NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

"Multidrug Resistance in Fluoroquinolone-Resistant *Enterobacterales* Isolates"

M.Sc. Thesis

Hafsa IQBAL

Nicosia

January, 2024

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Supervisor

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Approval

We certify that we have read the thesis submitted by HAFSA IQBAL titled "**Multidrug Resistance in Fluoroquinolone-Resistant *Enterobacterales* Isolates**" and that in our combined opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

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Declaration

I hereby declare that all the information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

HAFSA IQBAL

31/01/2024

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Özet

Flurokinolon Dirençli *Enterobacterales* İzolatlarında Çoklu İlaç Direnci

Hafsa Iqbal

Yüksek Lisans, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı

Danışman: Doç. Dr. Emrah Ruh

31 Ocak 2024, 57 sayfa

Amaç: Bu çalışma, siprofloksasin dirençli *Enterobacterales* izolatlarında çoklu ilaç direnci (ÇİD) oranlarının incelenmesi ve olası risk faktörlerinin değerlendirilmesi amacıyla yapılmıştır.

Gereç ve Yöntem: Çalışmada ÇİD oranlarının belirlenmesi için toplam 73 izolat çalışıldı. Bu bakteriler, Yakın Doğu Üniversitesi Hastanesi'ne kabul edilen hastaların (n=46) ve toplumdaki bireylerin (n=27) dışkı örneklerinden izole edilmiştir. Duyarlılık testleri siprofloksasin (5µg), ampisilin (10µg) amoksisilin-klavulanat (30µg), sefotaksim (30µg), seftazidim (30µg), sefepim (30µg), piperasilin-tazobaktam (110µg), gentamisin (10µg),trimetoprim-sulfametoksazol (25µg) ve tigesiklin (15µg) antibiyotik diskleri kullanılarak yapılmıştır.

Bulgular: Toplam 73 örnekten 48 (65.7%)'inde çoklu ilaç direnci saptanmıştır. ÇİD oranı hasta grubunda 34 (73,9%) ve kontrol grubunda 14 (51,9%) olup, hasta ve control grupları arasında istatistiksel bir fark saptanmamıştır. Bu çalışmada, yaş (p=0,016) ve eğitim (p=0,049) ÇİD ile ilişkili olan faktörler olarak belirlenmiştir.

Sonuç: Bu çalışma, siprofloksasine dirençli izolatlar arasında ÇİD oranının yüksek seviyede olduğunu göstermektedir. Bu nedenle Kuzey Kıbrıs'ta antibiyotik direnci dikkatle incelenmelidir.

Anahtar Kelimeler: *Enterobacterales*, siprofloksasin direnci, çoklu ilaç direnci.

Abstract

Multidrug Resistance in Fluoroquinolone-Resistant *Enterobacterales* Isolates

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Aim: This study was conducted to search for multidrug resistance (MDR) rates in ciprofloxacin-resistant *Enterobacterales* isolates and to evaluate potential risk factors.

Materials and Methods: A total of 73 isolates were studied in order to find the rates of MDR in the study. The fecal samples of patients (n=46) hospitalized in the Near East University Hospital, and also community members (n=27) were included in the study that are our control samples. Antibiotic discs containing piperacillin-tazobactam (110µg), gentamicin (10µg), trimethoprim-sulfamethoxazole (25µg), tigecycline (15µg), ampicillin (10µg), amoxicillin-clavulanate (30µg), cefotaxime (30µg), ceftazidime (30µg), and cefepime (30µg) were used in the susceptibility tests.

Results: Of the 73 samples, 48 (65.7%) were MDR positive. The rate of MDR was 34 (73.9%) in the patient group and 14 (51.9%) in the control group. In this study, age (p=0.016) and education (p=0.049) were identified as the only significant factors associated with MDR.

Conclusion: This study demonstrates that a high percentage of ciprofloxacin-resistant isolates were MDR positive. Therefore, antibiotic resistance should be carefully monitored in Northern Cyprus.

***Keywords*:** *Enterobacterales*, ciprofloxacin resistance, multidrug resistance.

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List of Abbreviations

AM: Ampicillin

AMC: Amoxicillin-clavulanic acid

ASHP: American Society of Health-System Pharmacists

CAZ: Ceftazidime

CDC: Centre for Disease Control and Prevention

CDT: Combined Disc Test

CIP: Ciprofloxacin

CLSI: Clinical and Laboratory Standards Institute

CN: Gentamicin

CTX: Cefotaxime

DAEC: Diffusely-Adherent *E. coli*

DNA: Deoxyribonucleic Acid

EAEC: Entero-Aggregative *E. coli*

EHEC: Entero-Hemorrhagic *E. coli*

EIEC: Entero-Invasive *E. coli*

EMB: Eosin Methylene Blue

EPEC: Enteropathogenic *E. coli*

ESBL: Extended Spectrum Beta-Lactamase

ETEC: Entero-Toxigenic *E. coli*

FEP: Cefepime

GIS: Gastrointestinal Syndrome

ICU: Intensive Care Unit

MBL: Metallo Beta-Lactamase

MDR: Multidrug Resistance

MIC: Minimum Inhibitory Concentration

MNEC: Meningitis-associated *E. coli*

MOA: Mechanism of Action

mRNA: Messenger Ribonucleic Acid

MRP: Multidrug Resistance Protein

PABA: Para aminobenzoic Acid

PAE: Post Antibiotic Effect

PBP: Penicillin Binding Protein

rRNA: Ribosomal Ribonucleic Acid

SBL: Serine Beta-Lactamase

SXT: Trimethoprim-sulfamethoxazole

TGC: Tigecycline

TPT: Piperacillin-tazobactam

tRNA: Transfer Ribonucleic Acid

UTI: Urinary Tract Infection

WHO: World Health Organization

XDR: Extensively Drug Resistance

CHAPTER I

Introduction

A vast order of diverse germs, or bacteria, known as *Enterobacterales* is commonly responsible for infections in medical environments. *Escherichia coli (E. coli),* and *Klebsiella pneumoniae* are two examples of *Enterobacterales.* As bactericidal antimicrobial agents, fluoroquinolones prevent bacterial cells from replicating their DNA. Resistance to fluoroquinolones is often caused by mutations in the DNA gyrase and topoisomerase genes; in Gram-negative bacteria, GyrA mutations are the most common mechanism.

Antibiotics are medications that are used to treat and prevent bacterial infections. Bacteria become resistant to antibiotics when they become accustomed to their use. Resistance to antibiotics is acquired by antibiotic-resistant microorganisms, not by humans or animals. Antibiotic resistance is a condition when germs become resistant to drugs intended to destroy them.

Multidrug-resistant organisms are bacteria that have become resistant to one or more treatments, making them unable to be eliminated or treated by the medications. Antibiotics are essential pharmaceuticals. MDR sometimes known as "superbugs," are resistant to several antibiotics due to one or more resistance mechanisms. In the instance of cross-resistance, resistance to numerous antimicrobial drugs is conferred by a single resistance mechanism. Multidrug-resistant organisms develop when antibiotics are administered for longer than necessary or when they are not needed. It's possible that few germs can resist antibiotic therapy at first. The more often antibiotics are used, the higher the risk of developing resistant germs.

Bacteria are typically common multidrug-resistant organisms: Enterococci resistant to vancomycin (VRE) resistant to methicillin *Staphylococcus aureus*.

With the exception of *S. pneumoniae* and *H. influenzae*, which cause respiratory illnesses, that are acquired in the community, studies on global surveillance indicate that resistance rates to fluoroquinolones have risen recently in practically all bacterial species. Nevertheless, first-level alterations resulting in low-level fluoroquinolone resistance were

seen in 10–30% of these isolates. In *Enterobacterales* that cause intra-abdominal infections and urinary tract infections, either acquired in the community or as a result of medical care, fluoroquinolone resistance has grown and has reached 50% in certain regions of the world, mostly in Asia. One to two thirds of *Enterobacterales* that developed extended-spectrum β-lactamases had fluoroquinolone resistance.

Aim of the study

This investigation aimed to look into the multidrug resistance of fluoroquinolone-resistant *Enterobacterales* by employing multiple antibiotic classes. This study assessed socioeconomic factors in addition to potential risk factors linked to MDR, such as epidemiology.

CHAPTER II

Literature Review

2.1. General Characteristics

An order of gram-negative, rod-shaped, facultatively anaerobic, non-spore-forming bacteria is called *Enterobacterales*, that belongs to the class Gammaproteobacteria. Enterobacter is the genus for this order. Members of the *Enterobacterales* family are referred to as *Enterobacteria* because they are a natural component of the intestinal flora and because they inhabit the intestines of both humans and animals. Diarrhea is also a common side effect of certain species, as are urinary tract infections. They have the ability to penetrate the bloodstream, which can be extremely dangerous for survival (Ronald, 2003).

The broad order *Enterobacterales* contains a wide variety of bacteria that frequently cause diseases linked to healthcare. *Escherichia coli (E. coli) and Klebsiella pneumoniae are two examples of Enterobacterales bacteria.* The bacterium *Klebsiella* is found in both people and the natural world. It typically exists in the human mouth and intestine as part of the natural flora (Quereshi, 2018). Humans are *K. pneumoniae's* primary reservoir, and infections with the bacteria are typically obtained in hospitals and affect immune compromised individuals. *Klebsiella* species are rarely transferred through contact with the skin; the population's transmission rates of *K. pneumoniae* are 5–38 percent in stool samples and 1–6% in the nasopharynx.

In 2020, the name "*Enterobacterales*" was chosen to represent a new taxonomic scientific order. The "*Enterobacteriaceae*" are now a family inside the order "*Enterobacterales,*" along with *Erwinaceae*, *Pectobacteriaceae*, *Yersiniaceae*, *Hafniaceae, Morganellaceae,* and *Budvicaceae.*

Like other bacteria, *Enterobacterales* can become resistant to medicines, especially carbapenem medications, which are used as a last resort when treating organisms that are resistant to antibiotics. *Enterobacterales* are referred to as carbapenem-resistant *Enterobacterales* (CRE) when they become resistant to the carbapenem class of antibiotics. Since CRE do not react to common antibiotics, they are challenging to treat. Sometimes CRE develop resistance to every antibiotic on the market. The public's health is at risk from CRE.

2.1.1. The Family of *E. coli* Bacteria

E. coli is a well-known member of the bacterial family *Enterobacterales* and a member of the genus Escherichia. The term "etheric bacteria," or bacteria that may thrive in the gastrointestinal tract—which is made up of the digestive system's structures is often used to describe *Enterobacterales* (oral cavity, oesophagus, stomach, intestines, rectum and anus). Both aerobic and facultative anaerobic growths are possible in *E. coli*. The *Enterobacterales* also includes *Salmonella*, *Shigella*, and *Klebsiella*. *Salmonella* and *Shigella* are linked to food borne illnesses. UTIs can be brought on by *Klebsiella. Shigella* and *Salmonella* are uncommon commensals, while *Klebsiella* and *E. coli* are common.

Virulent strains of *E. coli* predominate. Nevertheless, some can result in intestinal illnesses such as gastroenteritis, meningitis in infants, and urinary tract infections (UTIs). Probiotic strains of *E. coli* exist as well; one such strain is the Nissle 1917 strain, which is obtained from feces and is used to prevent disease. Avirulent and virulent strains are distinguished by distinct genetic determinants; nonetheless, certain serotypes are more pathogenic than others (Robbens et al., 2014).

2.1.1.1. *Enteric E. coli*

Enteric *Escherichia coli*, or *E. coli*, is a common human natural flora as well as a serious disease that is responsible for a considerable amount of sickness and mortality throughout the globe. Enteric *E. coli* are traditionally divided into six pathotypes, while other pathotypes are frequently suggested. Enteropathogenic *E. coli* (EPEC), which can be transmitted fatally, is the most frequent reason why children in developing nations get diarrhea, according to Harry et al. (2004). Second, hemorrhagic colitis, also referred to as bloody diarrhea, is brought on by Enterohemorrhagic *E. coli* (EHEC), and hemolytic uremic syndrome (HUS), also known as non-bloody diarrhea. The third pathogen and the main cause of diarrhea in travelers is entero-toxic *E. coli*, or ETEC. In impoverished countries, it also makes youngsters sick with diarrhea.

In addition, Entero-Aggregative *E. coli* (EAEC) is increasingly recognized as the cause of persistent diarrhea in both industrialized and developing nations, having been linked to multiple international outbreaks. Although entero-invasive *E. coli* (EIEC) typically causes watery diarrhea, it can also induce dysentery and invasive inflammatory colitis (Harry et al., 2004). And lastly, children older than 12 months old who have diarrhea are affected by diffuse-adherent *E. coli* (DAEC) (CDC, 2020).

2.1.1.2. Meningitis/sepsis-associated *E. coli*

The primary cause of gram-negative meningitis in neonates is this particular serotype of *E. coli*. Urine and feces can spread various *E. coli* strains that cause urinary tract infections or intestinal infections. Disorders of the peripheral and central nervous systems do not appear to offer any distinct advantages for the selection and dissemination of highly pathogenic strains of MNEC (Morris, T. E., &Kahlmeter, G.,2023). The *E. coli* that causes meningitis spreads through the circulation (CDC, 2012).

2.1.1.3. UTI-associated *E. coli*

Urinary tract infections can be brought on by bacteria that enter the urethra from external sources, such as the nearby anus. The bacteria that causes UTIs most frequently is *Escherichia coli*, or *E. coli*. Although other bacteria can cause a UTI, *E. coli* is the primary culprit in about 90% of cases. Although *Escherichia coli* usually survive in the human digestive system without harm, if it gets into the urinary tract, it can lead to severe infections. The infection most likely starts when commensal flora and a uropathogenic strain enter the gut (Rossen, J. W. A. 2021).

2.2. Antibiotics

Antibiotics are potent drugs that can save lives. They are used to treat bacterial infections such as urinary tract infections and strep throat. However, they can have negative side effects including diarrhea and are not appropriate for many illnesses. Antibiotics can help you with as little danger as possible if you know when you need them and how to take them correctly.

Since they were first developed at a time when the only treatments for serious bacterial infections were surgical drains or home remedies, antibiotics were initially hailed as "miracle drugs". Trimethoprim and sulfonamides, penicillin, cephalosporins, chloramphenicol, tetracyclines, colimycins, macrolides, lincosamides, streptogramins, rifamycin, glycopeptides, aminoglycosides, fluoroquinolones, oxazolidinones, glycylglycines, lipoglycopeptides, and variations on these medications have been developed in the fifty or sixty years since their introduction.

Antibiotics are medications used to treat bacterial infections. They don't work well against viral illnesses like the flu or the common cold. Microscopic microorganisms called bacteria are present in your body, on your skin, and everywhere else. The majority of germs are harmless to humans. Certain types (such as those on your skin or in your stomach) support your overall health. However, some bacteria can cause illness, with symptoms varying from a minor infection to a serious infection requiring hospitalization.

The population has a longer life expectancy and infectious diseases are under control since the discovery of antibiotics. Antibiotics are crucial for this reason. They frequently save lives and can make you feel better.

However, excessive use of antibiotics is not always a bad thing. When antibiotics are used for conditions that don't require them, such as virus infections or minor bacterial infections that would go away on their own, needless side effects might result, which further exacerbates the worldwide issue of antibiotic resistance.

2.2.1. How do antibiotics work?

Antibiotics work by either completely eradicating or preventing bacterial development. For example, antibiotics have the power to destroy bacteria by removing essential elements like DNA or cell walls that are essential to their life. Antibiotics can stop bacteria from growing by preventing them from producing specific proteins that are required for them to proliferate. Most antibiotics that are used today are either natural compounds themselves or are derived from them. They may, nevertheless, also include artificial (lab-produced) materials. Using extracts from bacteria, animals, fungi, and

plants, scientists are continuously investigating and creating novel antibiotics to combat illnesses.

In 1941, Selman Waksman used the term "antibiotic" to refer to a tiny chemical that a bacteria created to inhibit the growth of other organisms. The discovery of penicillin, which is produced by a fungus, and the revelation that soil bacteria can produce tetracycline, streptomycin, and chloramphenicol in 1945 marked the beginning of the antibiotic era. These early antibiotics and the majority of their offspring are now essentially worthless due to the development of antibiotic resistance in serious human diseases, and the age of antibiotics is set to end if a replacement is not discovered.

Screening procedures are frequently used to find useful antibiotics. This kind of screening involves the cultivation of isolates from a broad range of bacteria, which are then screened for the production of diffusible chemicals that hinder the growth of the organisms being tested. Antibiotics discovered by such screenings must be disregarded because the vast majority of them are already well-known. The most promising compounds can be filtered and possibly modified once the other antibiotics have been tested for therapeutic action and selective toxicity.

Semi-synthetic synthesis is a prevalent method used in current antibiotic manufacture. Antibiotics are produced semi-synthetically by combining natural fermentation with laboratory processes to increase the antibiotic. The drug's own effectiveness, the quantity of antibiotic generated, and its potency can all be maximized. What you are trying to manufacture will depend on the medicine that needs to be made and the antibiotic's final application. One novel molecule can be created by chemically altering the active component of a naturally occurring antibiotic. Chemical modification and improvement of a natural product yields novel antibiotics with increased therapeutic efficacy.

2.2.2. The mechanism of action of antibiotics

Antibiotics cause disruptions to the fundamental structures or functioning of the bacterial cell. This inhibits the growth of bacterium or eliminates it. An antibiotic is said to either bacteriostatic or bactericidal based on these properties.

The precise biochemical interaction via which a medicinal ingredient exerts its pharmacological effect is referred to as the mechanism of action (MOA) in pharmacology. A mechanism of action often describes the precise molecular targets, such as an enzyme or receptor—that the medicine interacts to. Because of the unique activity that occurs at receptor sites and the chemical makeup of the drug, medicines have a particular affinity for certain receptor sites.

Drugs that do not attach to receptors nevertheless have therapeutic effects because they interact with the body's chemical or physical makeup. Antacids and laxatives are common examples of medications that function in this manner. The main mechanism of action of antimicrobial medicines used to treat bacterial infections is often used to categorize them. There are six main ways to go about things:

Impairment of cell wall synthesis

1. Inhibition of protein synthesis
2. Impairment of nucleic acid production
3. Blocking metabolic pathway
4. Inhibition of membrane function
5. Blocking ATP synthase

Gram-positive bacteria have a thick or hard coating of polysaccharides called the cell wall that surrounds their cytoplasmic membrane. An outer membrane is present in gram-negative bacteria, which is a second lipid membrane, covering a thin layer of peptidoglycan. The periplasmic membrane is the space between the cytoplasmic and outside membranes. Gram-negative bacteria's outer membrane acts as an additional barrier to keep a range of contaminants out. Contrarily, medications can flow through the porins in this membrane.

2.2.2.1. Impairment of cell wall synthesis

The polymer of peptides and glycan called peptidoglycan is exist in the bacterial cell walls. Two types of antibacterial drugs function by inhibiting or delaying the target bacteria's cell wall union. Since creature cells lack cell walls, anti-toxins often target the growth of bacterial cell walls, of which peptidoglycan plays a major role. The stability of

the cell wall beneath depends on the peptidoglycan layer, which is the most distant and crucial part of the wall.

The class of antimicrobial drugs known as β-Lactam anti-infection agents (beta-lactam anti-microbials) is at the top and includes all anti-toxin specialists whose subatomic designs have a β-lactam core. This includes monobactams, carbapenems, cephalosporins (cephems), and subordinates of penicillin called penams. β-Lactam anti-microbials function by inhibiting the bacterial cell wall's peptidoglycan layer from combining, making them bactericidal. PBPs, or penicillin-binding proteins, help with the last stage of peptidoglycan synthesis. PBPs vary in their preference for limiting the use of penicillin or other β-lactam antibiotics. β-lactamase, a substance that breaks down the β-lactam ring, is often combined by microbes to promote resistance against β-lactam anti-toxins. β-lactamase inhibitors, such as clavulanic corrosive, are frequently administered with β-lactam anti-microbials to overcome this barrier.

Based on glycosylated cyclic or polycyclic non ribosomal peptides, glycopeptide anti-toxins are an inferior class of antibacterial drugs that block cell wall union. Vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, and decaplanin are significant glycopeptide antibiotics. These medications block the formation of peptidoglycan, which stops vulnerable microorganisms from developing cell walls. They attach themselves to the cell wall's amino acids, stopping more units from sticking to the peptidoglycan.

2.2.2.2. Inhibition of Protein Synthesis

The multitasking process of protein production involves structural interaction; substances that hinder the processes involved in protein formation are known as inhibitors of protein synthesis. The ribosomal level is where these inhibitors operate, interfering with bacterial mRNA translation into protein at various stages. They are very specific to 70S ribosomes in prokaryotic cells since ribosomes are not the same in size, sequence, structure, or protein/RNA ratios in eukaryotic cells. Prokaryotes use translation to read nucleotide sequences by assembling a large (50S) and a small (30S) component to build a ribosome that binds to the mRNA. During the initiation, elongation, and

termination phases, tRNA attaches to the A, P, and E sites to translate the polypeptide sequence. Antibiotics can be changed if resistance arises by focusing on distinct phases of mRNA translation. The following protein synthesis inhibitors are listed:

- Aminoglycosides
- Tetracyclines
- Glyccycline
- Chloramphenicols
- Linezolids
- Kacrolides
- Ketolides
- Streptogramins

2.2.2.3. Metabolic Pathway Inhibitors

A class of antibiotics called "bacterial metabolic pathway inhibitors" targets the biosynthesis of nucleic acids and amino acids. Tetrahydro folic acid (TH4) is an important coenzyme required for the production of nucleic acids and certain amino acids in all living species. The precursor that bacteria use to produce folic acid is called para-aminobenzoic acid (PABA). Bacterial metabolism inhibitors change the metabolism of bacteria by blocking the synthesis of TH4. Antibiotics that prevent the metabolism of folate Sulfonamides, and their derivatives, work by preventing bacteria from synthesizing folic acid. Since all bacterial species require folic acid production, sulfonamides are broad-spectrum bacteriostatic antibiotics. Since human cells are unable to manufacture folic acid, they are unaffected. Sulphonamide antibiotics have two main structural features;

1. All sulfonamide antibiotics include a free aminobenzene ring (N4) at the para position of the sulfonyl group.
2. A nitrogen ring with five or six members is frequently joined to sulfonylamino (N1) in sulfonamide antibiotics.

Sulfonamides and para-aminobenzoic acid (PABA), a crucial antecedent and constituent of folic acid, are structurally identical. The making of folic acid (DHF) involves two stages. Dihydropteroic acid is first produced when PABA combines with the pteridine derivative. The reaction with glutamic acid comes next. The mechanism by which sulphonamide antibiotics function is to impede the activity of dihydropteroate synthase (DHPS), an enzyme that catalyzes the conversion of PABA and dihydropterin pyrophosphate into dihydropteroate. Due to their structural resemblance, PABA and sulfonamides engage in competition for binding to the enzyme's active site.

2.2.2.4. ATP Synthesis Inhibitor

ATP synthase is the primary source of cellular energy synthesis in all plants, animals, and most microorganisms. Numerous diseases have been linked to ATP synthase complex failure, and this enzyme can be targeted therapeutically to treat a wide range of illnesses.

An enzyme that can convert adenosine diphosphate and inorganic phosphate into adenosine triphosphate is known by the broad term ATP synthase. It's among the most ancient and well-preserved enzymes. Membrane-bound transporters known as ATP synthase molecules link the creation or hydrolysis of an ATP nucleotide with the passage of ions across the membrane. It has a molecular mass of roughly 530 kDa overall. In the F1, ATP is synthesized and hydrolyzed at three different catalytic sites.

2.2.2.5. Inhibition of nucleic acid inhibitors

Antibiotics can prevent the synthesis of nucleic acids. These antibiotics are known as nucleic acid inhibitors. Quinolones are an important class of antibiotics that obstruct topoisomerases, particularly topoisomerase II (DNA gyrase), an enzyme involved in DNA replication, hence interfering with DNA synthesis. In the supercoiled turns of closed circular DNA, DNA gyrase relaxes supercoiled DNA molecules, bridging phosphodiester linkages and causing transient fractures. This makes it possible for DNA or RNA polymerase to copy the DNA sequence. Levofloxacin, norfloxacin, and

ciprofloxacin are a few examples of second-generation quinolones, often known as fluoroquinolones, that are effective against gram-positive and gram-negative bacteria. Prokaryotic and eukaryotic cells have topoisomerase; however, quinolones specifically inhibit the bacterial topoisomerase II. Anticancer medications like irinotecan and etoposide, which are efficient inhibitors against mammalian topoisomerases, are used to kill cancer cells.

2.2.3. Fluoroquinolones

A class of broad-spectrum systemic antibacterial drugs known as fluoroquinolones are being used extensively to treat urinary and respiratory tract infections.

Gram-negative and positive aerobic bacteria of various kinds are effectively combated by fluoroquinolone. It is believed that they work by inhibiting type II DNA topoisomerases, commonly referred to as gyrases, which are essential for bacterial DNA transcription and replication. Their safety record is excellent and their ability to inhibit the enzymes of their human hosts is minimal.

Fluoroquinolone prescriptions are used to treat a variety of bacterial infections, such as typhoid fever, anthrax, bacterial gastroenteritis, urethritis, gynecological infections, urinary tract infections, sepsis, and intra-abdominal infections. They can also be used to treat infections of the skin, soft tissues, and joints, as well as a few other infectious illnesses. Fluoroquinolones such ofloxacin, ciprofloxacin, gemifloxacin, levofloxacin, moxifloxacin, and norfloxacin are readily available in the United States. These drugs are very well absorbed, well tolerated, and hardly have negative side effects when taken orally. The removal of several quinolones and fluoroquinolones, including temafloxacin (1992), gatifloxacin (2006), and trovafloxacin (1999), occurred in response to unprompted complaints of serious side effects, including hepatotoxicity. One in 100,000 people exposed to fluoroquinolones at this time are thought to have an idiosyncratic liver damage.

2.2.3.1. *Mechanism of Fluoroquinolone Resistance*

Resistance to fluoroquinolones arises from two primary mechanisms:

- modifications to target enzymes of drug and
- change in the accessibility of drug target enzyme.

Topoisomerase IV mutations and DNA gyrase mutations are two categories for alterations in medication target enzymes. The proportional influence of these changes on the emergence of fluoroquinolone resistance varies depending on the kind of bacteria (Gram-positive or Gram-negative).

2.2.3.2. Fluoroquinolone Generations

From their antibacterial spectrum, quinolones can be categorized into generations. There are no set standards for classifying medications into different generations, despite the fact that they frequently have a more limited range of action than more recent drugs. For all intents and purposes, only the first-generation group of non-fluorinated medicines (quinolones) meets the universal criteria. Consequently, depending on the author's approach, the literature has significant variances. First generation is used rarely. Medications including ciprofloxacin, levofloxacin, and moxifloxacin are frequently prescribed. Flumequine has further uses in veterinary medicine in addition to these.

There are several **first-generation** drugs that are structurally related, but formally 4-quinolone is not included: • Cinoxacin • Nalidixic acid • Piromidine acid • Pipemidic acid

Second Generation. There are instances when type 1 and type 2 fluoroquinolones are distinguished. nadifloxacin, norfloxacin, ofloxacin, pefloxacin, rufloxacin, ciprofloxacin, fleroxacin, lomefloxacin, and rufenloxacin. Enoxacin is a second-generation medication that shares structural similarities with 4-quinolones but is not recognized as one.
Third Generation. The third generation, in contrast to the first and second, is active against streptococcus. Third-generation fluoroquinolones include levofloxacin, temafloxacin, grepafloxacin, pazufloxacin, sparfloxacin, and balofloxacin..Tosufloxacin (Ozex, Tosacin) is a third-generation medication that shares structural similarities with 4-

quinolones but is not recognized as one.

Fourth Generation. Fourth-generation fluoroquinolones have an impact on topoisomerase IV and DNA gyrase. Resistance builds more slowly as a result of this dual action. Clinafloxacin, gatifloxacin, moxifloxacin, sitafloxacin, prulifloxacin, besifloxacin, and delafloxacin are among the fluoroquinolones in the fourth generation. Some third-generation drugs that are structurally related but are not 4-quinolone drugsare

• Trovafloxacin and Gemifloxacin (discontinued in clinical use)

*Table 2.1 Generations of Fluoroquinolones and their Antibiotics**

Generations	**Quinolone Antibiotics**
First Generation	Nalidixic acid, cinoxacin, flumequine, oxolinic acid, piromidic acid, pipemidic acid, rosoxacin
Second Generation	Lomefloxacin, norfloxacin, ciprofloxacin, ofloxacin, fleroxacin, pefloxacin, rufloxacin
Third Generation	Levofloxacin, sparfloxacin, temafloxacin, grepafloxacin, balofloxacin, pazufloxacin, tosufloxacin
Fourth Generation	Moxifloxacin, gemifloxacin, trovafloxacin, gatifloxacin, clinafloxacin, garenoxacin, sitafloxacin, prulifloxacin, finafloxacin

Some fluoroquinolones and their mechanisms of action are discussed separately below;

Ciprofloxacin. A common second-generation fluoroquinolone antibiotic used to treat mild to moderate respiratory and urinary tract infections brought on by susceptible bacteria is ciprofloxacin. Ciprofloxacin has been linked to a few, credible reports of liver damage that could be fatal or extremely serious. Ciprofloxacin is an antibiotic that belongs to the fluoroquinolone class and has bactericidal properties. It stops DNA replication by inhibiting bacterial DNA topoisomerase and DNA gyrase. Ciprofloxacin, which includes *Enterobacterales* like *Escherichia coli, Salmonella spp., Shigella spp.,* and *Neisseria,* is the most effective fluoroquinolone against gram-negative bacilli. Certain gram-positive bacteria can also be effectively combatted by ciprofloxacin. Ciprofloxacin has the strongest anti-Pseudomonas aeruginosa activity among the

quinolones. *P. aeruginosa* susceptibility has been steadily shown to decrease in hospitals and nursing homes where risk factors are known, namely in Europe, North America, and South America. One of the few oral medicines that can be used to treat *P. aeruginosa* infections is ciprofloxacin. Ciprofloxacin's actions vary with concentration; at low levels, it solely inhibits topoisomerase (II). When given at high quantities, it can also induce topoisomerase (IV) (Laponogov et al., 2009).

The infection that this medication is intended to treat also dictates its primary target. Drugs target topoisomerase (IV) in cases where gram-positive bacteria are the source of the infection; topoisomerase (II) is the target in cases where gram-negative bacteria are the cause. The infection that this medication is intended to treat also dictates its primary target. Drugs target topoisomerase (IV) in cases where gram-positive bacteria are the source of the infection; topoisomerase (II) is the target in cases where gram-negative bacteria are the cause.

Levofloxacin. Levofloxacin is an antibiotic belonging to the fluoroquinolone class that has the ability to directly prevent the synthesis of bacterial DNA. By blocking DNA gyrase in organisms that are susceptible, levofloxacin encourages breaks in DNA strands by preventing supercoiled DNA from relaxing. Levofloxacin is the fluoroquinolone that is most active against Streptococcus pneumoniae and other penicillin-sensitive and resistant Gram-positive bacteria. In comparison to ciprofloxacin, it is less active against Pseudomonas aeruginosa and other Gram-negative bacilli.

Levofloxacin is a successful treatment for other common respiratory pathogens, including Haemophilus influenzae, Moraxella catarrhalis, Legionella spp., Mycoplasma spp., and Chlamydia pneumoniae. As a second-line anti-tuberculosis treatment, levofloxacin is chosen above other fluoroquinolones due to its better in vitro action against tuberculosis Mycobacteria. Globally, there is increasing worry over fluoroquinolone resistance, which can result from plasmid-mediated or chromosomally encoded processes.

Ofloxacin. The inhibition of bacterial DNA gyrase is the principal method of action of the fluoroquinolone ofloxacin. It has a wide spectrum of action in vitro against aerobic gram-positive and gram-negative bacteria; however, its efficacy against anaerobic

bacteria is limited. Unlike the majority of other broad-spectrum antibiotics, ofloxacin is able to be given intravenously or orally. It is quite efficient in penetrating bodily fluids and tissues. Ofloxacin has been shown in clinical trials to be useful in treating a range of infections, both oral and intravenous, and is frequently just as successful as conventional therapies in these cases.

Compared to other fluoroquinolones currently on the market, ofloxacin is less likely to result in medication interactions that are clinically relevant and is well tolerated. For this reason, ofloxacin offers a useful oral treatment (with the possibility of intravenous administration if necessary) for a range of clinical infections, but it is especially beneficial in these infections. infections that are more severe or persistent when reliant on traditional medicine. Typically, a broad-spectrum injectable medicine is needed, which lowers expenses and makes outpatient therapy even easier.

Ampicillin. Ampicillin, commonly known as extended-spectrum penicillin, is an aminopenicillin that was created as a semisynthetic β-lactam antibiotic that works against both Gram-positive and Gram-negative bacteria. By attaching penicillin to an amino group or side chain, it becomes aminopenicillin. The drug's effectiveness against specific bacteria is markedly changed by the addition of side chains. At first, Proteus mirabilis, *E. coli*, Shigella, Salmonella, Hemophilus, and Neisseria species were all successfully combatted by these antibiotics. However, unless culture and sensitivity data showed no sensitivity, ampicillin is no longer the antibiotic of choice in the treatment of certain polymicrobial diseases, due to changes in susceptibility.

Ampicillin functions by binding to penicillin-binding proteins (PBPs), preventing the synthesis of peptidoglycans in cell walls, and deactivating inhibitors that bind to autolytic enzymes in order to obstruct the formation of cell walls.

Piperacillin/tazobactam. Extended-spectrum penicillin derivative; β-lactamase inhibitor (tazobactam). By attaching itself to the membranes of bacteria, piperacillin prevents the formation of cell walls. Bacterial beta-lactamase is neutralized by tazobactam. For susceptible bacteria, piperacillin has bactericidal properties. Tazobactam increases the range of action, inhibits bacterial growth, and shields piperacillin from enzymatic breakdown.

Cefepime. Cefepime functions similarly to other beta-lactam antibiotics. Cefepime binds covalently to the enzymes that catalyze the last metabolic stage of peptidoglycan production, thereby inhibiting the growth of bacterial cell walls. The binding leads to defects in the cell wall, which in turn cause autolysis and eventually kill the organism. Compared to third-generation cephalosporins, cefepime is more stable against beta-lactamases and has a higher coverage of Gram-negative bacteria through a number of methods.

Penicillin-binding enzymes' decreased affinity for cefepime is one of these ways. The presence of a side chain substitution in the chemical structure, which sets it apart from previous generations and increases its effectiveness against staphylococcal species, is another factor. Cefepime's gram-negative coverage is greater than that of the third generation because it is a zwitterion, which has the benefit of allowing gram-negative bacteria to enter the cell wall more quickly.

Gentamycin. One type of aminoglycoside antibiotic is gentamycin. Gentamicin is a great choice for treating a number of common infections since it demonstrates bactericidal action against aerobic gram-negative bacteria. Systemic, topical, and ocular forms of gentamicin are commonly administered parenterally due to their low absorption from the gastrointestinal tract. The aminoglycoside antibiotic gentamicin has bactericidal properties. Through an oxygen-dependent active transport mechanism, gentamicin penetrates gram-negative membranes. Anaerobic bacteria cannot be killed by aminoglycosides since they require oxygen to survive.

Like all aminoglycosides, gentamicin possesses deadly action that varies with concentration. Greater antibiotic killing is correlated with higher concentrations. Because of these factors, during systemic usage, doctors should regularly monitor peak and trough levels. Furthermore, studies have shown that aminoglycosides have a synergistic effect on gram-positive bacteria when taken in combination with other medications, however the exact mechanism is yet unknown.

Tigecycline. Tigecycline is a bacteriostatic glycylcycline antibiotic that is delivered parenterally. It is structurally similar to tetracycline, but it has a binding affinity that is five times stronger. Tigecycline binds reversibly to the helical region (H34) on the

30S subunit of the bacterial ribosome, where it inhibits peptide chain elongation, or bacterial protein translation. Like other tetracyclines, this one acts in a similar way. By preventing amino acid residues from being incorporated into peptide chain elongation, tigecycline binding inhibits peptide synthesis and bacterial growth. The incorporation of a glycyclamide moiety at position 9 of minocycline enabled the development of tigecycline, which was intended to circumvent important molecular processes of tetracycline resistance, including the acquisition of tetracycline-specific efflux pumps [tet(A)], as well as the defense of ribosomes [tet(M)].

Sulfamethoxazole. Sulfamethoxazole is a sulfonamide that, because of its structural resemblance to the natural substrate, para-aminobenzoic acid (PABA), inhibits the synthesis of dihydrofolic acid by bacteria. Unlike animals, which need external sources of folic acid, the majority of bacteria obtain the nutrient they need by synthesizing it from PABA. The enzyme responsible for the bacterial conversion of PABA to dihydrofolic acid is called dihydropteroate synthase, is competitively inhibited by sulfamethoxazole. The production of tetrahydrofolate is inhibited, which ultimately stops the creation of bacterial purines and DNA and has a bacteriostatic effect.

2.2.4. Antibiotic Screening Tests

To find the right antibiotic for a specific bacterial strain isolated from a clinical sample, disc diffusion antibiotic susceptibility testing is carried out.

2.2.4.1. Disk Diffusion Test

The technique most frequently employed in labs to assess a bacterial isolate's antibiotic susceptibility. This technique involves inoculating agar plates containing a culture of the bacteria to be tested with portions that have been impregnated with an antibiotic at a specified concentration. For 18 to 24 hours, the plate should be incubated at 37°C. The concentration of antibiotics usually stays high close to the antibiotic disc after diffusion, but it gets lower as one gets farther away. The zone of bacterial growth inhibition surrounding the disc is measured to ascertain an antibiotic's susceptibility.

Medium Selection. To determine if bacteria are susceptible to antibiotics, a medium that both supports the test and control strains of bacteria is selected. For instance, blood agar is used to test for Streptococcus and Enterococcus species, and Mueller-

Hinton agar is used to screen for staphylococci and Gram-negative bacteria. Chocolate agar is used for H. influenza and for sulfonamides and cotrimoxazole, use Wellcoat test medium.

To prepare the medium, pour it over a 100 mm Petri dish's flat horizontal surface, filling it up to a 4 mm depth. The pH of the medium is kept between 7.2 and 7.4. Tetracyclines, novobiocin, and fusidic acid exhibit increased activity at alkaline pH values, whereas macrolides, including aminoglycosides and erythromycin, exhibit decreased activity at acidic pH values. Plates can be kept for up to a week at 4 °C after preparation.

Preparation of Inoculum. On solid media, bacteria are first isolated and grown in pure culture. Incubate for four to six hours at 37°C after inoculating the appropriate culture media and contacting three or four morphologically identical colonies of the bacteria to be studied. By comparing the bacterial suspension's turbidity in the broth to that of a standard tube with 0.5 McFarland opacity, the density of the suspension is adjusted to 1.5×10 8 CFU/ml. Distribute the culture throughout the medium to inoculate it by using a sterile cotton swab. Extra broth can be eliminated by submerging a sterile cotton swab in the soup and rotating it on the tube's side above the liquid level.

Antibiotic Disc. Antibiotic susceptibility testing tests only for clinically relevant antibiotics. Antibiotic discs of 6 mm filter paper can be made in the laboratory from pure antimicrobial agents or purchased commercially. Apply the disk to the surface of the medium using sterile tweezers, a dispenser or a sharp needle to coat it with the test bacterial strain, place the plate in an aerobic environment at 37°C for 18 to 24 hours and then record the reading.

2.2.4.2. Types of disc diffusion tests

The following categories of disc diffusion tests exist:

1. Kirby–Bauer disc diffusion method
2. Stokes disc diffusion method.

1. Kirby–Bauer disc diffusion method

The Kirby-Bauer test, which is commonly used to determine a bacteria's sensitivity or resistance to multiple antibiotic drugs, uses Mueller-Hinton agar. Being a non-selective and non-differentiating medium, Mueller-Hinton agar can be used to cultivate a wide range of microorganisms. This is a "loose" agar that helps control the diffusion rate of antimicrobial agents more effectively than other types of media. Wipe the bacteria onto the agar and place the antibiotic disc on top. Antibiotics diffuse from the disk into the agar and decrease with distance from the disk. Antibiotic concentrations have the effect of killing or inhibiting bacteria, which leads to NO growth in the surrounding areas of the disc. We refer to this as the inhibitory zone. The size of the zone is searched on a standardized chart, giving results of "susceptible," "resistant," or "intermediate." The Kirby-Bauer test uses Mueller-Hinton medium, which has a high protein content.

2. Stokes Method

This method has numerous variables under built-in control. This technique involves dividing a Petri dish with Mueller-Hinton agar into three horizontal sections. Arrange the test strain in the center of the plate, and the control strain in the upper and lower thirds. Using the modified Stokes method, the test strain is injected into the upper and lower thirds of the plate, and the control strain is injected into the center of the plate. Examine the area of bacterial inhibition surrounding the disc after incubating the plate at 37°C. A 100 mm Petri dish can hold up to 6 antibiotic discs.

In the disc diffusion test, an antibiotic-carrying disc (shown as a white disc) is introduced to a plate of solid growth media containing bacteria. A region of clear medium surrounding the disc, formed by the bacteria expanding overnight, suggests that the antibiotic is preventing the bacterium from multiplying. The farther you are from the infection source, the less antibiotic concentration diffuses into the media. Consequently, the larger the clear space free of bacteria that grows around the antibiotic-containing disc, the more vulnerable a bacterium is to that particular antibiotic.

Interpretation of Disc Diffusion Tests. The interpretation of disc diffusion test results, including Kirby-Bauer and Stokes, is as follows:

- Sensitive (S): Infections should be treated with normal doses of antibiotics.
- Moderate (I): More dosages may be effective in treating infection.
- Resistance (R): May not respond to normal doses of antibiotics.

Alternative Methods

Many variations of the disc diffusion method have been developed, including the E-test and Oxford penicillin cup procedures used in hospital diagnostic laboratories, the well diffusion method, the cylinder diffusion method, and bio-autography techniques.

Oxford Penicillin Cup Method. The bacterial flora on the agar surface is inoculated with antibiotic-containing discs, and the plate is then incubated. From the edge of the disc to the edge of the free zone, the zone size is calculated. Interpretation becomes more challenging in populations with mixed susceptibility. These are represented as squared distances or linear dimensions as a function of the antibiotic concentration in the slice's natural logarithm. The intercept is the logarithm of the minimum inhibitory concentration (MIC), which is determined from the zero point of a linear regression fit across the data. Each antibiotic's diffusion coefficient in the agar determines the regression line's slope.

2.2.5. Multidrug Resistance

The antimicrobial resistance that a microbiological species displays to one or more antimicrobial drugs from three or more antimicrobial categories is known as multidrug resistance. Multidrug-resistant bacteria are those that do not respond to treatment with more than one antibiotic (MDRO). There are different types of multidrug-resistant (MDR), eXtensive drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria that have been used in the literature to describe the varied resistance tendencies identified in antibiotic-resistant bacteria linked with healthcare settings. Extensive antibiotic resistance, or XDR, refers to bacterial isolates that are only susceptible to one or two antibacterial groups which is characterized as not being susceptible to any antibiotic in any category other than two or fewer. Pan-drug resistance (PDR) is the inability to respond to any medication across all antimicrobial classes.

Vancomycin-resistant Enterococcus species (VRE), Methicillin-resistant Staphylococcus aureus (MRSA), Carbapenemase producing *Enterobacterales* and extended-spectrum beta-lactamase (ESBL) producing gram-negative bacteria are among the most prevalent MDROs.

Mechanisms of Multidrug Resistance

Bacteria can become resistant to various drugs by accumulating genes on resistance plasmids (R) or transposons that encode resistance to individual drugs, or by accumulating different kinds of multidrug efflux pumps. It occurs through action. Any drug present can be pumped out. The current situation poses the greatest concern due to the rise in MDR bacteria and the shortage of new antibiotics that can eradicate them.

The primary goal of research at this time is to develop novel treatments for such MDR. The WHO's publication of a list of all fatal MDRs resistant to all known treatments was the most significant move in this direction.

Additionally, WHO calls on all countries to develop new drugs and other treatments to successfully combat MDR. According to WHO, several new treatments for such MDR are urgently needed.

Vancomycin-resistant Enterococcus faecium (VRE), methicillin-resistant Staphylococcus aureus (MRSA), and β-lactamase-resistant Streptococcus pneumoniae are the three primary Gram-positive bacteria associated with multidrug resistance in these lethal infections. Numerous differences exist between the composition of the cell walls of Gram-positive and Gram-negative bacteria.

Gram negative bacteria do not have an LPS coating on their cell membranes, but Gram-positive bacteria do have a thick peptidoglycan layer protecting them from the damaging effects of the environment. The two fundamental kinds of gram-positive bacteria that are primarily recognized are cocci (like *Staphylococcus*) and bacilli (like Bacillus). Furthermore, lengthy anionic polymers, teichoic acids, membrane proteins that permit the entry and exit of different molecules, and capsular polysaccharides covalently connected to peptidoglycan are present in Gram positive bacteria.

Antibiotic resistance in Gram positive bacteria arises from two major pathways. Among them are:

• the β-lactamases enzymes release in the breakdown of antibiotics by enzymes.

• via lowering the sensitivity and affinity of the target site, such as PBP (penicillin binding protein), either through mutations in native PBP genes or through the acquisition of exogenous DNA.

There are different types of antibiotic resistance, the mechanisms of which are explained below.

Restriction of Antibiotic Entry. Antibiotics spread within cells through the appearance of mutations in genes that specifically encode outer membrane porin proteins, resulting in changes in the less permeable porin mutant OMPK36 in *Klebsiella pneumoniae*. Down regulation of major porin proteins or recruitment of the plasma membrane by selected alternative protein channels reduces membrane permeability to antibiotics in some bacteria, such as *Escherichia coli* and *Acinetobacter.*

Combinations of various Chromosome- and Plasmid-associated Efflux Pump Genes. Powerful efflux pumps remove numerous antimicrobial agents from cells. Their over expression allows resistance to previously effective antibiotics, like the MDR efflux pumps seen in Escherichia coli and Pseudomonas aeruginosa.

Antibiotic Target Mitigation and Protection. Antibiotics can have their binding affinity decreased by moving the target. It is well known that *K. pneumoniae* and *Staphylococcus aureus* are resistant to linezolid. The allele encoding the ribosomal 23s rRNA subunit is mutated to accomplish this. Erythromycin ribosomal methylases and its family of enzymes methylate the binding sites of medicines, including macrolides, lincosamines, and streptogramins, causing the development of drug resistance.

Using the enzyme chloramphenicol-florfenicol resistance methyl transferase, A2503 in 23s rRNA can have CH3 incorporated into it to confer resistance to a number of different drug groups, including penicillin, proiromutilin, lincosamide, and oxazolidone. The genetic basis of methicillin resistance in *Staphylococcus aureus* has

been identified as chromosomal mec A. PBP2a, a single binding protein with a decreased affinity for β-lactam antibiotics in general, is encoded by this chromosome.

Antibiotic Resistance by Hydrolytic Enzymes. Chromosome detection is the means by which resistance is attained, and plasmid-mediated encoded genetic enzymes are employed to identify antibiotics, such as β-lactamases, which comprise cephalosporinase, which breaks down cephalosporins, and penicillinase, which only breaks down penicillin. It inactivates phosphorus and aminopenicillin and is degraded by proliferation. Total β-lactam digestion is significantly aided by β-lactamases, whereas carbapenemases and carbapenems inactivate total β-lactamases.

Mitigating Antibiotic Resistance. Antibiotic detection of genetic enzymes is rendered ineffective by the addition of active functional groups. For instance, phosphorylation results in the nucleotidylation, acetylation, and acetylation of -OH and CO-NH groups by acetyl transferases, phosphor transferases, and nucleotidyl transferases, which causes the microaerophilic Gram-negative bacterium Campylobacter coli (*E. coli*) to be resistant to aminoglycolides.

2.2.6. Fluoroquinolone-resistant in the Salmonella genus

Salmonella, or gram-negative bacteria, are classified into two types: typhoidal and nontyphoidal, both of which are harmful to humans.

The use of FQ-ciprofloxacin with the third-generation cephalosporin ceftriaxone was prompted by reports of Salmonella MDR for ampicillin, chloramphenicol, and sulfamethoxazole; nevertheless, resistance to the medication quickly emerged. This has been mentioned as one of the primary causes for the World Health Organization's 2017 classification of his FQ-resistant Salmonella as a pathogen significant for the creation of novel antibiotic mutations in the quinolone resistance-determining areas of the chromosomal Gyr and Par genes have been shown to be the cause of quinolone resistance. This results in a lower affinity of quinolénes for binding to the enzyme topoisomerase.

Plasmid-mediated quinolone resistance (PMQR) is an additional technique that is worth mentioning. Thus, genes like Qnr offer physical protection, the Aac-60-lbcr gene lessens the action of FQ and oqxAB and qepA encode the quinolone efflux pump.

CHAPTER III

Materials and Methods

3.1. Participants and Study Design

Samples from earlier research projects conducted by different teams were employed in the microbiological examination. The first group (n = 46) consisted of patients who had been hospitalized to Near East University Hospital; the controls (n = 27), on the other hand, were individuals who had not been admitted during the six months before to the study. This study was ethical approved, and the approval for the study was achieved from Near East University Ethics Review Board (Project no: YDU/2019/65-717).

3.2. Samples, Data of Participants, and Isolated Bacteria

Bacterial were isolated from stool samples collected from participants for this investigation, one sample per person. During stool collection, a survey was carried out to look at a number of variables related to the spread of bacteria resistant to antibiotics. These variables include intensive care, hospitalization, GIS, history of antibiotic usage, diarrhea, UTIs, travel to other countries, and socioeconomic variables like age, gender, education, and marital status.

A number of hospital-related factors (such as antibiotic use, surgery, unit stays, and urinary catheters) exist. A phenotypic confirmation test employing the mixed disc approach was previously used to evaluate ESBL production. Isolates of *Enterobacterales* that were also shown to produce ESBLs were kept in stock medium from earlier research at -20°C.

3.3. Purification and Isolation of Bacterial Isolates

In order to purify ciprofloxacin-resistant isolates, Eosin-Methylene Blue (EMB) medium was inoculated for 24 hours at 37°C. Sterile 0.9% NaCl was used to create a

standard 0.5 McFarland suspension from bacterial colonies that were collected on culture media plates. Next, using a sterile cotton swab, each bacterial suspension was moved to Mueller-Hinton medium.

3.4. Multidrug Resistance Determination

In this research study, different classes of antibiotics were tested to determine the MDR of bacterial isolates. I have tested 8 different drugs (5 classes) of throughout my career so far. Antibiotics included amoxicillin-clavulanic acid (AMC: 30 μg), piperacillin-tazobactam (TPZ: 110 μg), cefepime (FEP: 30 μg), ciprofloxacin (CIP: 5 μg), trimethoprim-sulfamethoxazole (SXT: 25 μg), gentamicin (CN: 10 μg), ampicillin (AMP: 10 μg) and tigecycline (TGC: 15 μg) was evaluated (using the disk diffusion test method) against *Enterobacterales*.

After growing the bacteria on EMB agar the previous day, use a cotton swab to transfer the bacterial isolates to Mueller-Hinton agar, ensuring that the entire surface or agar is covered with bacterial isolates. After that, incubate the plate for 24 hours at 37 °C with the antibiotic disc on top of the agar. You can put three or four antibiotics on the plate, but make sure to a distance of 2 cm is required between the objects. The inhibition zones of ≤13 for AMC and AMP; ≤21 for CIP, ≤10 for SXT, ≤14 for CN, ≤18 for cefepime, ≤20 for TPZ and ≤14 for TGC were all observed as resistant. The antibiotic's inhibition zone diameter was then measured (in millimeters mm) and evaluated in compliance with the recommendations and from the Clinical and Laboratory Standards Institute (CLSI, 2023) except tigecycline that was evaluated according to the Food and Drug Administration (FDA) guidelines.

Table 3.1. Antibiotics' inhibition zone diameter by CLSI, 2023 (except tigecycline)

Antibiotics		Susceptibility (S)	Intermediate (I)	Resistance (R)
Ciprofloxacin	5 µg	≥26	21-30^	≤21
Ampicillin	10 µg	≥17	14-16^	≤13
Trimethoprim-sulfamethoxazole	25 µg	≥16	11-15	≤10
Gentamicin	10 µg	≥18	15-17	≤14
Cefepime	30 µg	≥25	19-24	≤18
Piperacillin-tazobactam	110 µg	≥ 25	21-24	≤20
Amoxicillin-clavulanate	30 µg	≥18	14-17^	≤13
Tigecycline*	15 µg	≥19	15-18	≤14

***** Tigecycline was evaluated according to the FDA guidelines.

3.5. Statistical Analysis

For every variable in the survey, descriptive statistics were acquired. The frequency and percentage of the categorical data were given, and for the continuous variables, the arithmetic mean, standard deviation, median, minimum, and maximum were calculated. The Pearson Chi-square or Fisher's exact test was performed, depending on sample size, to look at the relationships between categorical data. Every statistical calculation was performed using the Macintosh version 2.3.21.0 of the Jamovi statistics program. A 0.05 level of significance was selected.

CHAPTER IV

Results

4.1. The Study Population

Data from many patients was gathered for the earlier investigations. A total of 73 volunteers enrolled 27 members of the public and 46 patients from the Near East Hospital. 46 (63%) and 25 (34.2%) of the 73 samples in total were male. 47.54 ±20.63 was the mean age and 47 (19.00-86.00) was the median age.

Moreover, participants were categorized by age, education and marital status. According to age, they are arranged into two groups; age 19 to 40 were 32 (43.8%) while the age 41 and above were 41 (56.1%) participants. Education indicates that there are 50 people in the group with a high school diploma or less, and 23 people with a university degree or more. Of the 48 participants, 48 were married, and the remaining 22 were single and three were widows.

4.2. Distribution of Species and Antibiotic Susceptibility Data

Among all the samples, *E. coli* (n=68/73; 93.1%) was the predominant species and then *Proteus mirabilis* (1/73; 1.3%) while 4 isolated are unidentified.

The predominant species in all of the samples is *E. coli*, and not a single one of them responded to any of the many antibiotic classes that we tested. All the 73 isolates show high resistant rate against ciprofloxacin that’s the highest rate against all the isolates tested. While 40 isolates are resistant against AMC, 45 isolates are resistant against AMP. The antibiotic that is resistant to least numbers of isolates are TPZ (5 isolates) and TGC (3 isolates). Here are the results we got from our evaluations.

Table 4.1. Antibiotic susceptibility rates in the study group (n = 73)

Antibiotics	**Susceptible (S) n (%)**	**Intermediate (I) n (%)**	**Resistant(R) n (%)**
Ciprofloxacin	0 (0.0)	0 (0.0)	73 (100)
Ampicillin	25 (34.2)	3 (4.1)	45 (61.6)
Amoxicillin-clavulanic acid	19 (26)	14 (19.1)	40 (54.7)
Piperacillin-tazobactam	61 (83.5)	7 (9.6)	5 (6.8)
Gentamicin	39 (53.4)	13 (17.8)	21 (28.7)
Trimethoprim-sulfamethoxazole	32 (43.8)	3 (4.1)	38 (52)
Tigecycline	59 (80.8)	11(15)	3 (4.1)
Cefepime	43 (58.9)	16 (21.9)	14 (19.1)

4.3. Frequency of MDR Bacteria

As far as we are aware, microorganisms that exhibit resistance to three or more types of antimicrobial medicines are considered multidrug resistant. Following analysis, it was discovered that 48 (65.7%) of the isolates tested positive for MDR. The numbers of MDR are 34 and 14 in patient and control samples respectively. The rates of MDR were 73.9% (n=34/46) in patient group and 51.9% (n=14/27) in control group. The difference of MDR rates between patient and control samples are not statistically significant.

Table 4.2. Multidrug-resistant bacteria's frequency in the patient (n = 46) and control (n=27) groups

Participants	**MDR positive**	**MDR Negative**	**Total**	
Patients	34 (73.9%)	12(26.1%)	46 (100%)	
Controls	14(51.9%)	13(48.1%)	27 (100%)	$p = 0.055$
Total	48 (65.7%)	25(34.2%)	73 (100%)	

4.4. Association of Risk Factors with Multidrug Resistance

4.4.1. Demographic and Socioeconomic Factors Evaluation with Multidrug-Resistance

The age group of 41 and above had the greatest rate of MDR (n=41/73; 56.1%). Previous research indicates that the rate of MDR is unaffected by marital status or education level, and there is no statistically significant relationship between MDR and socioeconomic status. Furthermore, there is no discernible impact of gender on the MDR rate.

4.4.2. Epidemiological Factors and Multidrug-Resistance Association

Events, traits, or other measurable things that have the capacity to alter a health state or other specified outcomes are known as epidemiological factors. Understanding MDR's prevalence, risk factors, mortality, etc. is crucial to combating the disease, particularly in places with limited data and surveillance. Nonetheless, it appears that there have been temporal and regional changes in the MDR pathogen pattern that causes nosocomial infections. Even while a specific MDR could be endemic in one place, it might be uncommon in other places. Therefore, the best approach to preventing MDR depends heavily on the epidemiological features specific to the particular area. Previous studies show that no epidemiological factors altered the MDR rate in the study group.

4.4.3. Hospital-Related Factors and Multidrug Resistance Association

When the samples were collected, 23 of the patient group had been in the hospital for at least three days. Six (60.0%) had surgery (p=0.416), five (p=0.306) required a urinary catheter, and three (100%) of all patients brought to the hospital spent time in the intensive care unit (ICU) (p=0.557). Moreover, 21 (80.8%) of the patients took antibiotics while they were in the hospital (p=0.314). Based on statistical research, there was no correlation ($p>0.05$) between hospitalized associated variables and multidrug resistance.

Table 4.3. Demographic and socioeconomic factors evaluation with multidrug resistance in the study group (n=73)

Risk factors	MDR Positivity %	*p* value
Age		
19-30	10 (45.5%)	
30 and above	38(74.5%)	*p* =0.016
Total	48 (65.8%)	
Gender		
Male	30 (62.5%)	
Female	18 (72.0%)	*p*=0.417
Total	48 (65.8%)	
Education		
Lower than university	27 (77.1%)	
University and higher	21 (55.3%)	*p*=0.049
Total	48 (65.8%)	
Marital status		
Single	14 (56.0%)	
Married	34 (70.8%)	*p*=0.205
Total	48 (65.8%)	
Socioeconomic status		
Low and middle	40 (64.5)	
High	8 (72.7%)	*p*= 0.738
Total	48 (65.8%)	

Table 4.4. Epidemiological factors and multidrug resistance association in study group (n=73)

Risk factors	**MDR Positive n/N (%)**	***p* value**
GIS*		
Yes	9 (52.9%)	
No	39 (69.6%)	*p*=0.204
Total	48 (65.8%)	
History of antibiotics use**		
Yes	27 (71.1%)	
No	21 (60.0%)	*p*=0.320
Total	48 (65.8%)	
History of diarrhea**		
Yes	11 (57.9%)	
No	37 (68.5%)	*p* =0.401
Total	48 (65.8%)	
History of UTI**		
Yes	6 (50.0%)	
No	42 (68.9%)	*p* =0.318
Total	48 (65.8%)	
Travel history**		
Yes	29 (70.0%)	
No	19 (59.4%)	*p* =0.310
Total	48 (56.8%)	
Turkey or Europe Travel**		
Yes	24 (80.0%)	
No	5 (45.5%)	*P* =0.052
Total	29 (70.7%)	
Travel to Asia or Africa**		
Yes	5 (45.5%)	
No	24 (80.0%)	*P* =0.052
Total	29 (70.7%)	

*shows the sample collection time.

**shows last six months prior to the study.

Table 4.5. Hospital-related factors and multidrug resistance association in the study group

Risk Factors	MDR Positive	*p* value
ICU Stay*		
Yes	3 (100%)	
No	31 (72.1%)	*p* =0.557
Total	34 (73.9%)	
Surgery*		
Yes	6 (60.0%)	
No	28 (77.8%)	*p*=0.416
Total	34 (73.9%)	
Urinary catheter*		
Yes	5 (100%)	
No	29 (70.7%)	*p*=0.306
Total	34 (73.9%)	
Antibiotic use*		
Yes	21 (80.8%)	
No	13 (65.0%)	*p* =0.314
Total	34 (73.9%)	

*Indicates the current hospitalization.

CHAPTER V

Discussion

The broad order *Enterobacterales* contains a variety of bacteria, or germs, such as *K. pneumoniae* and *E. coli*, which are frequently responsible for infections in both community and medical settings.

In order to evade the antibiotics' effects, bacteria that are immune to them are constantly creating new "resistance mechanisms", or defense mechanisms. For example, some *Enterobacterales* produce what are referred to as extended-spectrum beta-lactamases (ESBLs). Some regularly used drugs are destroyed and degraded by these enzymes, like cephalosporin and penicillin, making them ineffective in treating infections.

Fewer antibiotics are needed to treat infections caused by *Enterobacterales* that produce ESBLs as a result of this resistance. Additionally, bacteria harbouring ESBLs possess the capacity to develop resistance to many types of antibiotics, including aminoglycosides, quinolones, tetracyclines, and trimethoprim. Common illnesses like urinary tract infections that are brought on by bacteria that produce ESBLs can occasionally require more involved treatments. Instead of taking oral antibiotics at home, patients with these illnesses might need to be hospitalized to the hospital and given intravenous (IV) carbapenem medicines.

Carbapenems are the only drugs that effectively combat bacteria that produce ESBLs; nevertheless, resistance enzymes that neutralize these antibiotics are also becoming increasingly common. The more we depend on these important class of antibiotics, the more likely it is that resistance will spread.

In hospitalized patients, 197,400 cases were ESBL-producing *Enterobacterales* and there were 9,100 deaths projected in the United States in 2017. Medicines are used to treat infections brought on by bacteria that produce ESBLs, although there may not be as many treatment choices available due to the bacteria's resistance to many routinely recommended medicines. Serious *Enterobacterales* infections that produce ESBL are

frequently treated with carbapenem antibiotics, which are generally only used for extremely resistant infections.

The Infectious Disease Society of America (IDSA) offers new guidelines for antibiotic-resistant infections. That states that on September 17, 2020, diseases caused by carbapenem-resistant *Enterobacterales* (CRE), extended-spectrum β-lactamase producing *Enterobacterales* (ESBL-E), and difficult-to-treat Pseudomonas aeruginosa (DTR-P. aeruginosa) were published. A literature study and this updated advice paper were prompted by the numerous publications from previous years that have enhanced our comprehension of CRE and ESBL-E management.

Of the 73 isolates in this investigation, 48 (65.1%) were MDR individuals. There are few available therapeutic options for *Enterobacterales* infections acquired in hospitals due to the elevated prevalence of MDR. A possible explanation for the degree of diversity observed in MDR isolates is the usage of antibiotics from distinct classes, studied populations, study times, and specimen types.

In this study, the *Enterobacterales* specie among all the isolates that have been found to be predominant is *E. coli* with the rate of 93.1%. *E. coli* has also been predominant specie according to previous publications. The high frequency of MDR (*E. coli* 82.3%) in *Enterobacterales* isolates have been reported by Mer Rouge Laboratory, Djibouti, from January to July 2019 as well as (Zavarla-Cerna et al., 2020; Onduru et al., 2021). Furthermore, the present literature review shows that other specie *Proteus mirabilis* were found at very low rate 1.3%. our studies agree with other past studies which reported that *K. pneumonia, Proteus mirabilis* and *Enterobacter spp*. etc. showed low rates among all the *Enterobacterales*.

In this present study, resistance rates of antibiotics are evaluated. The rate of MDR was ascertained by analyzing 73 isolates in total. The highest resistant rate of are all *Enterobacterales* isolates were found to be Ciprofloxacin (100%) then ampicillin (61.6%) followed by amoxicillin-clavulanic acid (54.7%) and trimethoprim-sulfamethoxazole (52%). The high level of resistance can be compared with the previous studies. Additionally, it was determined that among beta-lactam or beta-lactamase

inhibitors, piperacillin-tazobactam had the highest antimicrobial susceptibility rate (61%). Majority of isolates (65.7%) were found to be MDR. 64% of MDR *Enterobacterales* were found in earlier investigations.

In this study, demographic and socioeconomic data were investigated in addition to MDR evaluation. Age-based groupings were used to assess it; for example, there were statistically significantly more MDR isolates in the age group of 31 and older than in the 19 to 30 age group (p=0.016) (Table 4.3). This importance may result from a weakened immune system, ongoing antibiotic usage, hospital stays, long-term care institutions, nursing homes, and the use of medical equipment like feeding tubes and urine catheters, among other things. The elderly individuals are exposed to antibiotics more than younger individuals or may also forget to take their medicines. The likelihood of MDR is increased by the irregular routine.

MDR was observed in patients with university degrees or higher degrees at lower rates than in individuals without such a degree, although the difference was not determined to be statistically significant. According to Table 4.3, they have a weak correlation (p=0.049) with MDR. However, prior research (See et al., 2017) indicates that those with less education are more likely to do self-medication, which contributes to the establishment of MDR. This increased significance can be attributed to the fact that those with less education or none at all are more into self-medications, which helps to foster the growth of MDR. Also, people take the wrong prescription or don't adhere to the recommended dosage schedules.

Socioeconomic factor data in this study were shown to have no significant association with MDR isolates (p=0.738), indicating that they were not associated with MDR (Table 4.3). Moreover, previous studies by Kasim et al. (2020), explain evidence that poverty is important factor of MDR. Additionally, this study found no statistically significant link between hospital stay and MDR. Patients with long hospital stays had a statistically significant 1.7% higher rate of MDR.

Another category for patient grouping is GIS. When GIS (of any kind) was assessed, significant findings could not be obtained (p=0.204). Additionally, earlier

studies verified 3.7% GIS rate and were no proof that there was a meaningful association with MDR.

As stated by WHO (2014), usage of antibiotics is the primary cause of MDR globally. However, our research revealed no meaningful correlation (p=0.320) between antibiotic use and MDR (Table 4.3).

A statistical analysis revealed that among *Enterobacterales* isolates, travel, history of UTI, and history of diarrhea had no bearing on MDR. But it shows significant results when evaluated them specifically like travelling to Turkey, Asia and Africa gave significant results. The reason can be traveler's diarrhea. The factors that contribute to the increased occurrence of ESBL-E include the travel destination, duration of stay, seeing friends and family, and consuming pastries and ice cream.

The link between hospital-related factors and MDR in *Enterobacterales* was also examined in this study, and the usage of antibiotics and stays in the intensive care unit, surgery, or urinary catheters did not significantly correlate with MDR. Nonetheless, research by Grzegorz et al. (2020) demonstrates a connection between hospital-related parameters and MDR.

CHAPTER VI

Conclusion

Out of the 73 subjects in this study, 48 (or 65.7%) were found to be MDR positive. MDR rates were 51.9% (n=14/48) in control samples and 73.9% (n=34/46) in patient samples. The patient and control groups' MDR rates did not differ statistically significantly. *E. coli* (93.1%) have been the most common species among all the *Enterobacterales* isolates.

Furthermore, an assessment of the risk factors associated with multidrug resistance revealed that the only variables that affected the results was age ($p=0.016$) and education ($p=0.049$); demographic and socioeconomic factors, as well as those related to hospitals and epidemiology, had no statistically significant effect on MDR ($p>0.05$).

The study findings indicate that a large percentage of MDR is present in the ciprofloxacin-resistant *Enterobacterales* isolates. This suggests that antibiotic resistance should be carefully monitored in Northern Cyprus.

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