucity of previous research in this field as there is a lack of studies providing knowledge about antimicrobial resistance to aminoglycosides. This study can serve as a model for future research on the proper use of antibiotics to control and minimize drug resistance. A retrospective record review of aminoglycosides antibiotic resistance to NE-GNB pattern between August 2020 to June 2022. The data were obtained from patient files at the archive and electronic system records at Near East University Hospital (NEUH)study was conducted in Cyprus,

We need to do survey on the antibiotics resistance from time to other to make assessment on it we face this poroblem and find the management and the solution for that.

CHAPTER 2

Literature Review

2.1 Enteric and Non-Enteric Gram Bacilli

Enteric bacteria are commonly found in the intestines of both humans and animals (Donnenberg, M. S., 2000). GN -EB may either be pathogenic, which means they cause sickness, or they can be harmless, like gut flora or microbiota (Singh, P. et al., 2021).

Gram-positive or Gram-negative cell wall cell shape are the two main factors that categorize all bacteria (i.e., rod, circular, or spiral-shaped) (Cabeen, M. T., & Jacobs-Wagner, C., 2005). In contrast to gram-negative bacteria, which have only one-layer of peptidoglycan, a substance consisting of sugars and amino acids, gram-positive bacteria have many layers of this substance in their cell walls. Therefore, the kind and form of the cell wall can be used to categorize the many types of enteric bacteria further.

All animals and humans have a significant number of enteric GNB in their intestines. Most enteric bacteria, also known as gut flora or human microbiota, are benign and support the maintenance of a healthy digestive tract. Other intestinal bacteria, however, harmful affect and cause disease. For instance, whereas most *Escherichia coli* (E. coli) strains are benign, the pathogenic strains can release toxins that can cause various foodborne disorders. For instance, enterohemorrhagic *E coli* can make people sick with bloody diarrhea.

One of the Enterobacteriaceae species in the microbiota that has received the most research is *E coli* (Martinson, et al., 2019; Nyberg, S. D. et al., 2007). EGB have been stuied extensisly in medical literature.In addition to having a substantial impact on the intestines, Enterobacteriaceae can occasionally can cause urinary tract infections and can be isolated in the genitourinary tract. Other noteworthy Enterobacteriaceae species include Klebsiella, Proteus, and Enterobacter, Salmonella, Shigella, Yersinia *Campylobacter jejuni* (*C jejuni*) can be isolated from the human infections. Some gram posivtive bacilli, as well as *Clostridium difficile may also isolate from the infection,*

Most enteric micro-organisms are benign and support a healthy gut environment. However, certain intestinal bacterial strains may be harmful effectsto the human body . Some people's immune systems are capable of fighting of illness once micro-organisms have entered the body. However, in some people, the infection is not able to be cleared

by the immune system, and sickness results. Depending on the type of bacteria involved and the precise site of infection, different enteric bacteria might cause different symptoms. Fever, diarrhea, vomiting, and stomach discomfort are typical signs and symptoms. Maintaining hydration and contemplating antibiotic medication are the common forms of treatment for those exposed to pathogenic bacteria depending on the kind of bacterial illness.

Gram-negative Non-enteric bacteria belong to a varied category of bacteria that share several traits. Gram staining which displays the structure of the bacteria's cell wall, classifies them as Gram-negative bacilli. bacteria that are Gram-negative have a smaller layer of peptidoglycan as well as an outer membrane than Gram-positive bacteria. Non-enteric microorganisms on the other hand, are microorganisms that are not typically found in the gastrointestinal system.Gram-negative non-enteric bacteria are a diverse group of bacteria with varying characteristics as well as pathogenic potential. "*Pseudomonasaeruginosa, Acinetobacter baumannii, Burkholderia cepacia complex*, and *Stenotrophomonas maltophilia*" are a few instances. These microorganisms are frequently found in natural environments including soil, water, and vegetation, although they can also be discovered in hospital environments.

Gram-negative non-enteric microorganisms may generate a range of illnesses in human body, especially for people with weak immune systems or who have had extensive medical operations. They are well-known for their capacity to acquire resistance to numerous antibiotics, makes therapy difficult in some circumstances. These microorganisms might create enzymes which alter or inactivate antibiotics, have mechanisms for efflux that pump out the drugs, or experience genetic alterations that shift the target sites of antibiotics.

Considering their clinical importance and rising occurrence, infections triggered by "Gram-negative non-enteric bacteria" must be identified and managed correctly. For efficient treatment and prevention of the spread of these bacteria in clinical and community settings, proper diagnostic procedures, antibiotic susceptibility testing, and infection control measures are required.

2.2 Aminoglycosides

Aminoglycosides are actinomycete-derived natural or semi synthetic antibiotics (Krause, K.M.etal., 2016). A number of them have been licensed for use in humans. They were among the first antibiotics to be introduced or frequent clinical use. In the early days of antimicrobial chemotherapy, they were widely used as first-line drugs, but cephalosporins, Carbapenems, and Fluoroquinolones finally to place in the 1980s. A renewed interest in aminoglycosides, which are rapidly bactericidal and have abroad spectrum of activity, is due to their synergistic interactions with several other antibacterial classes, the rise of multidrug-resistant bacteria, and the potential for improved safety and efficacy of the class through optimized dosing regimens. Aminoglycosides attach to the A-site on the 30S ribosome's 16S ribosomal RNA to prevent the production of new proteins (Kotra, et al., 2000). Aminoglycosides enter bacterial cells in three steps (Madigan, et al., 2003), the first of which increases membrane permeability and the second and third of which are energy-dependent. The phospholipids and teichoic acids of Gram-positive bacteria and the phospholipids and lipopolysaccharide (LPS) of Gram-negative bacteria are examples of negatively charged components of the bacterial membrane that are electrostatically bound to the polycationic aminoglycoside in the first stage, which is followed by the displacement of magnesium ions (Davis, 1987; Ramirez & Tolmasky, 2010)Since streptomycin was originally extracted from Streptomyces griseus and used in clinical settings in 1944, the class has served as the foundation of antibacterial chemo therapy (Krause, K. M. et al., 2016). Over the years, several further members of the family were developed, including Neomycin, Kanamycin, Gentamicin, Netilmicin, Tobramycin, and Amikacin. As third generation cephalosporin, Carbapenems, and Fluoroquinolones were more widely available and were thought to be less toxic and/or offer broader coverage than the aminoglycosides, a movement away from systemic usage of the class of antibiotics started to occur. However, the introduction of novel aminoglycosides and increased resistance to these medications has sparked renewed interest in the previously used aminoglycosides.

Aminoglycosides are delivered intravenously or intramuscularly since their absorption via the GI system is low. Members of the aminoglycoside class have a volume of distribution close to that of the entire body, showing that they are widely distributed

throughout tissues, including the lung. Because of this property, aminoglycosides are frequently used in combination regimens to treat pneumonia. Because aminoglycosides pass through the urinary tract quickly, they are also excellent for treating urinary tract infections (Craig, 2011).

In patients with significant Gram-negative bacterial infections, empiric combination treatment with at least one antimicrobial medication to which the pathogen is sensitive results in decreased mortality and better out comes. In addition to ensuring that the pathogen is adequately covered by at least one active drug, the use of empiric aminoglycoside-b-lactam combination therapy has been proposed to contribute to improved outcomes by utilizing the in vitro synergy observed between these classes and preventing the emergence of resistance (Matsumoto, 2014; Tamma, et al., 2012).

2.3 Antimicrobial resistance

Antimicrobial resistance (AMR) is the capacity of a bacterium to fend against the effects of a medication that formerly had the power to eradicate it. AMR poses a severe danger to the global healthcare system and has a detrimental effect on people, animals, and the environment (Marston, H. D. et al., 2016). Antimicrobial resistance has evolved in many commensal and pathogenic species. Antimicrobial abuse or excessive usage and the introduction of AMR have raised death and morbidity rates and yearly health care expenses. The World Health Organization (WHO) has recognized the need to research the causes of AMR's growth and the necessity for sensible antibiotic usage policies (Dyar, et al., 2017)

Bacteria can become resistant to antibiotics in various ways, including intrinsic resistance, spontaneous gene changes, acquiring additional resistant genes, and intrinsic resistance to drugs (Nikaido, H., 2009). Horizontal gene transfer and antibiotic selection pressure have little effect on intrinsic resistance. It could be connected to an antibiotic's lack of affinity for the bacterial site, the drug's penetration into the bacterial cell, or the presence of enzymes that break down medicines (Jacopin, E. et al., 2020). Previously susceptible bacteria become resistant to drugs when they acquire resistance. This adaptation may result from the mutation of a specific gene or the conjugation or transduction of a resistant gene. Understanding the mechanism of resistance can help

clinicians utilize antibiotics effectively and reduce the chance of developing acquired resistance (Tenover, 2006). Antibiotics containing polymyxin, for instance, are frequently employed as the last drug in MDROs. However, *Acinetobacter baumannii and Pseudomonas aeruginosa* are GNBs that develop resistance to polymyxin antibiotics, but Proteus species, Serratia species, and Burkholderia species inherently resist the same medications.

The emergence of multidrug-resistant GNB in the hospital setting is increasingly recognized as a global problem, with ICU patients particularly vulnerable. Despite the fact that there are few prospective novel agents, the creation of new combinations may generate hope in the future.

2.3.1 Mechanisms of aminoglycoside resistance

Resistance to aminoglycosides can develop through a variety of methods which enable bacteria to avoid the effects of such antibiotics. Listed below represent a few of the most prevalent manifestations of aminoglycoside resistance:

1. Enzymatic Modification; Actinoglycoside-modifying enzymes (AMEs) are enzymes which chemically change the framework of aminoglycoside antibiotics. These alterations can involve the insertion of chemical groups or the deactivation of certain functionality of the moieties, causing the antibiotic's ineffectiveness. The bacterium may hinder the antibiotic from attaching to its target location and exhibiting its antibacterial action by altering it (Pradier, & Bedhomme, 2023; Bastian, et al., 2022).
2. Tempering with Focused Sites; Bacteria are capable of developing resistance to aminoglycosides by changing the binding sites of the antibiotics (Darby, et al., 2023; Llano-Sotelo, et al., 2002). One process includes the development of enzymes that may change ribosomal sites of binding, including "aminoglycoside-modifying enzymes or rRNA methyltransferases". This change lowers aminoglycoside affinities for their focus, making it more difficult for antibiotics to effectively suppress bacterial protein production.
3. Chromosomal Mutation; In the bacterial chromosome, unexpected genetic changes can arise, resulting in alterations in particular genes implicated in

aminoglycoside sensitivity (Maunders, et a., 2020). These changes may influence the chemical makeup or function of proteins targeted by aminoglycosides, lowering their efficacy. Mutations like these can arise in genes producing "ribosomal proteins, ribosomal RNA, or other protein-synthesis components" (Thacharodi, & Lamont, 2023).

4. Efflux Pumps; Bacterial efflux pumps are specialized carriers that aggressively push aminoglycosides out of the bacterial cell prior to begin to exert their antibacterial properties. Those efflux pumps function as a defense system, evacuating drugs as well as restricting their accumulation across the bacterial cell, lowering antibiotic concentration and potency (Bialek-Davenet, et al., 2015).

(Doi, Y. et al., 2016). Each of these processes affects distinct class members differently, and any particular resistant isolate frequently combines several of these mechanisms. Furthermore, except for Mycobacterium spp. and Borrelia spp., almost all prokaryotes encode multiple copies of rRNA. Hence the resistance to aminoglycosides via target site mutations has not been seen. However, current large-scale surveillance effort said in understanding phenotypic aminoglycoside resistance among significant pathogens, the epidemiology of particular resistance mechanisms has not often been the focus of these investigations. Aminoglycosides attach to the aminoacyl-tRNA recognition site (A-site) of the 16S rRNA, which makes up the 30S ribosomal subunit, inhibiting polypeptide synthesis and causing cell death. Resistance to aminoglycosides may occur based on several mechanisms:

(1) Enzymatic modification and inactivation of the aminoglycosides, mediated by aminoglycoside acetyl transferases, nucleotide transferases, or phosphotransferases and commonly observed across gram-positive and negative bacteria; (2) increased efflux; (3)

decreased permeability; and (4) modifications of the 30S ribosomal subunit that interferes with binding of the aminoglycosides. For the latter, both mutations (nucleotide substitution) and post transcriptional alterations have been linked to aminoglycoside resistance (Ramirez, & Tolmasky, 2010).

A study in Norway aimed to look at aminoglycoside resistance in Escherichia coli and Klebsiella pneumonia. Collection of 49 blood culture isolates with aminoglycoside resistance acquired between 2000 and 2009.The study investigated co-resistance to alternative antibiotics and the dynamics of aminoglycoside resistance; 67 isolates (mainly from urine) with resistance to both aminoglycosides and extended-range Beta-lactam antibiotics were included. 92% of the isolates were resistant to three aminoglycosides, and 60.3% were resistant to Gentamicin, Netilmicin, Tobramycin, and kanamycin. Resistance to amikacin was poor. Ciprofloxacin resistance was identified in 88% of the isolates with gentamicin resistance. Multiple resistance mechanisms coexist according to the patterns of aminoglycoside resistance. The usage of ciprofloxacin and third-generation cephalosporins probably influenced Norway's rise in aminoglycoside resistance (Lindemann, et al., 2012).

At the Minia university hospital in Egypt, researchers explored the aminoglycoside resistance of Gram-negative bacteria isolated from people who had ear, skin, urinary tract, and gastro intestinal tract infections. Escherichia coli (28.57%) and Pseudomonas aeruginosa (25.7%) were the two most common isolates, with Escherichia coli (mostly from the urinary tract and gastrointestinal tract infections) coming in second. Maximum resistance to streptomycin (83.4%), lowest resistance to amikacin (17.7%), and

Intermediate resistance to Neomycin, kanamycin, Gentamicin, and Tobramycin were all displayed by isolates. Older aminoglycosides were more resistant to bacterial growth than more recent ones. According to interpretive reading, streptomycin resistance, whether present as a single phenotype or in combination, was the most prevalent aminoglycoside resistance phenotype, followed by kanamycin neomycin. The resistant *Pseudomonas aeruginosa* strains could produce enzymes that alter aminoglycosides and use efflux as a defense mechanism. The selection pressure of aminoglycoside usage, which effective infection control methods might reduce, may be responsible for the high resistance rates to aminoglycosides displayed by Gram-negative bacteria in the research

(Gad, G. F., et al., 2011).

Over ten years, a worldwide sample of Gram-negative microorganisms was collected to evaluate the evolution of aminoglycoside resistance. The study examined medical facilities in North America, Latin America, Europe, and the Asia-Pacific to determine how resistant nine common species groups were to gentamicin, tobramycin, and amikacin. Between 1998–2000 (27,491 strains) and 2005–2007, isolates from the bloodstream and respiratory tract infections were gathered from38 countries (30,430 strains). *E coli,* Klebsiella, Enterobacter, Citrobacter, Serratia, *P mirabilis,* Indole-positive Proteae, *P aeruginosa*, and Acinetobacter were among the organisms found. Two central monitoring laboratories conducted susceptibility tests. This world-wide study concluded that the most frequently identified Gram-negative bacterium, E coli, is more resistant to AG-S across all geographical areas. The examination of this sizable sample of gram-negative bacteria revealed significant regional variation in AG-S, with Latin America and the Asia Pacific region having the greatest rates of resistance. It indicates that a new class of aminoglycoside drugs might be a good therapeutic option to Gentamicin, Tobramycin, Amikacin for gram-negative infections after the aminoglycoside resistance mechanisms associated with these isolates (Biedenbach, et al., 2009).

2.3.2Antimicrobial resistance in Cyprus

The spread of antimicrobial drug resistance may be facilitated by self-medication, which is a matter of concern (Grigoryan et al. ,2007). Self-treatment with an ineffective medicine against the causing organism or with an insufficient dosage increases the chance of selecting resistant, hard-to-get rid-of organisms. The community may then become exposed to these resistant organisms. In addition, studies show that using no needed medications such as Chloramphenicol, Doxycycline, and Aminoglycosides can exacerbate the negative effects of self-medication. Drug interactions, misdiagnosed conditions, and super infections are other issues associated with self-medication (Grigoryan, et al., 2007).

In North Cyprus, a study found that patients ranked antibiotics as the second most kind of drugs with leftovers; this is due to the fact that antibiotics are highly accessible in

Northern Cyprus. Therefore, the pharmacies inNorthern Cyprus, where it is possible to obtain antibiotics without a prescription, should be made aware of this situation and should remind patients who purchase antibiotics to finish the course of therapy (Gürman, et al., 2016).

Out of 168 pharmacies in Cyprus, 84 pharmacies were included in a survey to determine the prevalence of non-prescription antibiotics in pharmacies. In comparison to many developed nations, Northern Cyprus has been discovered to have a greater rate of non-prescription antibiotic use and sale (Süer, et al., 2019).

CHAPTER 3

Material and Methods

3.1 Study design

A retrospective study has been designed to detect aminoglycosides resistance among NE-GNB in our hospital between August 2020 to June 2022. The patient files have been collected from hospital archive and electronic recording system database.

3.2 Study Subjects

The selection of patient and and inclusion and exclusion criteria has been summarized in

Table 3.1 **Inclusion and exclusion criteria**

Inclusion	Exclusion
• Patients who hospitalized between August 2020 to June, 2022 • Patients of all ages • Patients received at least one antibiotic • Patients diagnose with Microorganisms.	• Patients with missing information or incomplete file • Patients who did not receive antibiotics

The documents were retrieved in an excel format and ran from January 2020, to December 2021, with a total of 659 patient recorded included. The data collecting devices were a planned technique that contained "patient demographic information such as name, age, gender, and admission department.

The clinical samples including aspiration fluid, sputum, abscess/wound material, CSF, bronchial lavage, catheter tip, pleural fluid, sperm, urethral discharge/swabs, vaginal discharge/swabs, urine, and blood" have been collected.

3.3 Scope of Study

This study was conducted in Northern Cyprus, Near East University Hospital (NEUH), a hospital closing area of 55,000s quartermasters,209 single-patient rooms, and Intensive care Units include 30 beds adults and 14 -beds neonatal intensive units, eight operational

theatres.

3.4 Materials

3.4.1 Gram staining material

Figure 3.1 **Gram staining material**

Crystal Violet	**g/100ml**
Crystal Violet	2
Ethanol	20
Ammonium oxalate	0.8
Distilled water	100ml
Gram Solution for Iodine	**g/100ml**
Potassium Iodate	2
Crystal Iodine	1
Distilled water	100ml
Decolorizer	g/100ml
Acetone	50ml
Ethanol	50ml
Counter Stain	**g/100ml**
Safranin	2.5
Ethanol	100
Ammonium Oxalate	0.8
Distilled Water	100ml

Table 3. 2 **Inoculation and Incubation Materials**

Instrumen	**Company**
Testing Tube	Greiner Bio-One
Vial	Qorpak
Measuring Cylinder	VITLAB GmbH
Pipettes	Eppendorf
Spectrophotometer	Thermo Fisher Scientific
Vortex Mixer	Eppendorf
Gloves	Ansell

Table 3.2 Continued

Lab coat	Medline Industries, Inc.
Goggles	GÜVEN İŞ Safety Equipment
Autoclave	Raypa
Agar powder	Genelabs
Distilled water	Brazil
Incubator	Memmert
Inoculating loops or swabs	Biomerieux
Blood agar plates	Biomerieux
MacConkey agar plates	Biomerieux

Table 3.3 **Media and Culture Used**

Medium	**Company**
Blood agar	Neogen
MacConkey agar	Condalab

3.4.2 Blood agar media

In microbiology, is a form of solid growth media used to cultivate and identify bacteria (Opota, et al., 2015). It is made comprised of nutritious agar mixed with sterile sheep or horse blood. The blood supplies important nutrients for bacterial development and aids in the differentiation of various bacterial species depending on their capacity to hemolyze the red blood cells in the agar. Based on their hemolytic patterns, blood agar medium is particularly effective in detecting pathogenic bacteria such as Streptococcus and Staphylococcus species.

3.4.3 MacConkey agar media

Refers to a selective and differential medium used to isolate and identify gram-negative bacilli (kim et al., 2023), especially members of the Enterobacteriaceae group. It contains bile salts and crystal violet, which hinder gram-positive bacteria development and enable for the selective separation of gram-negative bacteria (Singhal, et al., 2023). MacConkey agar (MAC), created in the twentieth century by Alfred Theodore MacConkey, is an important differential media used for microbiological investigation. It

is used to isolate and identify non-fastidious gram-negative rods, notably those belonging to the Enterobacteriaceae family and the genus Pseudomonas. To make MAC, 52 gram of dried MacConkey agar medium (Condalab) were mixed with one litre of distilled water and boiled until the media was completely dissolved. The resultant solution was then autoclaved for 15 minutes at 121°C and 15 lbs of pressure, as reported by Sonnenirth (1980)

3.4.5 VITEK 2 System Machine

For identification of heighted bacteria VITEK 2 system was utilized. The VITEK 2 system is an automated microbiological device that identification and susceptibility processes of gram positive and gram-negative bacterial species it has accurately and efficiently properties (Torres-Sangiao et al., 2022).

Table 3.4 **VITEK 2 System Equipment.**

Medium	Produced By	Country of Origin
VITEK 2 Compact Compact Work-settings Barcode Scanner Printer Uninterrupted Power Supply Power Conditioner Test Cassetles DensiCheck plus	bioMérieux	France

3.4.6 Isolation and culture Media.

Implemented media for isolation as well as identification of microorganisms is described in the table below;

Table 3.5 **Media Culture Equipment for Blood and MacConkey Agar**.

Equipment and Apparatus	Company
Blood agar plates	Biomerieux
MacConkey agar plates	Biomerieux
Inoculating loops or swabs	Biomerieux

Table 3.5 **Continue**

Bacterial suspension or culture	Bio-Rad Laboratorie
Incubator	Biobase
Sterile Petri dishes	BioLAB
Sterile spreader	BioLAB
Colony counter	BioLAB

3.4.7 Identification and susceptibility

3.4.7.1 VITEK-2 Compact Technology

Isolated bacterial were identification through the use of VITEK-2 compact machine in the laboratory, at Near East University

- As we mentioned before the Vitek2- Compact machine for identification and antibiogram had set the the machine according to McFarland between (0.50-0.70 cfu for gram negative bacteria tests.

VITEK2GN Frame for Identification process: The VITEK2-GN Frame is an element within the VITEK2 system as well, which is a clinical laboratory-based automated microbiological identifying system. The VITEK2GN Frame was created particularly for identifying Gram-negative bacteria. VITEK2GN was employed for microorganism detection and antibiogram testing. It most likely supplied automatic findings for the examined microorganisms, such as identifying as well as antibiotic susceptibility.

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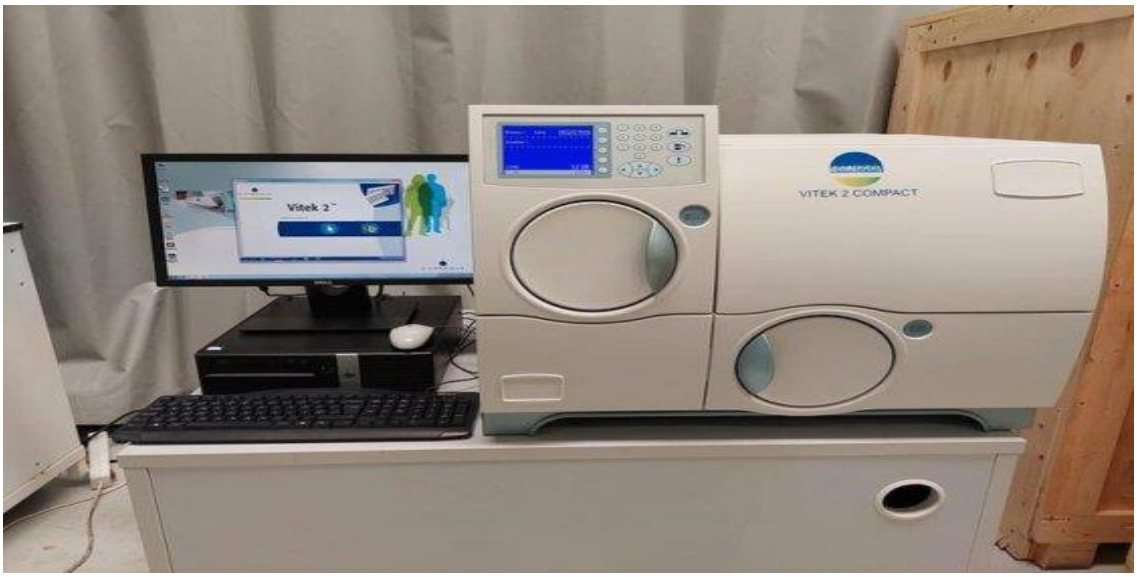


Figure 3.2 **VITEK-2 Compact System Work Station**

3.4.7.2 Cards for Antimicrobial Identification and Susceptibility Testing

Gain rapid, accurate susceptibility test results and resistance detection in clinically relevant bacteria with self-contained, disposable test cards for use with VITEK-2 instruments

Figure 3. 3 **VITEK-2 Gram Negative Identification Card. Source** (Pincus, 2006)

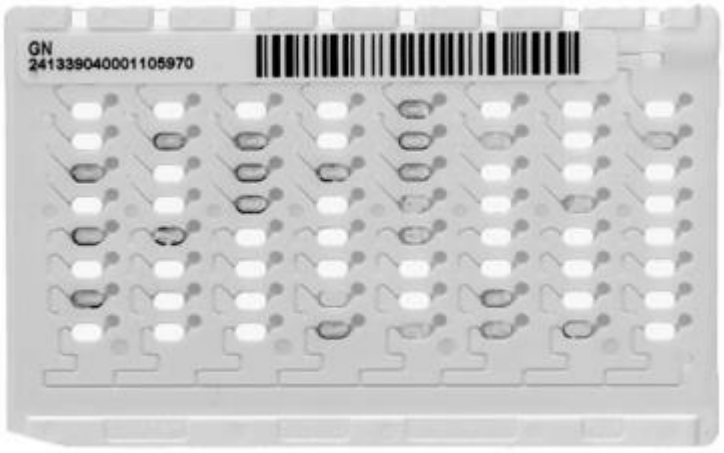


Figure 3.4 VITEK 2 **Compact: 10-card cassette, suspension tubes and data input bar code scanner. Source** *(Pincus, 2006)*

3.5 Methods

3.5.1 Gram Staining and culture and Isolation

These processes had done according to the Microbiological Standard for gram staining and culture and isolation in the lab.

3.5.2 Preparation of the Inoculum

The first step in identifying isolates using the VITEK-2 system was to do a Gram stain to choose the proper card for testing. A sterile brush was then used to transfer multiple colonies from pure cultures to a clear plastic test tube containing 3ml of sterile saline solution containing 0.45% to 0.5% liquid sodium chloride and a pH range of 4.5 to 7.0. According to the system's instructions, the suspension created for the VITEK-2 system was adjusted to a McFarland turbidity standard of 0.5 to 0.63.

3.5.3 Inoculation

The procedure of recognizing cards contaminated with bacterial suspensions required the use of an integrated vacuum system, which produced final identification findings in eight hours or less (Pincus, 2006). A transfer tube was carefully inserted into a similar suspension tube to accomplish the identification, while a test tube holding the bacterial suspension was placed into a cassette. An identity card was placed into the adjacent slot, and up to ten tests (VITEK-2 Compact) may be stored on the cassette. The inoculation solution was then delivered through the transfer tube, filling all of the microchannels on

the cassette. To finish the process, the filled cassette was manually put into the vacuum chamber station, and air was reintroduced into the station (Pincus, 2006)

3.5.4 Identification and susceptibility processes.

3.5.4.1 Sealing the Card and Incubation

Prior to inserting the inoculation cards into the carousel incubator, the inoculation cards were sealed. This entailed running the cards through a machine that sealed the transfer tube and closed the card. The carousel incubator could accommodate up to 30 or 60 cards. The cards were incubated at 35.51.0°C, with all card kinds cultivated in a linear orientation. Each card was retrieved from the carousel incubator and transported to the optical system for reaction reading every 15 minutes. The card was returned to the incubator after the reading until the next reading was planned. Throughout the incubation phase, data was gathered at 15-minute intervals (Pincus, 2006).

3.7 Statistical analysis

Intermediate resistant results from clinical isolates were considered resistant Statistical methods were used to analyze the data, including the calculation of descriptive statistics such as the frequency and percentage for categorical variables, the mean, the median, the standard deviation (SD), and the minimum and maximum for the continuous variables. A Pearson Chi-square test was performed to evaluate the associations between categorical variables. The level of significance was defined as $\alpha = 0.05$. All calculations and analyses were carried out with the SPSS

(Statistical Package of Social Sciences Demo Version 22.0) program.

CHAPTER 4

Results and Discussion

The number of patients with NE-GNB which applied to our hospital during our study period was 659.

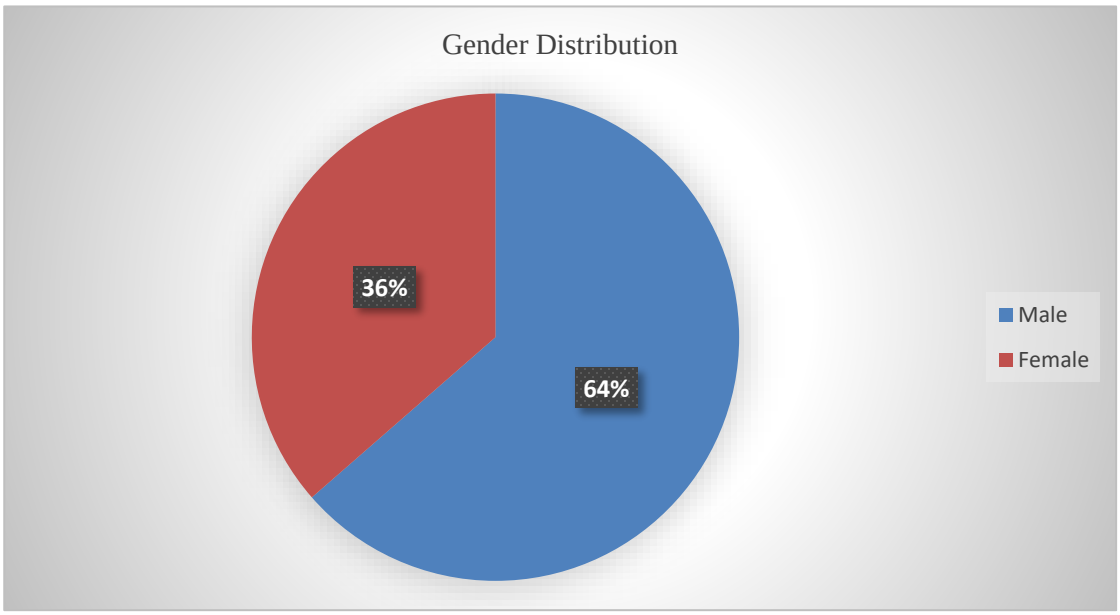
4.1 Distributions of Patients According to Gender

A below in the tabe 4.1 and figure 4.1 shows the characterization of patients. A total of 659 patients were admitted at the hospital. 419 which is (63.3%) were male. While, 240 amounted to (36.6 %) were females. We summarized these results in table and Figure

Table 4.1 **Gender Distribution**

Gender	Frequency (f)	Percentage (%)
Male	419	63.6
Female	240	36.4

Figure 4.1.**Graphical Representation According to Gender**



4.2. Distribution of the Non-enteric Gram-negative bacilli according their origin

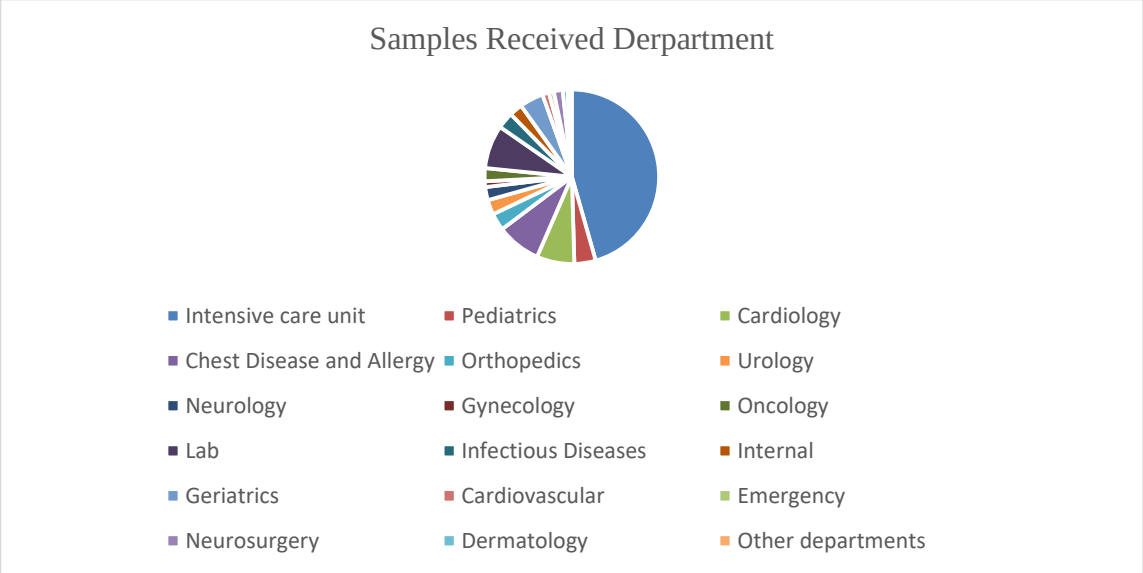
The distribution of the tested microorganisms according to the clinics their origin shown in Table 7. And Figure 6. According to our results, the analysis regarding patients

records according to the departments 300 (45.5%) patients were admitted at the intensive care unit as the highest, followed by 54 (8.2 %) admitted to chest disease and allergy unit, 53 (8.1 %) and the Cardiology department, 29 (4.4%) were admitted to geriatrics department, other departments has been outlined in the Table 4.2 and Figure 4.2 below.

Table 4.2 **Distribution of Samples According to their Department**.

Department	**Frequency (f)**	**Percentage (%)**
Intensive care unit	300	45.5
Pediatrics	26	4
Cardiology	46	7
Chest Disease and Allergy	54	8.2
Orthopedics	21	3.2
Urology	18	2.7
Neurology	16	2.4
Gynecology	7	1.1
Oncology	16	2.4
Lab	53	8.1
Infectious Diseases	20	3
Internal	16	2.4
Geriatrics	29	4.4
Cardiovascular	8	1.2
Emergency	6	0.9
Neurosurgery	11	1.7
Dermatology	6	0.9
Other departments	5	1

Figure 4.2. **Graphical presentation of Sample Received According to Department**.



4.3. Distribution of the Clinical Samples

Table 4.3 **The sample types for testing the presence of the microorganism**.

Sample	Frequency (f)	Percentage (%)
Aspiration fluid	220	33.4
Urine	159	24.1
Sputum	137	20.8
Abscess/Wound material	61	9.3
Blood	44	6.7
Catheter tip	24	3.6
Pus	5	.8
Bronchial lavage	5	.8
Pleural fluid	1	.2
Sperm	1	.2
Urethral discharge/swabs	1	.2
Vaginal discharge/swabs	1	.2

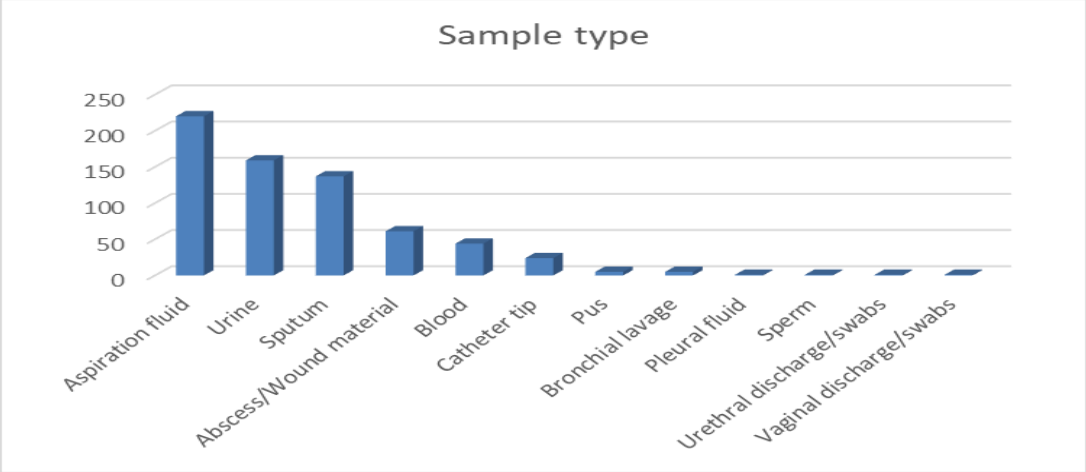
A as seen in the table 4.3 and figure 4.3. Aspiration fluid: was among the sample type with the highest largest frequency, contributing to 33.4% of all samples (220). Samples of urine were the second most frequent, with 24.1% of the total with 159 samples. Sputum: Sputum samples are the third most common, accounting for 20.8% of the total with 137 samples. Abscess/Wound material: This sample type occurs 61 times, accounting for 9.3% of all samples. Blood samples are quite uncommon, with 44 instances accounting for 6.7% of the total. Catheter tip: This sample type occurs 24

times, accounting for 3.6% of all samples.

Pus, bronchial lavage, pleural fluid, sperm, urethral discharge/swabs, and vaginal discharge/swabs are all examples of diagnostic procedures. These sample categories have extremely low frequencies, with only 1 or 5 occurrences apiece, accounting for less than 1% of all samples.

The table shows distribution of sample types used when testing for the presence of the microorganism. It shows that aspiration fluid, urine, and sputum are the most typically examined sample types, with others being less prevalent.

Figure 4.3 **Graphical Distribution of Cases According to Sample Type**



As we can see from the Table 5 and Figure 4 above, aspiration fluid is the most prevalent samples with 220 which is equivalent to 33.4%, the second highest frequent samples were urine with 159 (24.1%), and third highest was Sputum with 137 (20.8%), followed by Abscess/ wound material 61 (9.3%). and others.

4.4. Distribution of Non-enteric Gram-negative bacilli according to their strains

We isolated three genus of microorganisms in NE-GNBs from clinical specimens. *Achromobacter Xylosoxidans* was isolated from only one sample and this microorganism was ignored in the studies. Microorganisms of the genus Pseudomonas were isolated from 371 samples, and the genus Acinetobacter from 283 samples (56.3% and 44.8 respectively).

Species distributions of these two microorganism genera are summarized in Table 6.

As shown in the table, *Pseudomonas aeruginosa* and *Acinetobacter*

*baumanii/calcoaceticus complex, s*trains were the most prevalent strains according to our results. (56,3% and 41,7% respectively). The amount of the other *Pseudomons* and *Acinetobacter* strains isolated from clinical samples were summarized in Table 6.

Table 4.4 **Pathogenic Microorganism Strains Isolated from Clinical Samples**

Microorganism	**Frequency (f)**	**Percent (%)**
Acinetobacter strains	283	44,8
Acinetobacter baumanii/calcoaceticus complex	275	41.7
Pseudomonas strains:	371	56,3
Pseudomonas aeruginosa	362	54.9
Acinetobacter wifis	2	0.3
Acinetobacter Junii	1	0.2
Achromobacter Xylosoxidans	1	0.2
Pseudomonas fluorescens	6	0.9
Pseudomonas mendocina	2	0.3
Pseudomonas spp.	1	0.2

4.5. Aminoglycoside and the other group of antibiotic resistance in NE-GNB isolates

Firstly, aminoglycoside resistance was evaluated in NE-GNB isolates. In order to compare these results, other group antibiotic resistance results were also recorded.

In aminoglycosides group resistance rates were very close to each other (Min. was 35.4% in tobramycin and max. was 49.5 in netilmycin).

Aminoglycoside resistance and the other group of antibiotic resistance rates were summarized in Table 7.

Table 4.5. **Aminoglycoside and the Other Antibiotic Resistance Patterns in NE-GNB Isolates**

Antibiotic	R		S	
	N	%	N	%
Aminoglycoside				
Amikacin	236	36.1	418	63.9
Tobramicin	233	38.8	368	61.2
Gentamicin	287	46.3	333	53.7
Netilmicin	321	56.5	251	43.5
Beta-lactam group				
Piperacillin/Tazobactam	198	52.2	181	47.8
Piperacillin	159	43.5	245	56.5
Aztreonam	299	85.9	49	14.1
Cefepime	116	32.3	243	67.7
Ceftazidime	141	34.4	269	65.6
Imipenem	379	60.8	244	39.2
Meropenem	395	60.7	256	39.3
Quinolones group				
Ciprofloxacin	393	60.2	260	39.8
Levofloxacin	378	63.6	216	36.4
Other Antibiotics				
Colistin	41	6.4	603	93.6
Trimethoprim/Sulfamethoxazole	249	85.3	43	14.7

4.5.1 Aminoglycosides:

Amikacin resistance was found in 36.1% of bacteria, whereas 63.9% were sensitive. Tobramycin resistance was found in 38.8% of bacteria, whereas 61.2% were sensitive. Gentamicin resistance was found in 46.3% of bacteria, whereas 53.7% were sensitive. Netilmicin resistance was found in 56.5% of bacteria, whereas 43.5% were sensitive.

4.5.2 Beta-lactam:

Aztreonam resistance was found in 85.9% of bacteria, whereas 14.1% were susceptible. Cefepime resistance were 32.3% of bacteria, while 67.7% were sensitive. Ceftazidime resistance was 34.4% of bacteria, 65.6% were sensitive. Imipenem resistance was found in 60.8% of bacteria, and 39.2% were sensitive. Meropenem resistance was discovered in 60.7% of bacteria resistance, whereas 39.3% were sensitive.Penicillin Piperacillin/Tazobactam resistance was found in 52.2% of bacteria, whereas 47.8% were sensitive. Piperacillin resistance was found in 43.5% of bacteria, whereas 56.5% were sensitive.

4.5.3 Quinolones:

Ciprofloxacin resistance was found in 60.2% of bacteria, whereas 39.8% were sensitive. Levofloxacin resistance was found in 63.6% of bacteria, whereas 36.4% were sensitive.

4.5.4 Other Antibiotics:

Colistin resistance was found in 6.4% of bacteria, whereas 93.6% were sensitive. Trimethoprim/Sulfamethoxazole resistance was found in 85.3% of bacteria, whereas 14.7% were sensitive.

The table 4.6, below describes resistance and sensitivity of several antibiotics against a variety of bacterial strains, classified on gender basis, as well as the frequencies and percentages of resistant and sensitive cases, as well as the total number of cases, the Chi-Square values in the represent statistical significance of the results. Aminoglycosides: Male and female patients exhibit variable levels of resistance and sensitivity to the following antibiotics agent “Amikacin, Gentamicin, Tobramycin, and Netilmicin”. The Chi-Square 0.026 results, indicate that there may be a statistically significant gender difference in the response to these medicines ((Ayub et al., 2023) .

Piperacillin/Tazobactam, Piperacillin, Aztreonam, Cefepime, and Ceftazidime resistance and sensitivity patterns differ between males and females. The Chi-Square results show that there may be a substantial relationship between gender and antibiotic efficacy. Carbapenem: Imipenem and Meropenem exhibit gender variations in resistance and sensitivity. The Chi-Square results indicate that there may be a substantial link between

gender and antibiotic response. Ciprofloxacin and Levofloxacin exhibit different patterns of resistance and sensitivity in men and females. The Chi-Square results imply that there may be some relationship between gender and antibiotic efficacy, albeit this is not statistically significant for Levofloxacin. Tigecycline demonstrates gender variations in resistance and sensitivity, however the Chi-Square value implies that these differences are not statistically significant. Other drugs, such as Trimethoprim/Sulfamethoxazole and Colistin, exhibit differences in resistance and sensitivity between males and females. The Chi-Square values, however, show that these differences are not statistically significant.

Table 4.6 **Number(rate) of antimicrobial resistance profiles of hospital NE-GNB isolates toward 25 different antibacterial agents according to the gender**

Antibiotics	Gender	Resistant		Sensitive		Total	Chi-Square
		Frequency	Percentage	Frequency	Percentage		
		(f)	(%)	(f)	(%)	N	
Aminoglycosides							
Amikacin	Male	166	70.3	250	59.8	416	0.026
	Female	70	29.7	168	40.2	238	
Gentamicin	Male	200	69.7	199	59.8	399	0.015
	Female	87	30.3	134	40.2	221	
Tobramycin	Male	163	70	220	59.6	766	
	Female	70	30	149	40.4	436	0.011
Netilmicin	Male	216	66.3	149	59.4	365	0.068
	Female	110	33.7	102	40.6	212	
Beta-Lactams							
Piperacillin/Tazobactam	Male	138	73	142	58	280	
	Female	51	27	103	42	154	0.005
Piperacillin	Male	136	68.7	107	59.1	243	0.069
	Female	62	31.3	74	40.9	136	
Aztreonam	Male	192	64.2	28	57.1	220	
	Female	107	35.8	21	42.9	128	0.405
Cefepime	Male	77	66.4	149	61.3	226	0.028
	Female	39	33.6	94	38.7	133	
Ceftazidime	Male	101	38.7	160	59.5	261	0.028
	Female	40	28.4	109	40.5	133	

Table 4.6 **Continue**

Carbapenem							
Imipenem	Male	257	67.8	136	55.7	393	0.005
	Female	122	32.2	108	44.3	230	
Meropenem	Male	272	68.9	145	56.6	417	0.001
	Female	123	31.1	111	43.4	234	
Quinolones							
Ciprofloxacin	Male	266	67.7	148	57	414	0.006
	Female	127	32.3	112	43	239	
Levofloxacin	Male	249	65.9	129(59.7)	59.7	378	0.289
	Female	129	43.1	87	40.3	216	
Tetracyclines							
Tigecycline	Male	38	79.2	150	63.3	188	0.084
	Female	10	20.8	87	36.7	97	
Others							
Trimethoprim/Sulfamethoxazole	Male	168	67.5	24	55.8	192	0.137
	Female	81	32.5	19	44.2	100	
Colistin	Male	22	53.7	392	65	414	0.179
	Female	19	46.3	211	35	230	

4. 6 Antimicrobial Resistance of Isolated Samples According to Gender

We discussed the results in details above.

Table 4.7 **Resistance profiles of 10 hospital bacterial isolates toward 25 different antibacterial agents**

Antibiotic		P aeruginosaN(%)	P mendocinaN(%)	A baumaniiN(%)	A baumanii/calcoaceticus complex N(%)	P fluorescensN(%)	P lutealeN(%)	A lwoffiiN(%)	A juniiN(%)	Achromobacter xylosoxidansN(%)	PseudomonasN(%)
Amikacin	R	25(11.1)	-	4(100)	190(68.5)	1(16.7)	1(25)	-	-	-	-
	S	320(88.9)	2(100)	-	83(30.4)	5(83.3)	3(75)	2 (100)	1 (100)	1(100)	1(100)
Gentamicin	R	50(15.3)	-	3(75)	230(83.6)	1(33.3)	2(40)	-	-	1(100)	-
	S	276(84.7)	2(100)	1(25)	45(16.4)	2(66.7)	3(60)	2 (100)	1 (100)	-	1(100)
Tobramycin	R	27(8.1)	-	3(75)	200 (81)	1(20)	2(50)	-	-	-	-
	S	308(91.9)	2(100)	1(25)	47(19)	4(80)	2(50)	1 (100)	1 (100)	1(100)	1(100)
Netilmicin	R	112(33.6)	-	3(75)	203(92.4)	1(20)	1(25)	-	-	1(100)	-
	S	221(66.4)	2(100)	1(25)	17(7.6)	4(80)	3(75)	1(100)	1(100)	-	1(100)
Piperacillin/Tazobactam	R	121(33.9)	-	3(100)	59(96.7)	2(40)	2(50)	1(100)	-	1(100)	-
	S	236(66.1)	2(100)	-	2(3.3)	3(60)	2(50)	-	-	-	-
Piperacillin	R	160(48)	-	3(100)	30(100)	2(50)	2(50)	-	-	1(100)	-
	S	174(52)	2(100)	-	-	2(50)	2(50)	-	-	-	1(100)
	R	104(85.5)	2 (100)	-	4(100)	5(100)	3(75)	-	-	1(100)	1(100)

Table 4.7 **Continue**

Aztreonam	S	48(14.5)	-	-	-	-	1(25)	-	-	-	-
Cefepime	R	107(31.3)	-	-	5(100)	2(100)	1(20)	-	-	1(100)	-
	S	235(68.7)	2(100)	-	-	-	4(80)	1 (100)	-	-	1(100)
Ceftazidime	R	98(27.5)	-	-	36(94.7)	4(66.7)	3(75)	-	-	-	-
	S	259(72.5)	2(100)	-	2(5.3)	2(33.3)	1(25)	1(100)	-	1(100)	1(100)
Carbapenem											
Imipenem	R	127(36.5)	-	4(100)	244(95.7)	2(33.3)	2(50)	-	-	-	-
	S	221(63.5)	2(100)	-	11(4.3)	4(66.7)	2(50)	1(100)	1(100)	1(100)	1(100)
Meropenem	R	125(35)	-	4(100)	260(95.6)	1(20)	3(75)	-	-	-	-
	S	232(65)	2(100)	-	12(4.4)	4(80)	1(25)	2(100)	1(100)	1(100)	1(100)
Ciprofloxacin	R	120(33.3)	-	4(100)	260(96.3)	4(66.7)	2(50)	-	-	-	1(100)
	S	240(66.7)	2(100)	-	10(3.7)	2(33.3)	2(50)	2(100)	1(100)	1(100)	-
Levofloxacin	R	132(40)	-	4(100)	236(96.3)	4(80)	2(50)	-	-	-	-
	S	198(60)	2(100)	-	9(3.7)	1(20)	2(50)	1 (100)	1 (100)	1(100)	1(100)
Tetracycline	R	1 (50)	-	-	6(100)	-	-	-	-	1(100)	-
	S	1 (50)	-	-	-	-	-	-	-	-	-
Tigecycline	R	3 (75)	-	-	45(16.4)	-	-	-	-	-	-
	S	1 (25)	-	4(100)	229(83.6)	-	-	2 (100)	1 (100)	-	-
Trimethoprim/ Sulfamethoxazole	R	6(42.9)	-	3(75)	240(89.6)	-	-	-	-	-	-
	S	8(57.1)	-	1(25)	28(10.4)	2(100)	-	2 (100)	1 (100)	1(100)	-
	R	21(6)	1 (50)	-	15(5.5)	-	3(75)	-	-	-	1(100)

Colistin	S	329(94)	1 (50)	4(100)	260(94.5)	5(100)	1(25)	2 (100)	1 (100)	-	-

Table 4.7 shows the resistance (R) and sensitivity (S) of several bacteria species to antibiotics. *Pseudomonas aeruginosa*, *Pseudomonas mendocina*, *Acinetobacter baumannii*, *Acinetobacter baumannii/calcoaceticus complex*, *Pseudomonas luteale*, *Acinetobacter lwoffii*, *Acinetobacter junii*, *Achromobacter xylosoxidans*, and other *Pseudomonas species* are included. Each of the rows in the table indicates a different drug, and the columns show how resistant or sensitive the corresponding bacteria species are to that antibiotic. The percentages of resistance or sensitivity for each species are shown in parenthesis. As shown in the first row of table 4.7, the antibiotic agent Amikacin is resistant to Pseudomonas aeruginosa in 25 instances out of 225 (11.1%), whereas it is sensitive in the remaining 200 cases (88.9%). There is no information available on *Pseudomonas mendocina.* Amikacin is resistant to *Acinetobacter baumannii* in all four instances examined (100%). Similarly, resistance to *Acinetobacter baumannii/calcoaceticus* is strong (68.5%). Amikacin resistance and sensitivity varies among the other bacteria species in the table. Gentamicin, like Amikacin, has a high sensitivity among most bacteria species. When compared to other species, the *Acinetobacter baumannii/calcoaceticus* combination has a greater resistance rate (83.6%). Tobramycin: It has a high sensitivity among most bacteria species and a low resistance rate. The combination *Acinetobacter baumannii/calcoaceticus* has the greatest resistance rate (81%). Netilmicin is resistant to most bacteria, including *Acinetobacter baumannii/calcoaceticus complex* (92.4% resistance). Piperacillin/Tazobactam: It has high sensitivity rates for

the majority of bacteria species. *Acinetobacter baumannii* has the greatest rate of resistance (96.7%). Piperacillin has a high sensitivity to most bacteria species. *Acinetobacter baumannii* has the greatest rate of resistance (100%). Aztreonam has a high sensitivity rate for the majority of bacteria species. The *Acinetobacter baumannii/calcoaceticus* combination is completely resistant. Cefepime, Ceftazidime, Imipenem, Meropenem, Ciprofloxacin, and Levofloxacin: These medicines have high sensitivity rates against most bacteria. Tigecycline has a high sensitivity, especially against the *Acinetobacter baumannii/calcoaceticus* combination (83.6% sensitivity). Trimethoprim/sulfamethoxazole: It is moderately sensitive to most bacteria species, with increasing sensitivity rates. Colistin has a high sensitivity rate for most bacteria species and a low resistance rate.

CHAPTER 5

Discussion

The study investigated non-enteric gram-negative bacilli resistance to aminoglycoside. The study involved a relatively large sample of admitted and walk-in patients received from different departments at Near East University Hospital for period of two years. The data provides patients' demographics details, the diversity of samples received, including the resistance patterns for different antibiotics. Table 4.1 shows the patients' distribution of gender. The study comprised 659 patients, with men having the majority with 63.3% (419) while females with 36.6% (240). This distribution of males and females (Gender) correlates with the overall population, implying that the sample is typical of the hospital's patient population. Table 4.2 summarizes the distribution of patients per department. Intensive care unit department have the highest sample of patients (45.5%), while chest diseases and allergy followed with (8.2%), and lab department (8.1%). The distribution between departments reflects the wide range of medical problems and demands of the hospital's patients.

The results show the aminoglycosides percentage of resistance and sensitive as follow :Amikacin resistance was found in 36.1% of bacteria, whereas 63.9% were sensitive. Tobramycin resistance was found in 38.8% of bacteria, whereas 61.2% were sensitive. Gentamicin resistance was found in 46.3% of bacteria, whereas 53.7% were sensitive. Netilmicin resistance was found in 56.5% of bacteria, whereas 43.5% were sensitive. But it less than some group of B-Lactams for example Aztreonam resistance was found in 85.9% of bacteria resistance also for Trimethoprim/Sulfamethoxazole resistance was high resistance.

The kinds of samples collected for assessing the existence of microorganisms are listed in Table 4.3. The most frequent sample type collected was aspiration fluid (33.4%), as well as urine (24.1%) and sputum (20.8%). Because these samples are widely used to diagnose such diseases, our data imply that respiratory as well as bladder infections are widespread across the patients.

The pathogenic bacteria identified from clinical samples are highlighted in Table 6. *Pseudomonas aeruginosa* (54.9%) was the most frequently isolated pathogen (Marra, et al., 2011), followed by the *Acinetobacter baumannii/calcoaceticus* combination

(41.7%). These findings underline the relevance of these viruses in healthcare-associated illnesses and the necessity for effective anti-spread interventions. Table 4.5 shows the antimicrobial vulnerability of the isolated bacteria to various antibiotic agents medications. The findings reveal that resistance as well as susceptibility stages varies between medicines. For instance, substantial resistance rates for Ciprofloxacin (58%) and Levofloxacin (56.3%) were detected, showing that quinolone antibiotics were ineffective against the tested isolates. Amoxicillin/Clavulanate as well as Nitrofurantoin, on the contrary hand, had minimal resistance rates (0.2%), indicating their potential as therapy choices.

Table 4.6 shows the resistance characteristics of the isolates according to gender. The findings demonstrate that antibiotic resistance rates differed across gender (Edlin et al., 2013). The chi-square analysis, on the other hand, indicated very minor relationships between gender and antibiotic resistance.

Despite the large number of antimicrobial agent classes available today, the therapeutic options to treat NGB infections are restricted, which constitutes a serious treatment challenge for physicians and pharmaceutical companies. Therefore, extensive world-wide surveillance in addition, these programs may also be able to identify therapeutic candidates for future clinical studies amongst new or novel classes of antimicrobial agents via testing of characterized strains from their organism collections

The aminoglycoside, such as gentamicin consumption, developed resistance to amikacin in *Paeruginosa* and resistance to *Acinetobacter baumanii*, 48 (14.7%) and 230 (83.6%), respectively (Table 6). Although our finding was in contrast with similar studies that showed the most common resistance to gentamicin was *P aeruginosa*, with no resistance to in A. *baumannii*, this difference in the findings may be affected by different diagnoses and cultures (Lee, et al., 2015; Hsueh, et al., 2005). Increasing the use of extended-(particularly Meropenem) could develop meropenem resistance in *P aeruginosa* and *Acinetobacter baumanii*. Our findings showed similarity to other studies which supported the increment of ceftazidime resistance. (Bantar, et al., 2004; Neuhauser, 2003; Gentry, et al., 2002; Lesch, et al., 2001; Rice, et al.,1996).

CHAPTER 6

Conclusion and Recommendations

This research studied the resistance of non-enteric gram-negative bacilli to aminoglycoside antibiotics. The findings were based on a large sample of patients hospitalized to the Near East University Hospital over the course of two years. The data gave useful insights into patient demographics, sample diversity, and drug resistance tendencies. The sample's gender distribution closely matched that of the total population, indicating its representativeness. Distribution of patients between departments reflected the hospital's vast range of medical concerns. Aspiration fluid, urine, and sputum were the most often obtained sample types, reflecting the frequency of respiratory and bladder illnesses among the patients. The most frequently isolated bacteria were *Pseudomonas aeruginosa* and *Acinetobacter baumannii/calcoaceticus,* indicating their importance in healthcare-associated diseases. Resistance rates differed with antibiotic, quinolones showed greater rates and amoxicillin/clavulanate and nitrofurantoin showing lower rates. Gender did not appear to be associated with antibiotic resistance. The study emphasizes the scarcity of therapy alternatives for non-enteric gram-negative bacilli infections, underlining the importance of worldwide surveillance and the development of innovative antimicrobial medicines.

Recommendations

1. Improved Surveillance: Antimicrobial resistance patterns must be continuously monitored in order to identify developing trends and potential treatment problems. This will help clinicians and pharmaceutical businesses make educated antimicrobial treatment decisions.

2. Creation of New Agent of Antimicrobial medicines: Given the limited treatment options for non-enteric gram-negative bacilli infections, the discovery of new antimicrobial medicines is crucial. Additional research and development efforts should be directed on developing novel classes of antimicrobial drugs capable of successfully combating these illnesses.

3. Strict infection control measures, such as adequate hand hygiene, isolation protocols, and environmental cleaning, can help limit the development of resistant bacteria in hospital settings.

4. Team-work and Information Sharing: Sharing information, best practices, and surveillance data requires collaboration among healthcare institutions, researchers, and public health authorities. This will make it easier to address antimicrobial resistance in a coordinated manner.

CHAPTER 7

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