NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY

ANTIBIOTIC RESISTANCE RATES IN EXTENDED-SPECTRUM BETA-LACTAMASE-PRODUCING *ENTEROBACTERALES* ISOLATES

M.Sc. Thesis

Israel of God Chinemelum EZENWA-EDWIN

Nicosia
January, 2024

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Assoc. Prof. Dr. Emrah RUH

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Approval

We certify that we have read the thesis submitted by **Israel of God Chinemelum Ezenwa-Edwin** titled "**Antibiotic Resistance Rates in Extended-Spectrum Beta-Lactamase-Producing *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.

Israel of God Chinemelum Ezenwa-Edwin

31/01/2024

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Özet

Genişlemiş Spektrumlu Beta-Laktamaz Üreten *Enterobacterales* İzolatlarının Antibiyotik Direnç Oranları

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31 Ocak 2024, 68 sayfa

Amaç: Bu çalışma, genişlemiş spektrumlu beta-laktamaz (GSBL) üreten *Enterobacterales* isolatlarında çeşitli antibiyotiklerin etkilerini değerlendirmek ve yedi ilaç arasındaki direnç ve duyarlılık oranlarının belirlenmesi amacıyla yapılmıştır.
Gereç ve Yöntem: Çalışmaya, 52'si hasta ve 23'ü kontrol grubundan olmak üzere toplam 75 örnek dahil edilmiştir. Yakın Doğu Üniversitesi Hastanesi'ne başvuran hastaların ve toplumdaki bireylerin dışkı örneklerinden örnekler izole edilmiştir. Fosfomisin, kolistin, amikasin, aztreonam, tobramisin, tetrasiklin ve nitrofurantoin antibiyotikleri için duyarlılık testleri yapılmıştır. İstatistiksel analiz Pearson ki-kare testi kullanılarak yapılmıştır.
Bulgular: Fosfomisin: Bir izolat (%2) dirençli iken, kontrol grubunda iki (%9) dirençli izolat gözlenmiştir. Tobramisin: iki izolat dirençliydi (%4), kontrol grubu örneklerinde ise dirençli örnek yoktu (%0). Amikasine dirençli izolat (%0) bulunmazken, kontrol grubunda bir izolat (%4) dirençliydi. Aztreonam: altı izolat (%11) dirençliydi, kontrol grubunda ise dört izolat (%17) dirençliydi. Tetrasiklin: 21 izolat (%40) dirençli iken kontrol grubunda 10 (%44) dirençli izolat vardı. Nitrofurantoin: bir izolat direnç gösterdi (%2), kontrol grubunda ise dirençli izolat yoktu (%0). 2023 CLSI kılavuzunda kolistinin zon çapına ilişkin bir sınır değer bulunmadığı için kolistin direnci konusunda herhangi bir yorum yapılmamıştır.
Sonuç: İzolatların çoğunda bazı ilaçlara karşı çok az direnç vardı veya hiç direnç yoktu. İlaçların en etkilisi fosfomisindi. En yüksek direnç oranları tetrasiklin için gözlenmiştir. Çalışmada genel direnç oranları yüksek olmasa da Kuzey Kıbrıs'ta antibiyotik duyarlılık testi yapılmalı ve direnç oranları dikkatle izlenmelidir.

Anahtar Kelimeler: *Enterobacterales*, genişlemiş spektrumlu beta-laktamaz, antibiyotik direnci.

Abstract

Antibiotic Resistance Rates in Extended-Spectrum Beta-Lactamase-Producing *Enterobacterales* Isolates

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Aim: The present study was done to evaluate the effects of several antibiotics in extended-spectrum beta-lactamase (ESBL)-producing *Enterobacterales* and to determine rate of resistance and susceptibility amongst seven drugs.
Materials and Methods: A total of 75 samples (from 52 patient and 23 controls) were included in the study. The samples were isolated from the fecal samples of the patients admitted to the Near East University Hospital and the individuals from the community. Susceptibility testings for fosfomycin, colistin, amikacin, aztreonam, tobramycin, tetracycline, and nitrofurantoin were conducted. Statistical analysis was done using Pearson chi-square test.
Results: Fosfomycin: one isolate was resistant (2%), while in the control group two resistant isolates were observed (9%). Tobramycin: two isolates were resistant (4%), while the control group samples had no resistant isolates (0%). There was not any isolate resistant to amikacin (0%) while for the control group one isolate was resistant (4%). Aztreonam: six isolates were resistant (11%), for the control group four isolates were resistant (17%). Tetracycline: 21 isolates were resistant (40%), while the control group had 10 resistant isolates (44%). Nitrofurantoin: one isolate showed resistance (2%) while in the controls there were no resistant isolates (0%). Colistin did not have a breakpoint value for the zone diameter in the 2023 CLSI guidelines, therefore the no interpretation was done for colistin.
Conclusion: Majority of the samples had little to no resistance to some of the drugs. Fosfomycin was the most effective of the drugs. The highest resistance rates were observed for tetracycline. Although the overall resistance rates were not high in the study, antibiotic susceptibility testing should be conducted, and the resistance rates should be carefully monitored in Northern Cyprus.

Keywords: *Enterobacterales*, extended-spectrum beta-lactamase, antibiotic resistance.

Table of Contents

List of Tables

List of Abbreviations

AMT: Amikacin

CAZ: Ceftazidime

CD: Crohn's disease

CDC: Centre for Disease Control and Prevention

CDT: Combined Disc Test

CLSI: Clinical and Laboratory Standards Institute

CN: Gentamicin

CT: Colistin

CTX: Cefotaxime

DAEC: Diffusely-Adherent *E. coli*

DDST: Double Disc Synergy Test

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*

EUCAST: European Committee on Antimicrobial Susceptibility Testing

F: Nitrofurantoin

FEP: Cefepime

FF: Fosfomycin

GIS: Gastrointestinal Syndrome

GIT: Gastro intestinal tract

ICU: Intensive Care Unit

MBL: Metallo Beta-Lactamase

MDR: Multidrug Resistance

MIC: Minimum Inhibitory Concentration

MNEC: Meningitis-associated *E. coli*

mRNA: Messenger Ribonucleic Acid

PBP:	Penicillin Binding Protein
PDR:	Pan Drug Resistance
rRNA:	Ribosomal Ribonucleic Acid
SBL:	Serine Beta-Lactamase
TE:	Tetracycline
TOB:	Tobramycin
tRNA:	Transfer Ribonucleic Acid
UDP:	Uridine Diphosphate
UTI:	Urinary Tract Infection
WHO:	World Health Organization
XDR:	Extensively Drug Resistance

CHAPTER I

Introduction

Enterobacterales is an order/ group of non-spore making, facultative anaerobic bacteria rod-shaped and gram-negative within the class Gammaproteobacteria (Baldelli et al., 2021). Formerly known as *Enterobacteriaceae* prior to 2016, it consists of bacteria that act on the gastrointestinal tract of animals (Baldelli et al., 2021). These actions could be helpful/ non-pathogenic (gut microbiota) like *Salmonella, Escherichia coli, Klebsiella,* and *Shigella* while some are disease causing like *Enterobacter* and *Citrobacter*, although they can all be pathogenic if they over colonize in the GIT (Spellerberg et al., 2020). After 2016 *Enterobacteriaceae* was placed into the order *Enterobacterales* alongside environmental phytopathogens (plant pathogens), such as members of the genera *Dickeya, Pectobacterium, Brenneria, Erwinia* and *Pantoea* (Presti et al., 2019).

Enterbacterales are beneficial to animals and humans, being situated in the human gastrointestinal tract; they help boost the innate and adaptive immunity, out compete other pathogenic microbes for nutrition to maintain an equilibrium, assist in digestion by metabolizing foreign compounds, absorbing minerals, changing bile acid and steroids, and initiating and eliminating toxins, genotoxins, and mutagens (Presti et al., 2019). This helps with maintaining the integrity of the epithelial layer of the GIT (Presti et al., 2019).

Gut microbiota are able to ferment non-digestible dietary fibers and intestinal mucus which support the growth of special microbes (acetate, propionate, and butyrate) that make short chain fatty acids (SCFAs) like butyrate, propionate, and acetate (Presti et al., 2019). Acetate which is the most abundant SCFA is used by tissues to metabolize cholesterol, helping to regulate appetite (Valdes et al., 2019). Butyrate has many functions such as apoptosis of colon cancer cells, activation of intestinal gluconeogenesis which provides energy to intestinal cells, and maintaining oxygen balance among the epithelial cells of the GIT. Therefore maintaining both glucose and oxygen homeostasis in the GUT (Valdes et al., 2019).

Pathogenic bacteria is also prevented by gut microbiota by lowering the pH of the intestine with the secretion of lactate and short-chain fatty acids (Valdes et al., 2019). Another way is by secreting toxic metabolites and fatty acids to stop the growth

or kill pathogenic bacteria, therefore inhibiting the colonization of pathogenic bacteria in the gut (Valdes et al., 2019).

Aims of the Study

The present study was done to see the effects of new untested anti-biotics in extended-spectrum beta-lactamase (ESBL) producing *Enterobacterales* and to determine rate of resistance and susceptibility amongst 7 drugs.

To evaluate if there is a significance between patient and controls in terms of resistance.

To evaluate if antibiotic use in the last 6 months had a significant effect on resistance.

CHAPTER II

Literature Review

2.1. General Characteristics

Enterobacterales are beneficial to the host gut functions, they are still bacteria and therefore can become pathogenic or lead to complications such as inflammatory bowel diseases, if the flora compositions are altered (Valdes et al., 2019). Change like; diet, usage of probiotics, being immunocompromised and the improper use of antibiotics can lead to immune cell population imbalance (T-cells) which results in inflammations in the bowel (IBD). A study done by Presti et al., (2019) found over 160 loci in some hosts, leading genetic disorders where IBD may arise from an extreme immune response directed against their gut microbiota (Valdes et al., 2019). Another type of IBD Crohn's disease (CD), an autoimmune illness, in which the gastrointestinal tract is attacked and inflamed by the host's immune system. The study discovered that patients with both active and inactive colonic CD had much higher amount of *Enterobacterales* in their fecal microbiota compared to healthy individuals (*Pasteurellacaea, Veillonellaceae,* and *Fusobacteriaceae*) (Valdes et al., 2019).
However the negative effects of microbiota cannot be properly pointed out without talking about the direct infections they inflict to the host. Since *Enterobacterales* mainly either cause disease or are naturally found in the gastrointestinal tract (GIT) of the host, they cause disease such as urinary tract infections (UTI), sepsis, pneumonia, dysentery and diarrhea (Valdes et al., 2019).

E.coli. These are gram-negative motile facultative anaerobic rods. They can ferment glucose and lactose and have flagella as well (Walter et al., 2018). They serves as a source of vitamin K (menaquinones) and B-complex vitamins (Walter et al., 2018). Usually harmless, however it has the ability to acquire a variety of extensive mobile genetic elements (plasmid and direct transposons) from either a bacteriophage or a neighboring bacteria that encode virulence factors (Koirala et al., 2021). This can create different stains that cause disease, mainly *E. coli* O157:H7 that produce the cholera toxin (*ETEC*), is responsible for around 40% of *E.coli* illnesses and travelers' diarrhea (Koirala *et al.,* 2021). Various other stains cause urinary tract infections

(UTIs), meningitis in neonates, gastroenteritis, watery diarrhea (*EPEC* and *EAEC*), bloody diarrhea (*EHEC* and *EIEC*) (Koirala et al., 2021).

***Salmonella*.** These are gram-negative, non-spore forming, motile (but non-motile in culture) facultative anaerobes bacteria (Kimberlin et al., 2021). They are said to have come from the same linage as *E. coli*, there are two main species in the genus: *Salmonella bongori and Salmonella enterica*. *S. enterica* has many subspecies and over 2000 serovars that further divided into non-typhoidal (responsible for infection and inflammation only local in the GIT) and typhoidal (systemic "Typhoid fever") (Kimberlin et al., 2021).

***Shigella*.** This is in the group of gram-negative, non-spore producing and non-mobile. In *Shegella* genera there are some species: *Shigella dysenteriae* (group A), *Shigella flexneri* (group B), *Shigella boydii* (group C), and *Shigella sonnei* (group D). *Shigella* is also said to come from the same linage as *Escherichia*. They are so similar that scientists believe that they both should be in the same genus, however because of the difference in clinical and epidemiological manifestations their genera stays different. *Shigella* causes an illness called Shigellosis (bloody diarrhea with mucus), which is then followed by Stomach pain or cramps, fever, nausea or vomiting which last for five to seven days.

***Klebsiella*.** This genera are gram-negative, non-motile, some species are aerobic while others are facultatively anaerobic (Bengoechea et al., 2019). The anaerobic species are oxidase-negative, rod shaped and have a capsule which is polysaccharide based non-motile and a lactose fermenter. *Klebsiella* are part of the normal microbiota, but can still be pathogen and cause opportunistic infections like; it can seriously harm both human and animal lungs, especially the alveoli, which can result in bloody, brownish/yellow sputum that looks like jelly. *Klebsiella* main infections are pneumonia, UTI, peritonitis, meningitis, diarrhea, and other infections (Lam et al., 2018). There are many *Klebsiella* species but *K. pneumoniae* and *K. oxytoca* are the main species to cause infections. *Klebsiella* are usually a problem to individuals with a low immune system such as: the elderly, newborns and immunocompromised (Bengoechea et al., 2019). Most infections are found at a hospital settings particularly

in contaminated invasive medical device. Cephalosporins, pipercillin tazobactam, and aminoglycosides (Bengoechea et al., 2019).

***Proteus*.** A gram-negative bacteria, rod shaped, aerobic bacteria with an ammonia smell (sewage) due to the urease enzyme they produce (Jamil, 2023). They are flagellated and therefore motile, with the unique characteristic of moving across surfaces in a "swarming motion" (O'Keefe et al., 2019). They are usually non-pathogenic, however they can still cause opportunistic infections usually in hospital settings. There are three main species that cause infections to the human host: *Proteus vulgaris, Proteus penneri* and *Proteus mirabilis. Proteus mirabilis* mainly cause urinary tract infection and wound infection (O'Keefe et al., 2019). The urease enzyme is responsible for converting urea to ammonia and carbon dioxide, this results in alkalization of urine leading to kidney stones. *P. mirabilis* can be treated by ampicillin and cephalosporins (Jamil, 2023).

2.2. Beta-Lactam Antibiotics

They are antibiotics that contain beta-lactam rings in their structure. They usually work by interfering with the target bacteria's cell wall synthesis (Pandey et al., 2023). The first beta-lactam to be discovered was penicillin from a fungi stain *Penicillium notatum.* Bacteria are increasingly becoming resistant to β-lactam antibiotics. They do this by synthesizing a β-lactamase, which is an enzyme that hydrolyses the β-lactam ring. In order to deal with the resistance, β-lactam antibiotics can be given with β-lactamase inhibitors. The list of bacteria belonging to this class are penems (a penicillin derivative), carbapenems and carbacephems, monobactams, cephalosporins and cephamycins (cephems) (Pandey et al., 2023).

2.2.1. Mechanism of Action in Beta-lactam Anti-biotics

The actions of beta-lactamase producing bacteria can not to be fully understood without talking about the actions of the antibiotics it effects (Leone et al., 2019). They work in the periplasmic space of a bacteria during the formation of the peptidoglycan layer (Leone et al., 2019). Beta-lactams affect the enzyme transpeptidase/penicillin and d-alanyl carboxypeptidase binding protein during the cross linkage between the bacteria cell wall acceptors and peptidoglycan precursors (Leone et al., 2019). The

beta-lactam rings of the antibiotic then attaches at the active zone of the transpeptidase, this prevents the action of the enzyme and therefore preventing proper formation of a cell wall (Pandey et al., 2023). The weak cell wall and the internal osmotic pressure of the bacteria results in bursting and death of the single cell organism (Pandey et al., 2023).

Carbapenem. These are beta-lactams which serve as a broad spectrum antibiotic against bacterial infections resistant to drugs. The list of the drugs in this class are; meropenem, ertapenem, imipenem and doripenem (Nazneen, 2022). They are usually given intravenously, often in combination with aminoglycosides to boost the effectiveness of both drugs. They are used in the treatment of diseases such as; pneumonia, blood stream infections, complicated UTI, intra-abdominal infections (Nazneen, 2022). They are able to cover both gram positive and negative bacteria, however they have a better effectiveness against *enterabacterales* (Nazneen, 2022).

Monobactams. These are very unique drugs as they are made from bacteria found in the soil. Unlike the other drugs in the beta-lactam subgroup their beta-lactam ring is not fused to another ring. The first drug to be approve is aztreonam, although there are still others in investigation. They work similarly to aminoglycosides but they are less toxic to the kidney (Makii et al., 2022). They are not effective against gram positive bacteria, treating septicemia, pyelonephritis, skin infections, urinary tract infection, endometritis lower respiratory tract infection, peritonitis, cystic fibrosis in children and cystitis (Makii et al., 2022).

Because it is a monocyclic β-lactam antibiotic as opposed to other bicyclic β-lactam antibiotics, aztreonam is known as a synthetic monobactam antibiotic (Makii et al., 2022). Aztreonam likes to bind more tightly to penicillin-binding protein 3 (PBP 3) of sensitive gram-negative bacteria, which gives it its antibacterial action in addition to inhibiting mucopeptide formation in the bacterial cell wall. These bacteria's PBP 1a is another bacterium for which the medication has some affinity, but little to none at all for PBPs 1b, 2, 4, 5, or 6. In gram-negative bacteria, aztreonam induces the production of abnormally elongated forms because PBP 3 is involved in high levels of cell division. As a result, there is a decrease in cell division and cell wall rupture, which causes lyses and death (Makii et al., 2022).

Penicillin. This is not only the first beta-lactam made but also the first antibiotic discovered by Alexander flemming in 1929 from the fungus *Penicillum ruben* (Drug.com, 2022). Over the years of the creation of the drug, several genetic modifications have been performed on the drug to create synthesis variants of the penicillin drug; penicillin G benzylpenicillin (which as an intravenous/muscular route of administration) was the first to be discovered then penicillin V phenoxymethylpenicillin (taken orally) was generated by incorporating phenoxyacetic acid into the growth medium of a genetically engineered strain of the *Penicillium* fungus (Drug.com, 2022).

Mechanism of Action of Penicillin. Penicillin is a beta-lactam and so acts by preventing the completion of peptidoglycan creation, which is a needed structural elements of bacteria's cell walls. It only blocks the action of enzymes required for the last stage of cell wall production, which involves cross-linking peptidoglycans (Kimberlin et al., 2021). Pentapeptide and uridine diphosphate-N-acetylmuramic acid is made where the fourth and fifth amino acids are D-alanyl-D-alanin. It binds itself to penicillin-binding proteins via the penicillin molecule's β-lactam ring structure. DD-transpeptidase, is an enzyme that transfers D-alanine, (also known as penicillin binding protein) (Pandey et al., 2023). The cross-linking of N-acetyl glucosamine and UDP-MurNAc is important for the structural strength of the bacterial cell wall (Torres et al., 2022). Penicillin just like other beta-lactam antibiotics function similarly to D-alanine-D-alanine in UDP-MurNAc because of their similar conformations (Torres et al., 2022). The DD-transpeptidase does not bind to UDP-MurNAc but the four-membered β-lactam ring of penicillin (Drug.com, 2022). This means that DD-transpeptidase is inactivated, cross-links between UDP-MurNAc and N-acetyl glucosamine are prevented, and there is then an imbalance between the synthesis and breakdown of cell walls. Because there are less cross-links in the cell wall, this means that the cell walls are weak and therefore water pours into the cell unrestrained because the cell is unable to maintain the proper osmotic gradient. Cell death and lysis follows (Kimberlin et al., 2021).

Penicillin G is unable to survive the acidic nature of the human stomach and hence must be taken intravenously at a high dose of 2.4g (Torres et al., 2022). They treat syphilis, disseminated gonococcal infections, septicaemia, pneumonia, endocarditis, pericarditis meningitis, cervicofacial disease, *Listeria* infections,

clostridial infections, fusospirochetosis, botulism, gas gangrene, tetanus, erysipelothrix, endocarditis, anthrax, meningitis, endocarditis, *Pasteurella* infection, meningitis, abdominal infections, Haverhill fever; rat-bite fever and, actinomycosis, empyema and meningococcal meningitis (Kimberlin et al., 2021).

Penicillin V on the other hand does well against stomach acid hence is taken orally at a lower dose of 500 mg treats infections similar to that of penicillin G, however does poorly against endocarditis (Torres et al., 2022).

The drug is one of the most important antibiotic as it has a very large spectrum of activity, however over the years bacteria have become increasingly resistant to the drug class. The low levels of information on resistance and overuse has led to multi-drug resistance, particularly in *Staphylococcus aureus* (Pandey et al., 2023).

In this class the list are all related to penicillin G, with various side chains placed at the precursor 6-2APA (Pandey et al., 2023). There are 3 groups; amino penicillin/extended spectrum antibiotics, anti-staphylococcal penicillin and anti-pseudomonal penicillin. The anti-staphylococcal drug*s* target the gram-positive *Staphylococcus* spp. and are as follows; nafcillin, oxacillin, dicloxacillin, cloxacillin, methicillin and flucloxacillin. Penicillin G was not allow to treat the *pseudomonas* bacteria genus, so the group anti-pseudomonal penicillin was created, they are; azlocillin, mezlocillin, piperacillin, temocillin, carbenicillin and ticarcillin (Torres et al., 2022).

Cephalosporins. They are five generations of this drug class, each class are group based on the time they were discovered and on which bacteria they act on.

First-generation cephalosporins: act on most gram-positive cocci and some gram-negative bacteria, namely, *Escherichia coli, Proteus mirabilis*, and *Klebsiella pneumoniae* (Tao et al., 2023). They used to treat skin and soft tissue infections (cellulitis and abscesses), bloodstream infection, otitis media, respiratory tract infections, bone infections and genitourinary tract infections (Tao et al., 2023). They are also used as a surgical prophylaxis (Goldblatt et al., 2020).

Second generation cephalosporins: act on *Haemophilus influenza, Bacteroides. Moraxella catarrhalis* (Goldblatt et al., 2020). Two subgroups, the second generation and cephamycin second generation. Cefuroxime and cefprozil are used to treat *H. influenza* and lyme disease while cephamycin subgroup treats *bacteroides* infections (Goldblatt et al., 2020).

They treat respiratory infections (pneumonia) as well as bloodstream infection, otitis media, respiratory tract infections, bone infections and genitourinary tract infections (like first generation) (Stanaway, J. D., 2019)

Third-generation cephalosporins: don't act on many gram-positive organisms but have act largely on *Enterobacterales* species and gram negative bacteria resistant to 1st and 2nd generation cephalosporins (Stanaway, J. D., 2019). The examples are cefpodoxime, ceftazidime, cefdinir, cefixime, ceftriaxone, cefotaxime and cefoperazone (Stanaway, J. D., 2019). They are able to penetrate the blood brain barrier and cerebral spinal fluid if they are taken intravenously (especially ceftriaxone).

Fourth-generation cephalosporins: are similar to third-generation cephalosporins, however act on more gram-negative bacteria with antimicrobial resistance, example beta-lactamase (Spelerberg et al., 2020). Cefepime is the main drug and is a broad-spectrum antibiotic that can pass through the cerebral spinal fluid (Spelerberg et al., 2020). The quaternary ammonium group gives the drug the ability to bypass the outer membrane of gram negative better than other generations (Spelerberg et al., 2020).

Fifth-generation cephalosporins: ceftaroline have the important task of acting on penicillin-resistant Pneumococci and methicillin-resistant staphylococci. However does not treat *Pseudomonas aeruginosa* (Spelerberg et al., 2020).

Aminoglycosides. Although aminoglycosides are not in the beta-lactam group combination of a beta lactam and aminoglycoside could, be a more effective treatment of patients experiencing serious infection (Richard, 2022). These are drugs that act mainly on gram-negative bacteria, have a bactericidal action against aerobes and some anaerobic bacilli. They stop the production of proteins and contain a component of an amino-modified glycoside (sugar) (Richard, 2022). They inhibit the protein synthesis of bacteria by irreversible binding, to the membrane of bacterial 30S ribosome through the help of the aminoglycosides' energy. When the antibiotic enters the cytosol of the bacteria, they disturbs peptide elongation at the 30S ribosomal subunit, this results in incorrect mRNA translation (Richard, 2022). This can stop translation proofreading leading to the incorrect RNA read and wrong termination. This leads to incorrect protein production, a structurally weak cell and death. Examples are neomycin, tobramycin, amikacin, gentamycin and kanamycin (Richard, 2022).

Fluoroquinolones. This is a quinolone antibiotic, among many other groups of extended-spectrum antimicrobials. These antibiotic share a similar core structure related to the substrate 4-quinolone (Bethesda, 2020). These antibiotics can be used in treating humans, animals both domestic and farm animals. The most used and common quinolone is fluoroquinolone. This drug has a fluorine atom in its chemical structure and has a wide spectrum of treatment in both gram-positive and gram-negative bacteria (Bethesda, 2020).

They are mainly used to treat nosocomial infections associated with urinary catheters, therefore can also treat genitourinary infections. Infections that are not hospital-acquired (community-acquired) are recommended in situations where the infection has a possible for multi-drug infections and all other regiments have been exhausted. The only situation that fluoroquinolones are considered as first-line treatment is in the cases of serious pyelonephritis or bacterial prostatitis and the patient need serious hospital care. Other instances are when a bone entering drug is needed to treat *Salmonella* spp. related osteomyelitis in sickle-cell patients, with chelating the bone (like tetracyclines).

Mechanism of Action in fluoroquinolones. Quinolones are bactericidal antimicrobial treatments. By stopping bacterial DNA from unwinding and replicating, they block DNA replication (Bethesda, 2020). They stop nuclease activity from being impacted while blocking the ligase activity of DNA gyrase and topoisomerase IV, two type II topoisomerases that cleave DNA to cause supercoiling. DNA is released with single and double-strand breaks that cause cell death when the ligase activity is impaired. Eukaryotic type II topoisomerase, some quinolones with aromatic substituents at their C-7 locations show strong activity. Double-strand breaks caused by insufficient repair of closely spaced 8-oxo-2'-deoxyguanosine in the DNA may be the cause of bacterial cytotoxicity (Kuula et al., 2019).

There are many generations of fluoroquinolones, the first was discovered on 1962 for the treatment of urinary tract infections (Bethesda, 2020). While trying to synthesize an anti-malarial chloroquinoline, George Lester discovered nalidixic acid (Kuula et al., 2019). Most of the quinolones used are in the second generation of fluoroquinolones, this generation has the original quinoline chemical structure, the C-3 carboxylic acid group, and a fluorine atom to the all its carbon rings (Bethesda, 2020).

The fluoroquinolone Drug List. The first generation are not as common as the second generation. They are rosoxacin, flumequine and oxolinic acid (Kuula et al., 2019). Similar chemical structure as the first-generation, however without the 4-quinolones, include pipemidic acid, piromidic acid, cinoxacin and nalidixic acid (Kuula et al., 2019).

Second generation include fleroxacin, nadifloxacin, norfloxacin, lomefloxacin ofloxacin, pefloxacin, and the most common ciprofloxacin. Similar chemical structure as the second generation, however without the 4-quinolones is enoxacin.

Third generation have abilities not seen with the first and second generations, the third generation are used in treatment against *Streptococcus* bacterial species. They includes levofloxacin, temafloxacin, pazufloxacin, balofloxacin, sparfloxacin and grepafloxacin. Similar chemical structure as the previous generation, however without the 4-quinolones is tosufloxacin (Leone et al., 2019).

Fourth generation fluoroquinolones uniquely have DNA gyrase and topoisomerase IV functions. This reduces the rate at which resistance develops (Leone *et al.*, 2019). They include delafloxacin, sitafloxacin, gatifloxacin, besifloxacin moxifloxacin prulifloxacin and clinafloxacin. Similar chemical structure as the previous generation, however without the 4-quinolones are gemifloxacin and trovafloxacin (Leone et al., 2019).

2.3. Extended Spectrum Beta-Lactamase Producing Bacteria

A form of resistant mechanism in which gram-negative bacteria produces an enzyme to break down antibiotics that treat infections. Bacteria gain the ability through the over/improper use of antibiotics by the host. Since antibiotic indiscriminately kills bacteria in the host's body, this can result to both the helpful microbiota and pathogenic bacteria to undergo defensive mutations leading to resistance (Teklu et al., 2019). These are commonly spread in hospital settings such as the Intensive care unit, as these the places where really ill, immunocompromised patience's are subjected to multiple treatments for prolonged periods of time (Teklu et al., 2019). The medical devices create an environment for both growth and spread of highly resistant bacteria. Examples of ESBL producing bacteria are *E.coli*, *pseudomonas*, *Klebsiella*, *Proteus* and *Salmonella* to list a few (Teklu et al., 2019).

2.4. Beta-lactamase Enzymes

These are enzymes that are produced by bacteria in-order to hydrolyze the active site of a beta-lactam antibiotic. There are two systems of classification of the beta-lactamase enzyme; Ambler method and the Bush-Jacoby-Medeiros method (Teklu et al., 2019).

2.4.1. Bush-Jacoby-Medeiros Method

The technique places Substrates and inhibitors traits according to the phenotypes of their enzymes. Beta-lactamases are divided into classes (substrates and inhibitors) based on their functions (Bush, 2010). They are then further divided into four common groups. The enzymes are grouped into either 2be or the OXA-type 2d based on the enzymes produced. 2be subgroup shows that it was derived from the 2b beta-lactamases, with the 'e' in 2be indicating that it is an extended-spectrum (examples are TEM-2, SHV-1 and TEM1). ESBLs produced from the 2be group, are differentiated by adding one amino acid from their progenitors. This gives them the ability to hydrolyze third generation cephalosporins and monobactams, therefore giving it a wider range of activity than the progenitor enzyme (Bush, 2010).

2.4.2. Ambler system

In the ambler system, ESBLs are divided into four types: class A, B, C and D. They are grouped on the bases of their different patterns of sequences and the way they hydrolyze drugs. This system also differentiates using the different collection zinc metallo-enzymes in the active site serine enzymes called serine beta-lactamases (SBLs). Although the four groups of enzymes are found in a lot of different types of bacteria in the environment, only a few are found in the important pathogenic bacteria. CTX-M, SHV, KPC and TEM are examples of class A; NDM and VIM are class B; CMY and ADC are in class C; and class D are called oxacillinase (OXA) (Pandey et al., 2023).

Class-A Beta-lactamases. The most popular and investigated class of all beta-lactamases enzymes. The gram negative bacteria such as *S.aureus, E.faecium,* A. *baumannii, K. pneumoniae, Enterbacter* and *P. aeruginosa* produce the class A enzyme giving them resistance to cephalosporins and penicillin class. KPC and TEM

and CTX-M beta-lactamases they produce are increasingly becoming an issue due to the over usage of oxyimino-cephalosporins like ceftazidime (Pandey et al., 2023).

Class B Beta-lactamases. The enzymes contain MBLs which is zinc-dependent and related to penicillin binding protein, belongs to the metallohydrolase superfamily (Pandey et al., 2023). This function at a substrate level; in the nucleic acid, RNA process and repair of DNA mechanisms in eukaryotes. MBLs are distinguished by the His,Xaa, Asp pattern that belong in the metallic center of the crosslinking of the two beta-sheets. The unique subfamilies are due to the types of the residues that are found in the core, including the chemistry and architecture (named B1, B2, and B3) (Pandey et al., 2023). A zinc center with tri-His (Zn1) and Cys-His-Asp (Zn2) metal sites is present in the most significant B1 enzyme; in B2 enzymes, the first His of the defining pattern is altered to Asn. (Pandey et al., 2023). This will then result in a mononuclear enzyme with only the Zn2 site occupied, and in B3 enzymes, the Zn2 coordinating Cys is supplemented by an one more His residue (Pandey et al., 2023). In the binuclear enzymes water completes the metal coordination. One of them is related to An2 ("apical" water), while the other one is the bridge that connects the two metal ions ("bridging" water). Tetrahedral is the definition of Zn1, while Zn2 is known as deformed square pyramid. With the exception of IMP enzymes, MBLs are zinc enzymes even though the other members of the superfamily use a wide variety of metal ions and they may be reassembled as distinct metal states. The MBLs are known as beta-lactamases for the large spectrum of action they perform. This includes penicillin, carbapenems and cephalosporins, however they have little action against nonbactams (Pandey et al., 2023).

Class C beta-lactamases. The class c are found in large numbers in the genes of most gram negative bacteria. The important pathogenic gram-negative bacteria have a genetic code for the enzymes of class C, with the abbreviation AmpC (expressed in unexpected occasions). The depression of these genes, by change from certain beta-lactams, results in increased levels of expression and then increase in MICs for susceptible beta-lactams. When certain enzymes are present like; DHA, CMY and FOX on transposons of bacteria the actions of class C enzymes are increased (Pandey *et al.*, 2023).

Class D Beta-lactamases. Class D OXA enzymes are most complex and the less understood of all the classes of beta-lactamase enzyme (Pandey et al., 2023). Initially the enzymes were only effective against penicillin, however today the OXA family currently includes enzymes that hydrolyses carbapenems and cephalosporins and have a large sensitivity against inhibitors. The proliferation of enzymes that hydrolyses carbapenem in *A.baumannii* and cephalosporinases in *P. aeruginosa* have raised the importance of the class. OXA enzymes have recently become active against gram-positive bacteria as-well (Pandey et al., 2023). Even due the plasmid-mediated oxacillin resistance caused by OXA enzyme transport was discovered in the 60s, the first structure were not available the end of the 20th century. The active site lysine (same as the Lys 73 of Class A enzymes) carboxylation as the critical indicator of the interaction for the reaction with carbon dioxide in the atmosphere. This gave new in-site to the mechanism of action of an otherwise already complex class (Pandey et al., 2023).

2.5. How Bacteria Gain Antimicrobial Drug Resistance

This is when the drug develops a defense against the antibiotics that previously worked on the antibiotic. This has become an increasing problem around the world, killing around 1 million people and 5 million associated deaths in 2019 alone (CDC, 2023). The problem affects all countries regardless of their economic levels, the US experiences 3 million reports of drug resistance every year and 48 thousand deaths every year (CDC, 2023).

Antibiotics act on micro-organisms by blocking the metabolic pathway such as the activities in the DNA (transcription and translation), protein synthesis, cell membrane synthesis or by inhibiting the processes involved with cell wall synthesis (as seen in beta-lactam drugs) (Duin et al., 2017). It is important to note that a bacterial cell wall is very essential for the survival of bacteria as is maintains the osmotic pressure, houses all bacteria organelles and provides protection from external elements. Bacteria are able to undergo chromosomal mutations or obtain extrachromosomal DNA and genetic elements (Duin et al., 2017). This addition of genetic information gives the bacteria new defense against the drug, such as reduced susceptibility to inhibition, change in the shape of the drug binding site or formation of enzyme that neutralize the drug. The bacteria may now have the ability to

chemically change the drug, degrade the enzymes and hydrolyze chemical bonds (Duin et al., 2017).

The most common type of Multi-drug resistance (MDR) is facilitated using the drug efflux pump (Yang et al., 2021). This is where there is an over expression of genes that code ATP-binding cassette transporter membrane proteins. This has the ability to remove drugs from the bacterial cell, protecting the bacteria from the effects of the drug. P-glycoproteins changes the permeability of the cell membrane, and with the use of ATP there is an efflux of the antibiotic out of the bacterial cell, resulting in a decreased intercellular concentration of the drug (Yang et al., 2021).

If one is to treat and prevent the spread of these resistant organisms, the best way is to identify the potential carriers, typically the patients who have the infection but show no signs and symptoms of the disease. Extended spectrum beta-lactamase bacteria are found through plasmid transfer, therefore both ESBL and non ESBL strains can be found on the genes of a single bacterium. This means that the colonies of the primary culture plate of the patient must be properly screened before any antibiotic treatments are administered. ESBL production is usually linked to the bacteria found in the gut like *Enterobacterales* (Yang et al., 2021). Therefore it is important to note that only beta-lactam antibiotics are effected by the resistance, other class of drugs such as fluoroquinolones, sulfonamides and aminoglycosides are also affected.

2.6. Beta-lactam Antibiotics Verse Extended Spectrum Beta-lactamase Bacteria

The defensive enzymes have the ability to bind and hydrolyze the beta-lactam rings of the antibiotic, making it useless and unable to perform the actions on the bacteria. The gram positive bacteria produce the enzyme on the external surface while the gram negative bacteria produce within the periplasmic space of the bacteria making their actions stronger (Yang et al., 2021). This is important to note as most *Enterobacterales* are gram negative. The names of the enzymes are related to the drug they work on, example penicillinase works on penicillin class of antibiotics (Yang et al., 2021).

2.7. Methods Used in Detecting Extended-spectrum Beta-lactamase

There are two main ways to test for the presence of extended spectrum beta-lactamase (Wang et al., 2021). The first method is to perform a screening test using cephalosporin indicators to search for resistance and reduced sensitivity, finding samples that are

likely to contain ESBLs (Wang et al., 2021). The next method is distinguishing isolates with ESBLs by looking for any interactions between clavulante and an oxyimino cephalosporin (Wang et al., 2021).

2.7.1. Screening Tests for ESBL Production

Dilution Antimicrobial Susceptibility Test. The CLSI uses dilution methods to screen for ESBL production in bacteria (Schumacher et al., 2018). In *Proteus,* a screening concentration of 1 μg/mL of cefotaxime, ceftrazidime or cefpodoxime are used while for the other drugs use 4μg/ml (Schumacher et al., 2018). In the instance of a bacterial growth at/ above the screening dosage of the drug, this will mean that the bacteria produces ESBL and must then be checked using phenotypic confirmation (Schumacher et al., 2018).

Disk-diffusion Method. This involves the identification of zone diameter of the antibiotic disks to see for possible presence of ESBL (Balouiri, 2016). The labs that use this method can also check for ESBL synthesis. The disk can contain any antibiotic of choice, however in this case cefpodoxime, ceftriaxone, ceftazidime, cefotaxime or aztreonam were used (Balouiri, 2016). The sensitivity to drugs are different depending on the drug, so this improves the detection (Balouiri, 2016).

Confirmatory Test for The Presence of Extended-spectrum Beta-lactamase

Double-Disc Synergy Test

This process is performed on a Mueller-Hinton agar contained dish, antibiotic test discs of third generation cephalosporin and amoxicillin-clavulante are placed 20mm from each other, following the guidelines of EUCAST (Uyanga, 2019). The cephalosporin inhibitory zone's sides, which are around the amoxillin-clavunate disc, is recorded as positive for ESBL production (Uyanga, 2019). Double disc diffusion test has sensitivities that are approximately from 77% to 95% and specificities ranging from 90% to 100%. The test is simple, however the results are subjective (Uyanga, 2019).

Combined Disc Test. ESBL confirmation test using a combination disc method are recently been implemented (Kumar, 2019). The zones of inhibition around an

antibiotic disc of a cephalosporin and a disc of a similar cephalosporin with clavulanate is evaluated using this method (Kumar, 2019). If there is a difference of ≥15mm seen with the two diameters this means that the bacteria been test is producing ESBL enzymes (Kumar, 2019).

Commercially Available Methods for ESBL Detections

E-test. The E-test strip has a thin, plastic with a 60mm by 5mm scaling and has two short gradients in opposing directions within the same strip (biomereieux, 2022). The strip has two sides, containing a gradient of the oxyamino cephalosporins on a side and a combination of cephalosporin and clavulanic acid on the opposite end (biomereieux, 2022). The sample is then incubated, the MIC value is measured at the point where the inhibition intersects with the end of the E-test strip (biomereieux, 2022).

Vitek ESBL test. The VITEK 2 ESBL test9bioMe'rieux) is an antibiotic susceptibility testing method used for the rapid detection of the presence of ESBL digitally (Spanu et al., 2006). It observes the constant inhibitory effects of cefepime (1g/ml), cefotaxime and ceftazidime either as a single unit (0.5 g/ml) or combined with the ESBL inhibitor clavulanic acid (4g/ml) (Spanu et al., 2006). After the samples are incubated, cards are introduced into the VITEK 2 machine, and turbidity is constantly observed for all the antibiotic been studied. Is there are any reduction in growth in clavulanic acid wells when compared to the wells that don't contain clavulanic acid is observed, the sample is recorded as ESBL positive (Spanu et al., 2006). Using the physical appearance of the susceptibility patterns with different beta-lactam antibiotics, computer programs in the VITEK system are used to identify beta lactamase production in gram negative bacteria samples (Spanu et al., 2006). Tests conducted with this method are recorded as minimum inhibitory concentration (MIC) values and are read as either susceptible, intermediate, or resistant (Spanu et al., 2006).

Becton Dickinson Phoenix Automated Microbiology System. The BD phoenix system, was made by BD biosciences and is a device used in the observation of bacteria that produce ESBL enzymes (Funke, 2004). The Phoenix ESBL test for any presence of ESBLs (Funke, 2004). It does this by check for the proliferation reaction of ceftriaxone, cefpodoxime, cefotaxome and ceftazidime with clavulanic acid and

without the drug. Within 5-7 hours, the results can be observed. ESBL production is observed in about 90% of ESBL producing bacteria using BD Phoenix ESBL automated detection system. The process has being also used in detecting ESBL presence in *Proteus, citrobacter* and *Enterobacter* spp. With *Klebsiella* and *E.coli* (Funke, 2004).

2.8. Recent Relevant Studies Related To the Topic

2.8.1. Study on Mobile Fosfomycin Genes.

Fosfomycin is unlike most drugs, it does not belong to any of the commonly known class of drugs. Fosfomycin belongs to a new family of phosphonic antibiotics and even though its name ends in -omycin, it isn't a macrolide (Katrin et al., 2020). The fosfomycin has bactericidal effects on its target bacteria and works by deactivating the enzyme UDP-N-acetylglucosamine-3-enolpyruvyltransferase, which is a times is called MurA. This prevents the forming of bacteria's cell walls (Katrin et al., 2020). The most important part in peptidoglycan synthesis, which involves ligating phosphoenolpyruvate (PEP) to the 3'-hydroxyl group of UDP-N-acetylglucosamine, is catalyzed by this enzyme. The substance that connects the peptide and glycan portions of peptidoglycan is provided by this pyruvate moiety. By alkylating a cysteine residue in the active region of the enzyme, fosfomycin, a PEP analog, inhibits MurA (Katrin et al., 2020).

Since the main dangers to the health and lives of people and animals is the increasing case of antibiotic resistance there has to be a new solution. Due to a lack of new antimicrobial medications, previous antibiotics like fosfomycin are being reconsidered as a possibility to treat for multidrug-resistant bacteria, particularly *Enterobacterales* that produce carbapenemase and extended-spectrum beta-lactamases. A broad-spectrum antibiotic with bactericidal properties, fosfomycin stops the beginning stage of cell wall synthesis. The development of fosfomycin-modifying enzymes or mutations in the drug absorption system can both lead to fosfomycin resistance.

In a study conducted by (Katrin et al., 2020), they concentrated fosfomycin resistant genes transcribes glutathione-S-transferase, including fosA and its subtypes, fosL1-L2 and fosC2, which are the causes of fosfomycin resistance in *Enterobacterales*. They provided information of the many resistance determinants'

putative origins and stated the plasmid linked to the spread of fosfomycin-changing enzymes (Ramos, 2019). The IncF and IncN plasmids thus have a major function. In recent years, there has been an upsurge in the discovery of mobile fosfomycin-resistant genes in *Enterobacterales*.

In Europe, fosA3 is the most commonly found fosfomycin-resistant gene, which is also just like the one in Asia (Silva, 2017). The detection of mobile fosfomycin-resistant genes in isolates originating from humans, animals, food, and the environment has raised concerns about the potential for the spread of these bacteria, particularly *Salmonella* and *Escherichia coli*, at the interface between humans, animals, and the environment (Katrin et al., 2020). They found that FosA was linked to the composite transposon Tn2921's insertion sequence IS2921. According to a study they did in Spain, fosA is no more limited to hospital settings as it was found in the sewage from six places that they examined. Patient samples from three hospitals were shown to have the fosA gene by hybridization, and species of *Enterobacterales*, *Pseudomonas* and *Acinetobacter* contained the gene. The concluded that some fosA varieties (fosA3) are more common than others, and their presence is often associated with plasmids (like the IncFII family or IncN).

2.8.2 Study on tetracycline and nitrofurantoin

It is known that resistant nosocomial infections, (like enterococci) have the capacity to gain resistance to all medically available antibiotics. They have a broad range of intrinsic and gained resistance determinants. For this reason, multidrug-resistant enterococci are seen as a major problem to public health (Ayesha et al., 2022). Due to the limited range of available treatments and the quick development of resistance to every new agents, the laboratory is needed in implementing precise, scalable, and workable antimicrobial susceptibility testing techniques to direct the right course of care for patients suffering from intense enterococcal infections (Ayesha et. al, 2022).

The team of (Ayesha et al., 2022) gave a summary of the benefits and limitations of the current manual and automated techniques for testing the susceptibilities of *Enterococcus faecium* and *Enterococcus faecalis* to daptomycin, β-lactams, aminoglycosides, vancomycin, lipoglycopeptides, oxazolidinones, and new tetracycline compounds. Along with providing recommendations for laboratories to avoid specific issues, they also found some issues and flaws with the performance and clinical use of antimicrobial susceptibility testing for enterococci (Ayesha et al., 2022).

The team addressed possible future innovations that could solve many current problems in susceptibility testing (Ayesha, et al., 2022*).*

2.4% of 697 VRE (616 *E. faecium* and 81 *E. faecalis*) in a 2003 multicenter U.S. trial were resistant to nitrofurantoin (Zhaniel, 2003). the team reviewed nitrofurantoin's susceptibility, which decreased dramatically over time (from 100% in 2005 to as low as 60%) in a study conducted in the United Kingdom from the early 2000s and 2014 using 5,528 enterococcal isolates from urine cultures at a tertiary hospital, of which 542 were VRE. The study also found that nitrofurantoin had increased effect against *E. faecalisthan E. faecium* (Zhang, 2021). They then compared their data with one from India using 239 *E. faecalis* isolates over a 10-year period found MIC50/MIC90 values for nitrofurantoin and fosfomycin, respectively, with an agar dilution of 8/64 and 8/16 mg/L (Zhang, 2021).

2.8.3. Study on Colistin

Colistin belongs to the class of antibiotics called polymyxins, which are cationic polypeptides (Stefaniuk et al., 2019). The team of Stefaniuk *et al.* (2019) evaluated the utility of the drug in human and animal well-being, plant husbandry and animal husbandry. The drug is also being used more and more as a final option for patients with complicated infections caused by gram-negative bacteria that are resistant to carbapenem. They believe that since colistin is used more frequently to treat infections brought on by multidrug-resistant (MDR) bacteria, this means that it is important to keep an eye on the antibiotic's resistance. It is possible for transposable genetic elements—such as plasmids containing the mcr genes—to encode bacterial resistance to colistin. The mcr gene has nine variations that have been found thus far, numbered mcr-1 through mcr-9. Lipopolysaccharide (LPS) alteration is linked to chromosomal resistance to colistin (Stefaniuk et al., 2019). A number of tools, like molecular biology and classical microbiology, help to locate the bacterial strains resistant to colistin and to pinpoint the mechanisms of resistance (Stefaniuk et al., 2019).

With the data from ECDC (2014), Gundogdu et al., 2018, they found out that Greece, Manco, Spain, and, Italy the colistin resistance rate increased to an average of over 30% of CRE isolates in 2013. As a result, these countries contributed 43, 31, and 20.8% of the total (Stefaniuk et al., 2019). The infections with colistin-resistant strains are also associated with an elevated fatality rate. The utilization of treatment alternatives for colistin-resistant MDR isolates depends on how sensitive the isolates

is, and colistin resistance complicates the selection of antimicrobial drugs. The characteristics of the isolates, the kind and location of infection, the PK/PD characteristics of the antibiotics, and any possible adverse effects (Petrosillo et al., 2019). They believe that colistin resistance in *Enterobacterales*, especially in *K. pneumoniae*, is a result of colistin usage in human medicine.

2.8.4. Study on Tobramycin

Multidrug-resistant gram-negative bacteria are a treat in healthcare and community environments. Natural resistance to the recent antibiotics in gram-negative bacteria according to Gupta et al., (2019), is mostly caused by the overexpressed efflux pumps and the bacteria's protective outer membrane. The team ran a number of combination treatments, or the use of two or more antibiotics which they referred to as hybrid treatments and it was a successful approach. The initial subject drug benefited from the combined actions of many antimicrobial abilities. This reduced the chance of possible resistance developing, decreased mortality, and enhanced clinical outcomes.

Unfortunately when the drugs were tested on the patients, there were not too many improvements (Gupta et al., 2019). As a strategy to deal with the issue of multidrug resistance and to test for the possibility of improved effectiveness of the antibiotics, antibiotic hybrids idea started. According to their work hybrids are synthetic creations comprising two molecules joined by a covalent bond are known as antibiotic hybrids (Gupta et al., 2019). These might be two antibiotics or an antibiotic combined with an adjuvant for example a siderophore to improve the drugs' ability to reach the target. The majority of their research was focused on tobramycin, aminoglycosides and fluoroquinolone compounds.

They ran tests on *Pseudomonas aeruginosa* on 2017, due to its multi-drug resistance problem at the time. They created tobramycin hybrids; tobramycin-moxifloxacin hybrid, tobramycin-EPSs conjugate hybrid and tobramycin-lysine peptoid hybrid .The antibiotic tobramycin has the ability to strengthen the effects of older antibiotics by enhancing the activity of antimicrobial drugs to which multidrug-resistant gram-negative bacteria were previously resistant. This is because tobramycin enters the cytosol by self-promoted uptake, this can help the transport of a second hybridized antibiotic within the cell (Gilbert et al., 2010). Another reason is aminoglycosides disrupt the bacterial membrane at higher concentrations and at lower concentrations inhibit protein translation by interacting at the rRNA level (Domalaon

et al., 2018). Tobramycin hybrids possess some properties that lead to the "resuscitation" of antibiotic efficacy against MDR bacteria, particularly *P. aeruginosa*, to which resistance has previously been present (Domalaon et al., 2018).

CHAPTER III

Materials and Methods

3.1. Study Design and Participants

Stock samples of patients from another study group done on the year 2022 were used. The stocks were re-purified and frozen from patients admitted at the Near East University Hospital on March and July 2019. Patients who had been admitted to the hospital and the second group were the controls, patients not admitted within 6 months before the study. The focus of the study was to test various drugs on only isolates that were ESBL-positive, therefore this means that out of the total patient sample n=52 were tested while controls n=23 were tested. The patients were required to be over the ages 18 and to have lived in the Turkish republic of Cyprus for over 12 months. 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). All personal information that could be link to the identity of the study participants was not revealed at any point in the study.

3.2. Samples, Participants' Data and the Bacterial Isolates

Enterobacterales samples from stool samples of patient volunteers were collected screened and purified and frozen at -20ºC as stock media samples were used for the study. The patients were surveyed to see for any history of antimicrobial use within six months prior to the sample collection.

3.3. Purification and Inoculation of Bacterial Isolates

The stock media isolates had to be purified and grown in either blood or eosin-methylene blue (EMB) agar, however all our samples were done on EMB using the streaking method. The agar plates are then left in the incubator at approximately 32-36ºC for 24 hours. The isolates were then sub-cultured to ensure for pure colonies of our bacteria of interest for a more accurate evaluation. The samples were then collected using swabs and mixed in a sterile 0.9% NaCl (normal saline) solution to prepare for a standard 0.5 McFarland reading. The standard 0.5 McFarland suspension was then applied on two Mueller Hinton plates for each patient with sterile swabs and labelled according to the appropriate patient number. Following proper aseptic techniques,

each of the two plates received 3-4 antimicrobial discs each 2 mm apart from each other. The antimicrobial discs of fosfomycin, tobramycin, tetracycline, colistin, amikacin, aztreonam and nitrofurantoin were placed on the agar plates. The Mueller Hinton plates were then incubated for approximately 24 hours, and the zone diameters were read, recorded, and then interpreted according to the Clinical and Laboratory Standards Institue (CLSI) 2023 guidelines. All agar, tubes, swabs and other tools were sterilized in an autoclave at 121ºC for 1 hour.

3.4. Statistical Analysis

For the survey's variables, descriptive statistics were acquired. Frequency and percentage information was given for categorical data, while the arithmetic mean, standard deviation, median, minimum, and maximum were computed for continuous variables. The relationships between categorical data were examined using either the Pearson Chi-square or the Fisher's exact test, depending on the sample sizes. All statistical computations were performed using the Macintosh version of the Jamovi statistics program (Version 2.3.21.0). A significance threshold of 0.05 was used.

CHAPTER IV

Results

4.1. The Study Population

75 patient stock samples were evaluated in this study with 52 patients from the location of the study, Near East University and a total of 23 control samples. All the bacteria samples used in this study, tested positive for extended beta-lactamase production. Samples collected from stool of patient participants were analyzed, 43(57%) were male and 32(43%) were female. The mean and median values for the sample age were 49 ± 21.17 and 49.99 (19.00 – 93.00). The age of the subjects were grouped as; for the age 19-30 were a total of 20 (27%), and ages from 31 and higher were 55 (73%). They were also group according to educational background, based on with university degree and above 31 (41%) and below university degree 44 (59%). 20 (27%) were married, and 55 (73%) were single. 63 (84%) were in the middle class and 2 (3%) were in the low income status.

In the population, 25 (33%) patients stated that they were on some GIS when their samples were collected. The patients were also questioned on whether they were taking any antimicrobials in the past 6 months during sample collection and 44 (59%) confirmed to taking some. 21 (28%) stated that they had experienced diarrhea, while 10 (13%) stated that they had UTI in the past 6 months before the sample collection. Out of the total study population 39 (52%) patients had travelled to countries outside the Turkish republic of northern Cyprus. 28 (37 %) travelled to Turkey or European countries, 11 (15%) to Asia or African countries within 6 months before sample collection.

The number of patients that were hospitalized for 3 or more days were 13 (17%). Out of 13 hospitalized patients, 7 (54%) patients were admitted in the ICU, 6 (46%) had surgery been performed to them and 7 (54%) had urinary catheters on them. These hospitalized patients 1 (8%) had been on antibiotics were admitted.

4.2. The result of the disc diffusion test on patient samples

This study consisted of seven antimicrobials. Fosfomycin, colistin, tobramycin, amikacin, aztreonam, tetracycline and nitrofurantoin. Two plates of agar Mueller Hinton were used for each samples tested. In one of the duel plates, four antibiotic disc

were added and the other would have three. The diameters were measure and according to the CLSI guidelines, the drug was either designated Susceptible (S), intermediate (I) or resistant (R).

When fosfomycin was tested in the study samples, 51 samples were susceptible (98%), no sample had intermediate reading (0%) and one sample was resistant (2%), while for the control group 21 samples were susceptible (91%), no intermediate sample (0%) and two samples were resistant (9%) to the drug. Tobramycin had 42 susceptible samples (81%), eight samples (15%) were intermediate and only two (4%) were resistant to the drug, while in the control group 22 samples were susceptible (96%), one sample was intermediate (4 %) and no sample was resistance to the drug (0%). Amikacin According the patient sample readings 51 were susceptible (98%), one sample was intermediate (2%) and no sample was resistant (0%) while for the control samples 21 were susceptible (91%), one sample was intermediate (4%) and one resistant to the drug (4%).

Aztreonam 43 samples were susceptible (83%), 3 samples were intermediate (6%) and six samples were resistant to the drug (11%), while in the control group, there were 14 susceptible samples (61%), five intermediate samples (22%) and four resistant samples (17%). According to the tests done with tetracycline 23 (44%) samples were susceptible, eight samples (16%) were intermediate and 21 samples (40%) were resistant while in the control group, seven samples were susceptible (30%), six samples were intermediate (26%) and 10 samples showed resistance to the drug (44%). Nitrofurantoin disc diffusion test the patient sample readings show 51 susceptible (98%), no samples were intermediate (0%) and one sample was resistant (2%) while the controls show 21 samples to be susceptible (91%), two intermediate samples (9%) and no resistant samples (0%).

Colistin does not have a direct reading of zone diameter range in the 2023 CSLI guidelines. However the drug was test on all the samples and the zone diameter readings are available.

4.3 Statistical Analysis

According to the rate of resistance for drug in the sample isolates were done using Pearson Chi-square was used to examine the connections between categorical data. There were no statistical significance between patient and controls in terms of

resistance. Antibiotic use in the last six months had no significant effect on resistance as well.

The *p* values between patient and controls in terms of resistance are; for fosfomycin, *p* value was 0.221, the *p* value for tobramycin was 1.000, the *p* value for amikacin was 0.307, the *p* value for aztreonam was 0.443, the *p* value for tetracycline was 0.802 and the *p* value for nitrofurantoin was 1.000.

The *p* value for the association between drug resistance in the study samples and antibiotic use within the last 6 months; fosfomycin *p* value was 0.067, tobramycin *p* value was 0.168, amikacin *p* value was 1.000, aztreonam *p* value was 0.728, tetracycline *p* value was 0.929, nitrofurantoin *p* value was 0.413.

Table 1. Susceptibility Rates of Antibiotics among the Patient Group (n=52)

	Total count for each drug in patient samples		
ANTIBIOTIC DISC	S	I	R
Fosfomycin (FF 300)	51	0	1
Colistin (CT10)	N/A	N/A	N/A
Tobramycin (TOB 10)	42	8	2
Amikacin (AK 30)	51	1	0
Aztreonam (ATM 30)	43	3	6
Tetracycline (TE 30)	23	8	21
Nitrofurantoin (F 300)	51	0	1

Table 2. Susceptibility Rates of Antibiotics among the Control Group (n=23)

	Total count for each drug in control samples		
Antibiotic disc	S	I	R
Fosfomycin (FF 300)	21	0	2
Colistin (CT10)	N/A	N/A	N/A
Tobramycin (TOB 10)	22	1	0
Amikacin (AK 30)	21	1	1
Aztreonam (ATM 30)	14	5	4
Tetracycline (TE 30)	7	6	10
Nitrofurantoin (F 300)	21	2	0

Table 3. The count of the rate of susceptibility for colistin according to various ranges. This was done due to the absence of zone diameter breakpoint in the 2023 CLSI guideline.

Participants	Colistin (CT10) (1-9) n/N (%)	Colistin (CT10) (10-19) n/N (%)	Colistin (CT10) (20-29) n/N (%)
Patients	1/52 (1.9)	42/52 (80.8)	9/52 (17.3)
Controls	0/23 (0.0)	19/23 (82.6)	4/23 (17.4)
Total	1/75 (1.3)	61/75 (81.3)	13/75 (17.3)

Table 4. Identification of Enterobacterales species among ESBL-positive isolates

Participants	*Escherichia coli* n/N (%)	*Klebsiella pneumoniae* n/N (%)	*Enterobacter cloacae complex* n/N (%)	*Citrobacter freundii* n/N (%)	Unidentified n/N (%)
Patients	48/52 (92.3)	1/52 (1.9)	1/52 (1.9)	1/52 (1.9)	1/52 (1.9)
Controls	20/23 (86.9)	0/23 (0.0)	0/23 (0.0)	0/23 (0.0)	3/23 (13.0)
Total	68/75 (90.6)	1/75 (1.3)	1/75 (1.3)	1/75 (1.3)	4/75 (5.3)

Table 5. Resistance rates of fosfomycin in the bacterial isolates.

Participants	Fosfomycin Non-resistant n (%)	Resistant n (%)	Total
Controls	21 (91.3)	2 (8.7)	23 (100)
Patients	51 (98.1)	1 (1.9)	52 (100)
Total	72 (96.0)	3 (4.0)	75 (100)

The *p* value for fosfomycin was 0.221

Table 6. Resistance rates of tobramycin in the bacteria isolates.

Participants	Tobramycin Non-resistant n (%)	Resistant n (%)	Total
Controls	23 (100)	0 (0.0)	23 (100)
Patients	50 (96.2)	2 (3.8)	52 (100)
Total	73 (97.3)	2 (2.7)	75 (100)

The *p* value for tobramycin was 1.000.

Table 7. Resistance rates of amikacin in the bacteria isolates.

Participants	Amikacin Non-resistant n (%)	Resistant n (%)	Total
Controls	22 (95.7)	1 (4.3)	23 (100)
Patients	52 (100)	0 (0.0)	52 (100)
Total	74 (98.7)	1 (1.3)	75 (100)

The *p* value for amikacin was 0.307.

Table 8. Resistance rates of aztreonam in the bacteria isolates.

Participants	Aztreonam Non-resistant n (%)	Resistant n (%)	Total
Controls	19 (82.6)	4 (17.4)	23 (100)
Patients	47 (90.4)	5 (9.6)	52 (100)
Total	66 (88.0)	9 (12.0)	75 (100)

The *p* value for aztreonam was 0.443.

Table 9. Resistance rates of tetracycline.

Participants	Tetracycline Non-resistant n (%)	Resistant n (%)	Total n (%)
Controls	13 (56.5)	10 (43.5)	23 (100)
Patients	31 (59.6)	21 (40.4)	52 (100)
Total	44 (58.7)	31 (41.3)	75 (100)

The *p* value for tetracycline was 0.802.

Table 10. Resistance rates of nitrofurantoin in the bacteria isolates.

Participants	Nitrofurantoin Non-resistant n (%)	Resistant n (%)	Total
Controls	23 (100)	0 (0.0)	23 (100)
Patients	51 (98.1)	1 (1.9)	52 (100)
Total	74 (98.7)	1 (1.3)	75 (100)

The *p* value for nitrofurantoin was 1.000.

Table 11. Association of fosfomycin resistance with antibiotic use in the last six months before the study.

Antibiotics use for the last six months	Fosfomycin Non-resistant n (%)	Resistant n (%)	Total
No	28 (90.3)	3 (9.7)	31 (100.0)
Yes	44 (100.0)	0 (0.0)	44 (100.0)
Total	72 (96.0)	3 (4.0)	75 (100.0)

The *p* value was 0.067.

Table 12. Association of tobramycin resistance with antibiotic use within the last six months

Antibiotics use for the last six months	Tobramycin Non-resistant n (%)	Resistant n (%)	Total
No	29 (93.5)	2 (6.5)	31 (100.0)
Yes	44 (100.0)	0 (0.0)	44 (100.0)
Total	73 (97.3)	2 (2.7)	75 (100.0)

The *p* value was 0.168.

Table 13. Association of amikacin resistance with antibiotic use within the last six months.

Antibiotics use for the last six months	Amikacin Non-resistant n (%)	Resistant n (%)	Total
No	31 (100.0)	0 (0.0)	31 (100.0)
Yes	43 (97.7)	1 (2.3)	44 (100.0)
Total	74 (98.7)	1 (1.3)	75 (100.0)

The *p* value was 1.000.

Table 14. Association of aztreonam resistance with antibiotic use within the last six months

Antibiotics use for the last six months	Aztreonam Non-resistant n (%)	Resistant n (%)	Total
No	28 (90.3)	3 (9.7)	31 (100.0)
Yes	38 (86.4)	6 (13.6)	44 (100.0)
Total	66 (88.0)	9 (12.0)	75 (100.0)

The *p* value was 0.728.

Table 15. Association of tetracycline resistance with antibiotic use within the last six months

	Tetracycline		
Antibiotics use for the last six months	**Non-resistant n (%)**	**Resistant n (%)**	**Total**
No	18 (58.1)	13 (41.9)	31 (100.0)
Yes	26 (59.1)	18 (40.9)	44 (100.0)
Total	44 (58.7)	31 (41.3)	75 (100.0)

The *p* value was 0.929.

Table 16. Association of nitrofurantoin resistance with antibiotic use within the last six months

	Nitrofurantoin		
Antibiotics use for the last six months	**Non-resistant n (%)**	**Resistant n (%)**	**Total**
No	30 (96.8)	1 (3.2)	31 (100.0)
Yes	44 (100.0)	0 (0.0)	44 (100.0)
Total	74 (98.7)	1 (1.3)	75 (100.0)

The *p* value was 0.413.

CHAPTER V

Discussion

5.1 Fosfomycin

The drug had 52 samples susceptible out of 51 samples (98%), 0 intermediate (0%) and one (2%) resistant isolate, while the control samples 21 samples were susceptible (91%) no intermediate sample (0%) and two resistant samples (9%) (p=0.221). Just like the other drugs we tested, fosfomycin had no significant statistical association between the rate of resistance and antibiotic use (p=0.067), however unlike the other drugs that were tested fosfomycin had huge diameter differences when compared with the standard diameter range of the CLSI guidelines. To put in better words, the zone diameter breakpoints of fosfomycin is ≥ 16 mm (susceptible), 13-15 mm (intermediate) and ≤ 12 mm (resistant), however the drug had diameters consistently ranging for 33 to 45 mm. This is in contrast with the other drugs with diameters which were close to their zone diameter breakpoints. There were about nine patient samples which passed 40 mm, giving almost 29 mm difference from the susceptible range for the drug.

A study done by Ito and his team, in more than 18,000 genome sequences from 18 gram-negative species, they conducted an investigation to see if there were an existence and distribution of the fosA genes (Ito et al., 2017). They stated that the resistance that was found in fosfomycin, was due to the fosA gene in some bacterial genome. In their analyzes, they found high prevalence of fosA (more than 83%) in the genomes of *Klebsiella oxytoca, Pseudomonas aeruginosa, Enterobacter cloacae, Providencia stuartii, Enterobacter aerogenes, Providencia rettgeri,* and, *Morganella morganii*. They showed the chromosomal placement of fosA in these genomes (ito et al., 2017). However, fosA was barely (less than 7%) found in *E. coli*, *Citrobacter freundii* and *Acinetobacter baumannii.* Majority of our bacteria sample had metallic sheen appearance on EMB agar plate which suggests *E. coli*. Moreover, they could give a wide range of variability in FosA sequences within and through species. (ito et al., 2017)

Fosfomycin is unlike most drugs, it does not belong to any of the commonly known class of drugs. Fosfomycin belongs to a new family of phosphonic antibiotics and even though its name ends in -omycin, it isn't a macrolide. When compared to

other extended-spectrum antibiotic regimens for UTIs, fosfomycin is a very effective antibiotic for single-dose intake. It is a costly medication (about $89) because of its strong bactericidal abilities against both gram-positive and gram-negative bacteria. The benefits and applications of this drug much exceed any possible risks and precautions. This makes it less accessible to the public and thus not frequently used in the Turkish republic of Northern Cyprus. This however may be a factor that contributes to our *enterobacterales* samples not showing signs of resistance.

5.2 Tetracycline

Tetracycline like fosfomycin is an anomaly, it shows some unique results that are different from the other drugs on the list. However unlike fosfomycin, almost half of the samples from both the patient and the control group show consistent patterns of resistances. Tetracycline 23 (44%) samples were susceptible, eight samples (16%) were intermediate and 21 (40%) were resistant while the controls were seven samples susceptible (30%), six samples were intermediate (26%) and 10 resistance samples (44%) (p=0.802). Unlike the drugs, tetracycline also had numerous reads of 0 zone diameter, meaning the drug didn’t work on the sample at all. It also had no significant statistical association between the rate of resistance and antibiotic use (p=0.929).

This is interesting because tetracycline is not in the beta-lactam drug class and does not contain the beta-lactam ring and hence the samples being beta-lactamase producers should not be a contributing factor to their resistance. Being "broad-spectrum" antibiotics, tetracycline exhibit strong bacteriostatic action against a variety of infections, including *Ureaplasma* and *Mycoplasma* (who do not have cell walls) (Ahmadi, et al. 2021). Despite being separated among three generations, tetracyclines all share the same methods of action: first-generation drugs that come from biosynthesis, like traditional tetracycline (Ahmadi, et al. 2021). Semi-synthetic second-generation antibiotics with enhanced characteristics and a broader range of action include doxycycline and minocycline. The third-generation synthetic drug with the strongest and broadest action towards both gram-positive and gram-negative bacteria is tigecycline. They reach the target via piercing the bacterial membrane. Tetracycline antibiotics have the ability to connect to the bacterial ribosome's 30S subunit in a reversible manner. This contact prevents aminoacyl-tRNA from attaching to the ribosome's acceptor site, which results in inhibition of protein production (Ahmadi, et al. 2021).

A meta-analysis done by wen et al., (2023) where 26 studies were used to analyze proportions of tetracycline, doxycycline, and minocycline resistance in 15 countries. For tetracycline twenty experiments were reviewed to assess the tetracycline susceptibility of 19,424 isolates of *Ureaplasma* and *Mycoplasma*, including *U. urealyticum* (17,871 isolates), *U. parvum* (434 isolates), and *M. hominis* (984 isolates). Tetracycline had the highest prevalence of resistance among these three antibiotics, most likely as a result of widespread over and misusage of the drug. They reported that therapeutic failure leads to the spread of resistant strains and recurrent infections. According to other research, the rate of tetracyclines resistance is rising and fluctuating. Therefore, the effectiveness of treating subsequent infections with these antibiotics should be reduced, more difficult, and more expensive.

Bacteria resistant to tetracyclines placed resistance-encoding genes onto plasmids and transposon elements (Boujemaa et al., 2020). Because of their movement, these components allow bacteria to spread their resistance to other bacteria and pass on genes horizontally (Boujemaa et al., 2020). Two main processes are linked to the development of tetracycline resistance in *Mycoplasma* spp.; the tet (M) gene produces ribosome-protecting proteins and an active drug efflux pump. This transposon gene produces the tetM protein, which inhibits tetracycline binding and induces conformational changes in the 30S ribosomal subunit. Tetracycline resistance is also caused by a number of other processes, such as a reduction in the amount of antibiotics that enter the cell, antibiotic modifications caused by the bacteria, and changing the target site caused by a mutation in the tetracycline-binding unit of 16S rRNA (Boujemaa et al., 2020).

5.3. Tobramycin

The drug was susceptible in 42 samples which is 81% of the samples, eight samples (15%) were intermediate and only two samples (4%) were resistant while controls had 22 susceptible samples (96%), one intermediate sample (4 %) and no resistant sample (0%) (p=1.000). This shows the drug responded when to the samples. Unlike fosfomycin there were no, outlandish zone diameter sizes. There were close to the standard guideline diameters of the CLSI.

Tobramycin in an Aminoglycoside and not a beta-lactam, this means instead of containing a beta-lactam ring to disrupt bacterial cell wall, they stop the production of proteins and contain a component of an amino-modified glycoside (sugar) (Richard,

2022). They inhibit the protein synthesis of bacteria by irreversible binding, to the membrane of bacterial 30S ribosome through the help of the aminoglycosides' energy. When the antibiotic enters the cytosol of the bacteria, they disturbs peptide elongation at the 30S ribosomal subunit, this results in incorrect mRNA translation (Richard, 2022).

However multidrug-resistant gram-negative bacteria are a treat in healthcare and community environments. Natural resistance to the recent antibiotics in gram-negative bacteria according to Gupta et al., (2019), is mostly caused by the overexpressed efflux pumps and the bacteria's protective outer membrane. The team ran a number of combination treatments, or the use of two or more antibiotics which they referred to as hybrid treatments and it was a successful approach. The initial subject drug benefited from the combined actions of many antimicrobial abilities. This reduced the chance of possible resistance developing, decreased mortality, and enhanced clinical outcomes. Unfortunately when the drugs were tested on the patients, there were not too many improvements (Gupta et al., 2019).

5.4. Aztreonam

Aztreonam 43 samples were susceptible (83%), three samples were intermediate (6%) and six samples were resistant (11%), while the controls were 14 susceptible (61%), five intermediate samples (22%) and four resistant samples (17%) (p=0.443). The tests showed very unremarkable results at first glance, however aztreonam is a beta-lactam drug in the class of monobactams. This is unusual as the samples used in the study are all extended spectrum beta-lactamase producers, and so these results should show a lot more resistance.

According the drug description by drugbank.com, because the drug's chemical structure shows huge levels of resistance to break-down by beta-lactamases (enzymes such as cephalosporinases) created by almost all gram-negative and gram-positive bacteria, aztreonam is different from most other beta-lactam antibiotics in that it may not produce beta-lactamase activity (Drugbank.com, 2023). As a result, it sometimes works against gram-negative aerobic pathogens that are resistant to antibiotics hydrolyzed by beta-lactamases. The drug works against a variety of bacteria that are resistant to many antibiotic classes, including aminoglycosides, penicillin, and some cephalosporins (Makii et al., 2022). A study from (Morroni et al., 2021) MBLs, or metallo-β-lactamases, which are amongst the hardest bacterial enzymes to combat

when present in bacteria. The only β-lactam that is not hydrolyzed by MBLs is aztreonam (ATM), yet co-produced extended-spectrum β-lactamases (ESBL) frequently inactivates it (Morroni et al., 2021). However MBLs are only produced in 10% of all beta lactamase producing bacteria, but still interesting to note.

5.5. Amikacin

According the patient sample readings 51 were susceptible (98%), one sample was intermediate (2%) and no resistant samples (0%) while for the control samples 21 were susceptible (91%), one intermediate sample (4%) and one resistant sample (4%) (p=0.307). The readings showed no unusual results amikacin an aminoglycoside so beta-lactamases should not contribute to resistance. Kanamycin A is the drug where amikacin originates from, this makes it a semi-synthetic aminoglycoside antibiotic. Label l-(-)-γ-amino-α-hydroxybutyryl side chain is acylated at the C-1 amino group of the deoxystreptamine moiety of kanamycin A to produce amikacin. (Remirez, 2017)

One of amikacin's special qualities is that it works against gram-negative bacteria that are more resistant, examples are *Acinetobacter baumanii* (Nesbitt, 2023). Moreover, amikacin has special effectiveness against the aerobic gram-negative bacteria belonging to the *Enterobacterales* family, as well as *Nocardia* and some *Mycobacterium* (Remirez, 2017). This could explain the high susceptible rates in the result readings.

5.6. Nitrofurantoin

The patient sample readings show 51 susceptible samples (98%), no intermediates (0%) and one resistant sample (2%) while the controls show 21 susceptible (91%), two intermediate samples (9%), no resistant isolates (0%) (p=1.000). The result show little to no resistance for the drug, because up until the 1970s, when trimethoprim-sulfamethoxazole and beta-lactam antibiotics became accessible, nitrofurantoin was a commonly used treatment for lower urinary tract infections (Langoya, 2023). The drug was no longer used as much, however prescriptions for nitrofurantoin have increased again due to rising resistance to newer antibiotics and an increase in the frequency of bacteria that produce extended-spectrum beta-lactamase (ESBL).

Lower urinary tract infections that are not too complex are treated with the antibiotic nitrofurantoin (Langoya, 2023). It works well against the majority of both

gram-positive and gram-negative bacteria. A synthetic antibacterial called nitrofurantoin is made from furan, an additional nitro group, and a side modification that contains hydantoin (Langoya, 2023).

5.7. Colistin

Colistin like previously mentioned does not have a direct reading of zone diameter range in the 2023 CSLI guidelines. However the drug was test on all the samples and the zone diameter readings are available. In Table 4.3, the readings were written according to a range of numerical values. The values were 1-9, 10-19 and 20-29, the amount of samples that had fallen in these ranges were stated and the percentages were calculated. The zone diameter are still not known, so it is still difficult to tell what the susceptibility of the drug.

CHAPTER VI

Conclusion

Majority of the samples had little to no resistance to some of the drugs, fosfomycin being the most effective of the drugs. Tetracycline was the most resistant of the drugs with consistent readings on both the controls and the patient samples, although it isn't a beta-lactam drug. Monobactam the one beta-lactam on the list didn't show much resistance, however this could be due to its natural resistance to some beta-lactamase enzymes. Overall the drugs tested all had their unique results, however with the readings one can say the extended spectrum beta-lactamase producing samples susceptible to most of the drugs tested.

The drugs show a high susceptibility rate and there are little resistance amongst them. This could be because of the infection control practices that are applied routinely in the near east university hospital.

However due to reports from studies, their rates of resistance are increasing with the years. This means that even though these drugs have a low rate of resistance, they should still be prescribed by doctors with care. Proper diagnostic and antibiotic susceptibility tests must be done before giving these drugs to patients, in order to minimize the chance of drug resistance developing in the future.

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