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Study the Role of Carbonyl Cyanide 3-Chlorophenyl-hydrazine as efflux pump inhibitor among Enterobacteriaceae isolates in Babylon, Iraq

A Thesis

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بسم الله الرحمن الرحيم

{ يَرْفَعِ اللَّهُ الَّذينَ آمَنُوا مِنْكُمْ وَ الَّذينَ أُوتُوا الْعِلْمَ

دَرَجاتٍ وَ اللَّهُ بِما تَعْمَلُونَ خَبير}

صدق الله العلي العظيم

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Supervision Certification

I certify that this thesis entitled **" Study the Role of Carbonyl Cyanide 3-Chlorophenyl- hydrazine as efflux pump inhibitor among Enterobacteriaceae isolates in Babylon, Iraq "** was prepared under my supervision by " **Hadeel Qassem Jabr Al_Issawi** " at the Department of Microbiology, College of Medicine/ University of Babylon, as a partial fulfillment of the requirements for the degree of Master of Science in Microbiology.

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Dedication

To ...

To my master the owner of the matter, Imam **Al-Hujjah Ibn Al-Hassan** (May God hasten his reappearance)

To my master, **Aba al-Fadl** (peace be upon him)

To the light that illuminates my path **(my father)**

To the candle of my life that eased my hard days **(my mother)**

To my brothers and sisters

To everyone who loves science and teaches it honestly

I dedicate to them the fruits of my humble labor.

Hadeel 2023

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Summary

Multidrug-resistant (MDR) Enterobacteriaceae are an emerging and a major concern for the medical community. Reported rates of Multidrug-resistant Enterobacteriaceae are increasing, and infections with these organisms are no longer limited to those associated with healthcare in the severely ill or infirm. Community-acquired infections are now described. The purpose of this study was to investigate the role of active efflux system to Aminoglycoside and Quinolones resistance in clinical isolates of Enterobacteriaceae using the efflux pump inhibitor CCCP.

A total of 300 different clinical samples were collected from patients (females 172:57.3%; males 128:42.6%) with urinary tract infection (urine), reproductive system (swabs), patients with bacteremia (blood) and lower respiratory tract infection (sputum) in Al-Mahaweel General Hospital, Marjan Teaching Hospital, and private laboratories during the period from November 2022 to March 2023.

The results found that out of 300 samples 280(93%) were culture-positive and 20(6.6%) culture-negative clinical samples. The results found that out of 280 clinical samples, only 134 (47.85%) samples were belonged to Enterobacteriaceae. The remaining 146 (52.14%) samples were belonged to other types of bacteria.

After observing the cultural and morphological characteristics of the bacterial isolates were as follows: E.coli n=94 isolates belonging to were obtained *Klebsiella. pneumonia*e n=24 , *Proteus*.spp n=7 and *Enterobacter*.spp n=9 .

The identification of all Enterobacteriaceae isolates was confirmed by Vitek-2 automated identification system.The results of isolation of Enterobacteriaceae revealed high percentage of *E.coil* 94 isolates distributed as urinary tract infections (UTIs) (43 isolates), lower respiratory tract infection

(LRTIs) (20 isolates), (17 isolates) for Bacteremia, (14 isolates) for bacterial vaginitis. results of isolation of *K.pneumoniae* 24 isolates from (UTIs) (4 isolates), (RTIs) (8 isolates), (12 isolates) for Bacteremia. *Enterobacter.spp* 9 isolates, from (UTIs) patients (7 isolates), (2 isolates) for Bacteremia. *Proteus.spp* 7 isolates,6 from (UTIs) (1 isolate) for Bacteremia.

Regarding age groups, the results showed that infection with *E. coli* and *K.pneumonieae* among female more than male. However, the results showed that infection with *Enterobacter*.spp and *Proteus*.spp among male more than female.

The antibiotic susceptibility (AST) of all Enterobacteriaceae isolates was performed by Vitek 2 system and also disk diffusion test (using 8 antibiotics).The results of the present study showed that in general all Enterobacteriaceae isolates were resistant to fluoroquinolones more than aminoglycosides.

The Antibiotic Resistance patterns (ARP) of Enterobacteriaceae isolates was determined depended on the results of Vitek 2 system (Index-1).The results showed that ARP of Enterobacteriaceae isolates found that out of 134 isolates, 61 were sensitive and 73 showed different antibiotic resistance patterns as follows: 48 were multidrug resistant, 17 were extensive drug resistance, and 8 were pandrug resistant.

In this study, oqxAB genes were detected in selected clinical isolates of Multidrug resistant, extensive drug resistance and pandrug resistant Enterobacteriaceae. The selected isolates were as follows: 12 XDR *E. coli* isolates ,one PDR *K. pneumoniae* , and one MDR *Enterobacter* spp. isolate. Analysis of PCR results showed that 6 (42.8%) out of 14 isolates were positive for oqxA gene, and 8 (57.1%) of the isolates were negative for *oqx*A gene, Howerver, no isolates showed positive results for the *oqx*B gene.

In order to confirm the effective role of the efflux pump in our isolates, the MICs of 3 antibiotics were compared with and without the efflux pump inhibitor (CCCP). The results of MIC for 14 Enterobactriecae isolates against these three antibiotics showed that all isolates had MIC ≥128 μg/mL in the absence of CCCP for Levofloxacin, Ciprofloxacin, and Gentamycin. The results showed the MIC of Levofloxacin and Ciprofloxacin were reduced for isolates, and the growth of bacteria was inhibited in presence the inhibitor (CCCP). However,all Enterobacteriaceae isolates showed high MIC values (≥ 128) even in the presence of the CCCP which indicates no effect the inhibitor in reducing the MIC of the isolates for Gentamycin.

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List of Abbreviations

Symbol	Description
ABC	ATP-binding cassette
AK	Amikacin
ANT	Adenylation Nucleotidyltransferases
AST	Antibiotic Susceptiblity Test
ATP	Adenosine TriphosPhate
BP	Base pair
CCCP	Carbonyl Cyanide-m-ChloroPhenylhydrazone
CDC	Center for Disease Control and prevention
CFU	Colony Forming Units

CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CN	Gentamicin
COPD	Chronic Obstructive Pulmonary Disease
EMB	Eosin Methyline Blue
EPIs	Efflux pumps inhibiters
EPs	Efflux pumps
GAT	Gatifioxacin
HLS	Streptomycin
ICU	Intensive Care unit
IDSA	Infectious Diseases Society of America
K	Kanamycin
LE	Levofloxacin
LPS	Lipopolysaccharide
MATE	Multidrug And Toxic compound Extrusion
MDR	Multi-Drug Resistance
MFP	Membrane Fusion Protein
MFS	Major Facilitator Superfamily
MIC	Minimal Inhibitory Concentrations
MRSA	Methicillin-resistant Staphylococcus aureus
NBDs	Nucleotide-Binding Domains
NX	Norfloxacin
PAβN	Phenylalanyl Arginyl β-Naphthylamide
PDR	Pan Drug Resistant
PMQR	Plasmid- Mediated Quinolones Resistance
QRDR	Quinolone Resistance-Determining Region
RND	Resistance Nodule Division
RTIs	Respiratory Tract Infection
SDS	Sodium Dodecyl Sulfate
SMR	Small Multidrug Resistance

TBE	Tris-Borate-EDTA
UTIs	Urinary Tract Infections
WHO	World Health Organization
XDR	Extensively drug resistant

Chapter one

Introduction and Literature Review

1.1 Introduction

Members of the family Enterobacteriaceae are responsible for a wide variety of nosocomial and community-acquired infections which were significantly associated with the increasing hazard of death and excess length of stay and costs, certain strains of Enterobacteriaceae have developed resistance to many commonly used antibiotics, and multidrug resistance is often the cause of antibiotic treatment failure (Wang *et al.*, 2022).

One of the antibiotic resistance mechanism Decreased drug accumulation due to adventitial membrane impermeability or overexpression of active efflux pumps. (Smithers *et al.*, 2021).

The efflux system consists of transporters that pump various toxic substrates, such as antibiotics and bactericides, from bacteria in an energy-dependent manner. In these cases, the intracellular concentration of the antibiotic decreases and the bacteria become less sensitive to the compound. Avakh *et al.*, 2023).

Strains overexpressing efflux pumps typically have antibiotic minimum inhibitory concentrations (MICs) two to eight times higher than susceptible strains of the species.

To study the role of drug efflux mechanisms in bacteria, efflux pump inhibitors (EPIs) are often used to completely block the efflux of various molecules .(Nguyen *et al.*, 2023).

One such compound is carbonyl cyanide 3-chlorophenylhydrazone (CCCP), an oxidative phosphorylation uncoupler that disrupts the proton gradient of membranes, This has often been found to increase the susceptibility of many multidrug-resistant bacteria, including members of the family Enterobacteriaceae. (Meka 2023; Roy 2023).

Aim of the study:

The aim of this study was to investigate the role of active efflux system to Aminoglycoside and Quinolones resistance in clinical isolates of Enterobacteriaceae using the efflux pump inhibitor CCCP.

Objective of the study:

To achieve this aim, the following objectives should be followed:

1. Isolation and identification of Enterobacteriaceae from different clinical samples.
2. Determination of antibiotic susceptibility (AST) and antibiotic resistance patterns of the Enterobacteriaceae isolates using disk diffusion test (DDT), and Vitek 2 system.
3. Phenotypic detection the presence of efflux mechanism using CCCP in Aminoglycoside and Quinolones resistant Enterobacteriaceae isolates.
4. Genotypic detection of and prevalence of oqxA and oqxB genes of efflux pumps among MDR Enterobacteriaceae isolates.
5- Effect of the inhibitor CCCP in reducing the MIC of antibiotics for Enterobacteriaceae isolates.

1.2 Literature Review

1.2.1 Enterobacteriaceae

The enteric family includes a large variety of bacterial species that live in the intestines of humans and animals naturally and its members are rod-shaped, Gram-negative, aerobic, or facultative aerobic anaerobic, and its members can cause many diseases in humans and animals, including wound infections and hospital-acquired infections and nosocomial infections, respiratory infections, urinary tract infections, and reproductive tract infections (Oliveira *et al*., 2017). It has different virulence factors as toxins ، enzymes also ferment a wide variety of carbohydrates, most of which are fermented into the sugar lactose. and has the ability to reduce nitrate to nitrite for energy production, non-spore, optimum temperature for its growth (Nasiru *et al.*,2022).

The natural habitat, human and animal intestines, including the family Many pathogenic genera, the most important of which are *Shigella Salmonella, Klebsiella*, and others, and some of them It is a natural flora such as *E. coli* (Dinev *et al.*,2023)**.**

All have small Straight or curved, up to 1.5 µm in width. Some are immobile, while others are active and have peri-flagellates. Catalase (+) and Oxidase (-). Enterobacteriaceae are always oxidase (-) and are therefore distinct from oxidase (+) bacteria such as *Pseudomonas, Aeromonas*, *Vibrio, Flavobacterium*, *Cardiobacterium.* Species with similar morphology.

The best known is *Escherichia coli,* a unique member of the normal gut flora of mammals and an important pathogen of intestinal and urinary tract infections (Pakbin *et al.*, 2021).

They all have a lipopolysaccharide (LPS) outer membrane, which is responsible for some of the disease-causing symptoms. Toxic properties are

often associated with a portion of this LPS layer. This region of LPS is called endotoxin. Binds to cells and is only released upon cell lysis (Gourama, 2020; Paracini *et al.*,2022).

1.2.2 Antigenic properties of the Enterobacteriaceae

This group of organisms has a complex antigenic structure, and the strains are differentiated in their seropositive behaviour.

1.2.2.1 O Antigen: a somatic antigen. Heat stable antigen. O antigens are lipopolysaccharides and are found in the cell wall of most Gram-negative bacilli. With sera containing anti-O antibodies, these antigens accumulate slowly in granular clumps. Antibodies to O antigens are mostly IgM(Svennerholm *et al.*, 2022) .

1.2.2.2 H antigen: Flagellar antigen: This antigen is thermostable and can be inactivated by heating above 60 °C. With sera containing anti-H antibodies, these antigens build up rapidly. Within a single species of *Salmonella*, flagellate antigens may occur in either or both forms, termed phase I and phase II. An organism tends to change from one stage to another; This is called a phase change. H antigen antibodies are mostly IgG (Sundaresan, and Rathinavelan, 2023; Saeed *et al.*, 2022).

1.2.2.3 "Vi" antigen: (K) capsule antigens located in the extremity of bacteria. The agglutination of freshly isolated strains is often interfered with by sera containing mainly O agglutinins. VI antigens are destroyed by heating for 1 hour at 60 °C (Farmer 2022; Ghaderinia *et al.*, 2022).

1.2.3 Genera of Enterobacteriaceae

1.2.3.1 *Escherichia coli*

Escherichia coli grows as a normal flora in the gastrointestinal tract and is considered an opportunistic pathogen because it can cause diarrhoea, in which case it can be called *E. coli*. It is responsible for approximately 90% of urinary tract infections in young women (Chahal, and Chaurasia, 2021).

Bacteria were first discovered by Theodore Eschrich , in 1885. They were coli, or "coli bacteria In 1894, it was isolated from the feces of healthy infants and deemed non-pathogenic (Farré-Maduell, and Casals-Pascual, 2019). and certain species of *Escherichia coli* was the leading cause of diarrhea among infants (Gomes, *et al.*, 2016).

1.2.3.1.1 Characteristics of *Escherichia .coli*

A rod-shaped, non-spore, Gram-negative bacterium that wields a flagellum moves around. coli colonies are generally convex and have a smooth surface. on MacConkey agar, colonies appear flat, dry and pink as they appear to have a colored 'gloss' on Eosin Methylene Blue (EMB) agar. *Escherichia coli* O157:H7 can be distinguished from other *E. coli* serotypes using MacConkey's sorbitol (Ahmed, 2021).

Since O157:H7, unlike other serotypes, sorbitol cannot be fermented. coli H2S strains are not produced in ferric triglyceride agar. Most strains are capable of producing the enzyme β-glucuronidase. The optimum temperature for growth is 36-37°C and pH 4.4-9 is suitable for growth. Bacteria give a negative result for oxidase, urease, and nitrite tests with a positive result for the catalase test, and for the indole test, most strains give a positive result (Verma *et al* ., 2022).

1.2.3.1.2 Epidemiology of *Escherichia coli*

Escherichia coli is the most important family of intestinal bacteria that are able to invade the intestine and live in it as a normal flora. Some *Escherichic* strains are opportunistic - they can transform into pathogens when conditions or opportunities are met (Holland *et al.*, 2020; Bonten *et al.*, 2021)

According to the Centers for Disease Control and Prevention (CDC), the most recent *E. coli* outbreak occurred on March 11, 2021, resulting in a total of 22 deaths and recent examination of *E. coli* outbreaks in recent years. The number of outbreaks per year has increased in the past several years.

The infection rate varies from person to person and environment to environment depending on health conditions and geographical location. The presence of closely related UPEC clusters is responsible for a large number of urinary tract infections regardless of antibiotic resistance. The bacteria mostly affects humans in the age group of 30 to 39 years, and the infection rate is higher in women than in men. (Öztürk *et al.*, 2020; Harahap *et al.*, 2021; Ait-Mimoune *et al.*, 2022).

1.2.3.2 *Klebsiella*

The genus *Klebsiella* belongs to the Enterobacteriaceae family, and this bacterium was isolated for the first time before Friedlander was diagnosed with pneumonia in 1882.

And I knew a reason epidemic and endemic infections in hospitals during the fifties of the last century, these bacteria grow at a temperature of (12-43)C°, and the optimum temperature for their growth is about (37) C°, and they are killed at the temperature of (55 C) for half an hour.

These bacteria are widely distributed in nature and isolated from different habitats such as parts of human body, animals, sewage, soil, lakes, salt water, fresh water, In general, they are opportunistic pathogens for

humans and animals (Russo *et al.*, 2023; Bitar, 2016; Jamshaid *et al.*, 2021; Cholewińska *et al.*, 2022).

1.2.3.2.1 Classification of *Klebsiella*

Klebsiella bacteria have been classified according to different classification systems, including: Cowan, Orskov, Bascomb and the name varies from country to country, as it is called *Klebsiella.pneumoniae* in the United States according to the Orskov classification, and is called Klebsiella aerogenes in Britain, which prefers the Cowan classification, but most European countries follow Orskov's classification is the dominant one worldwide (Priyanka *et al.*,2020).

1.2.3.2.2 Pathogenicity of *Klebsiella pneumoniae*

Klebsiella. pneumoniae is the second cause of bacteremia and fatal sepsis (septicemia) in children and children Newborns, and acute diseases, especially in people who suffer from organ weakness immune system due to diabetes mellitus, chronic heart disease, and pulmonary vasoconstriction, especially in Elderly people and newborns, and most *K.pneumoniae* infections are in hospitals ,Mortality is high if not treated properly (Bazaid *et al.*,2022).

Infections of the middle ear and infections of the soft mucous membranes This bacteria also causes Nose necrosis and lung infections (Hidayat *et al.*, 2022).

Klebsiella. pneumoniae is an opportunistic pathogen that accompanies people with Immune suppression disease and those suffering from various urinary tract infections Pyogenic Liver (Arato *et al.*, 2021).

And also caused abscesses, and that 60-80% of *K. pneumoniae*. isolates causing this disease contain K2 capsular type contains 10-14% of them and K1 capsular type contains 10-14% of them (Raj *et al.*, 2022).

1.2.3.2.3 Epidemiology of *Klebsiella pneumonia*e

These bacteria are widely spread in nature, as they have two types of common habitats, the first habitat represents the environment, as it flows into surface water, sewage, soil, and on the surfaces of fruits , cereals and vegetables (Zhu *et al.*, 2022). and the other habitat is environment hospitals and mucosal surfaces in breast milk, and the intestine is the most important place for the availability of these bacteria in a form natural (Ndlovu *et al.*, 2023).

There are three ways to get infected with *Klebsiella pneumoniae* ,through contact between patients and people and the environment around them, or through the air through Patients spray, or by swallowing the bacteria with contaminated food and drinks in the hospital (Hussain *et al.*, 2022).

The widespread use of broad-spectrum antibiotics on an irregular basis has led to the emergence of Strains of *Klebsiella* bacteria have multiple antibiotic resistances, and one of the reasons may be: They produce broad-spectrum beta-lactamase enzymes (Sarshar *et al.*, 2021).

Statistics show that *Klebsiella* bacteria cause 3% of epidemic diseases. ,bacteremia cases of 14% of bacteria (Schwaber *et al.*, 2022).

1.2 3.3 *Proteus* spp.

This bacteria belongs to the intestinal family (Girlich *et al.*, 2020) and spread in the feces and soils, and is present in the intestines of a human being characterized by its well-known creeping movement. The Swarming phenomenon, which is considered the most important characteristic of it, is positive for catalys and negative for examination, and it is not fermented for the sugar of lactose, and its colony is characterized by a pale color on the center of the maconkey and is productive. For H_2S, it is positive and appears in different shapes, such as the stringed shape, with active movement, or it has spherical bacilli, and has a distinct edge on the solid medium, due to the

movement of anthial. On urea analysis to CO2 and ammonia (El-Tarabili *et al.,* 2022; Al-Obaidi and Mohammed, 2022; Saleh *et al.,* 2019).

Proteus is the third type that causes hospital infections, including adverse factors, including hemo lysine, ures, and bacterosin, and the movement of adhesives and adhesion factors, which help it stick to robbery. *Proteus* is one of the most important types that cause common urinary tract infections that are related to the linear and are formed (Funjan 2021).

1.2.3.3.1 Pathogenesis of *Proteus* spp.

After *Escherichia coli*, *proteus* spp. is the most common cause of UTIs especially the young and the elderly of both sexes. The capricious types were too he recovered from infected wounds, abscesses, from cases of otitis media, meningitis and septicemia, osteomyelitis, and focal lesions of diabetic patients (Khalily *et al*., 2022; Isaac, 2019).Proteolytic species produce urease, which leads to rapid hydrolysis of urea with liberation mmonia and carbon dioxide, and therefore in urinary tract infections with protein becomes urine alkaline, promotes stone formation.The rapid movement of these bacteria may Contribute ontribute to the invasion of the urinary tract. *P.vulgaris* is an important hospital Pathogens (Agarwal and Radera, (2019).

1.2.4 Antibiotics

They are chemical compounds produced by microorganisms that are effective against antimicrobials .Others kill bacteria and inhibit their growth, but do not affect host cells (Koch *et al.,* 2021). Antibiotics have been used with great success to control most of the epidemics, and other infectious diseases. Antibiotics can be classified according to their effect on living organisms, (Shoeb *et al.,* , 2023).

Among the most important antibacterials used at the present time are penicillins, sulfonamides, tetracyclines, aminoglycosides, chloramphenicol, and first- and second-generation cephalosporins.

It depends on its mechanism of action or its chemical composition depending antibiotics can also be divided the cell wall is the place of action of the antagonist, an example of which is penicillin, and some of which have action on cell membrane as polymyxins antagonists, and others that inhibit protein making, including tetracycline Aminoglycosides, while anticoniolone inhibits DNA synthesis (Hu *et al.,* 2022).

1.2.4.1 Aminoglycosides

Aminoglycosides are a group of antibiotics used to treat a wide spectrum of bacterial infection (Dagur *et al.,*2023) . The therapeutic mode of action of aminoglycoside antibiotics is that they bind to RNA, namely the 16 S RNA of ribosomes, and thereby hinder translation. The binding motif at the RNA side is a stem-loop structure (Rubio *et al.,* 2022) they were shown to be transported:

1-by a few narrow spectrum efflux pumps of the MFS superfamily, who also transport sugars .

2-wide spectrum efflux pumps of the RND superfamily, such the AcrAD-TolC pump of *E. coli* or the MexXY-OprM pump of *P. aeruginosa* (Sionov *et al.,*2022).

The spectrum of activity of AGs includes Gram-negative bacteria, mycobacteria ,staphylococci, and leptospira. They have poor efficacy against anaerobic bacteria, streptococci, and intracellular bacteria. streptococci and Enterococci generally show a degree of intrinsic resistance to AGs due to impermeability of their cell wall, but penetration into the bacterial cell can be enhance by other antimicrobials which inhibit cell wall synthesis such as the beta -lactam antibiotics. Therefore, AGs are often used in combination with beta-lactams.

This combination also broade spectrum of activity (Dagur *et al.*, 2023). Serio show AMEs are composed of three classes that render aminoglycosides

inactive via (acetylation acetyltransferases; AAC), (adenylation nucleotidyltransferases; ANT), or phosphorylation (phosphotransferases; APH). The genes encoding these enzymes are frequently found on mobile elements (i.e., plasmids or transposons) that encode other antibiotic resistance genes, such as extended-spectrum β-lactamases (ESBLs) and carbapenemases, and thus are often found in MDR Gram-positive and Gram-negative bacteria (Yu *et al*., 2022; Abdul-Mutakabbir *et al.,* 2019).

Gentamicin and tobramycin are the major aminoglycosides; they have an extended spec-trum, which includes Enterobacteriaceae, and of particular importance, *P aeruginosa.* They are sometimes beneficial in treating serious infections caused by gram-positive pathogens such as *S aureus* and *enterococci,* but only when combined with other drugs. Streptomycin and amikacin are now primarily used in combination with other antimicrobial agents in the therapy of tuberculosis and other mycobacterial diseases.Neomycin, the most toxic amino-glycoside, is used in topical preparations and as an oral preparation before certain types of intestinal surgery, because it is poorly absorbed (Laxson, 2022; Nye *et al.*, 2022; Cohen *et al.*,2020).

1.2.4.2 Fluoroquinolones

Fluoroquinolones are a class of widely prescribed antibiotics with a broad range of activity against Gram-positive, Gram-negative, and some atypical microbes ,The antibacterial mechanism of action involves disruption of the catalytic mechanism of type-II topoisomerases in bacteria, namely topoisomerase IV and DNA gyrase. Fluoroquinolones inhibit the ability of the enzymes to ligate cleaved DNA and result in single and double-stranded DNA breaks (Fief *et al.*,2019), Two main mechanisms of resistance described: (i) point mutations in the so-called quinolone resistance-determining region (QRDR) of the gyrA gene parC genes (Bhatnagar *et*

al.,2019) and (ii) nonspecific, low-level resistance by modified or alter expression of the OmpF protein, resulting in decreased uptake of antimicrobial substances (Vergalli *et al.*,2020)) In the past, quinolones resistance was believed to be mediated by bacterial chromosomal mutations till 1998 when the researchers discovered plasmid mediated genes in *K. pneumoniae*, named "qnr" encoding to pentapeptide repeat family which play a role in binding and protecting DNA gyrase, and topoisomerase IV from repression by ciprofloxacin,Then four major groups of qnr genes (qnrAqnrBqnrC and qnrS) and two plasmid- mediated quinolones resistance (PMQR) genes aac(6')-Ib-cr and another qepA encoding various aminoglycoside transferases that modify ciprofloxacin to facilitate an efflux pump protein were identified (Kareem *et al.*,2021).

PMQR genes are often on the same plasmid as the ESBL genes. Resistance plasmids with genes encoding ESBLs can be transferred by the conjugation that helps dissemination of PMQR determinants in different Enterobacteriaceae species. Due to MDR establishment, co-existence of ESBLs and PMQR genes are a major concern (Azargun *et al.*,2018) .

Also note the high prevalence of fluoroquinolone (levofloxacin) non-susceptibility in community-associated MRSA isolates in the US in recent years (Sader *et al.*, 2016) resistance to fluoroquinolones in *E. coli* had the strongest association with septicemia hospitalization rates in adults aged 50-64y, 65-74y, 75-84y, and 85+y. *E. coli* is a major source of Gram-negative septicemia in the US (Moftian *et al.*,2023), and prevalence of fluoroquinolone resistance in *E. coli* isolates in both urinary tract and bloodstream infections in the US is high (Mills *et al.*,2022).

1.2.5 Multidrug-resistant (MDR) bacteria

Multi-Drug Resistance (MDR)Pathogenic organisms have mechanisms of resistance to combat the harmful effect of many classes of antimicrobials. MDR is defined as the resistance of pathogenic organisms to one or more antimicrobial agents.Many health organizations such as the WorldHealth Organization (WHO), the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) have warned that MDR bacterial infections are becoming difficult and costly to treat using expensive medications, andrequire prolonged treatment regimens (Alenazy, 2022).

One of the most effective mechanisms that confer MDR to bacteria is multi-drug efflux pumps, which prevent the accumulation of antibiotics inside the cell, thereby reducing their concentration to subtoxic levels. Many countries are facing an increased incidence of infections resulting from MDR Gram-negative bacteria, where these organisms caused 80% of all serious bacterial infections (Abalkhail *et al* ., 2022; Mitiku *et al.,* 2022; Samreen *et al.,* 2019).

In 2017, the WHO revised its list of MDR pathogens in need of urgent attention. This list indicated that the most critical pathogens are Gram-negative bacteria, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and Enterobacteriaceae, to which which *E. coli* belongs .There are several issues that underlie the serious danger posed by the emergence of MDR bacteria. First, and most importantly, results are skewed in patients infected with MDR bacterial be worse compared to patients with more susceptible organisms(Cillóniz *et al.*,2019).

In this way, Secondly, huge additional costs are associated with these infections. In the United States, the annual additional costs associated with the infections it causes resistant organisms compared to susceptible organisms are estimated at between $21 billion and 34 billion dollars 1Third, the

prevalence of MDR bacteria is closely related to use of broad-spectrum antibiotics, both for experimental and definitive therapy . this Increased use in turn leads to higher rates of MDR bacteria, leading to virulent formation turn.

Typically, MDR bacteria are associated with nosocomial infections. However, some MDR bacteria have become very common causes of community-acquired infections. This an important development as the prevalence of MDR bacteria in the community leads to a significant increase in population at risk, and thus an increase in the number of injuries caused by MDR bacteria. In addition, when a specific resistance pattern occurs in bacteria causing a community acquired infection that exceeds a certain threshold, broader spectrum antibacterial and/or combination antibacterial therapy is indicated for trial treatment of community acquired infections. In this review, we will identify trends in and epidemiology of community spread of different MDR bacteria.

Multidrug resistant (MDR) bacteria are well-recognized to be one of the most important current public health problems. The Infectious Diseases Society of America (IDSA) recognizes antimicrobial resistance as "one of the greatest threats to human health Worldwide" . (Rippon et al .,2021; Song *et al.,* 2020; Martin-Loeches *et al.,* 2018; Van Duin *et al.,* 2016).

1.2.6 Mechanisms of Antibiotic Resistance and Multidrug Resistance

Mechanisms of antibiotic resistance in pathogenic bacteria confer resistance to MDR to strongly different classes of antibiotics. This is because antibiotics are composed of different classes of chemicals, bacteria possess structures with different mechanisms of action and defense mechanisms, and these differ in their modes of action.

Mechanisms include inactivation of antibiotics, modification/alteration of drug targets to prevent entry of antibiotics, and a drug efflux pump (Zhang *et al.,* 2021;; Mone *et al.,*2021 Liu *et al.,* 2019). Drug efflux pumps are

important in medicine because of their efficient function in ejecting a variety of different chemical structures unrelated to the use of antibiotics or antimicrobials up to the concentrations needed to kill bacteria that are multi-resistant to more than one antibiotic or antiseptic (Farhat and Khan, 2022).

Bacteria function in the presence of specific external risks, in particular multiple antibiotic mechanisms that simultaneously compensate and secure these risks.for example, Enterobacteriaceae often use efflux pumps to dispose of antibiotics, but under certain conditions they use a different mechanism such as modulating drug targets (Dhanda *et al.*, 2023).

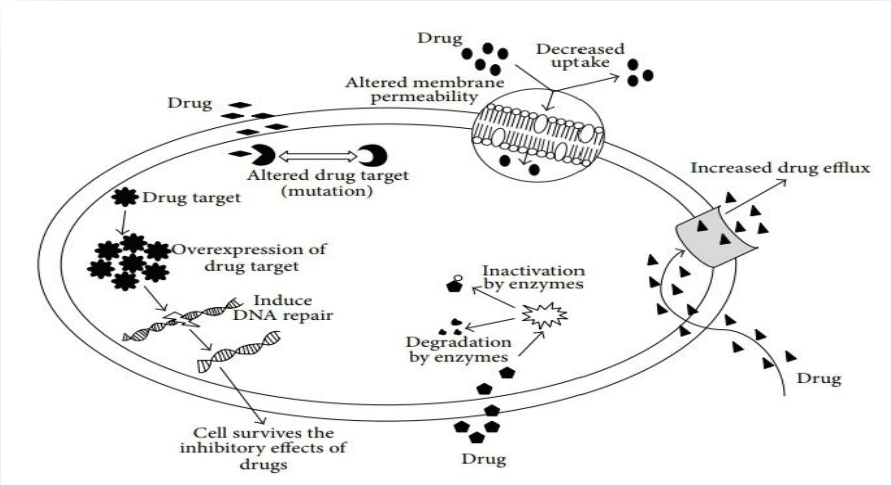


Figure 1_2: Mechanisms of MDR

1.2.7 Efflux Pumps

Efflux pumps are membrane proteins involved in the export of noxious substances from the bacterial cell interior to the external environment . efflux proteins are found in both Gram-negative and Gram-positive bacteria as well as in eukaryotic organisms.

1.2.7.1 Efflux pump Families.

They were the five major families of efflux transporters: the resistance-nodulation-division (RND) family,the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), the multidrug and toxic compound extrusion (MATE) family and the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily (Mahapatr *et al.*,2022).

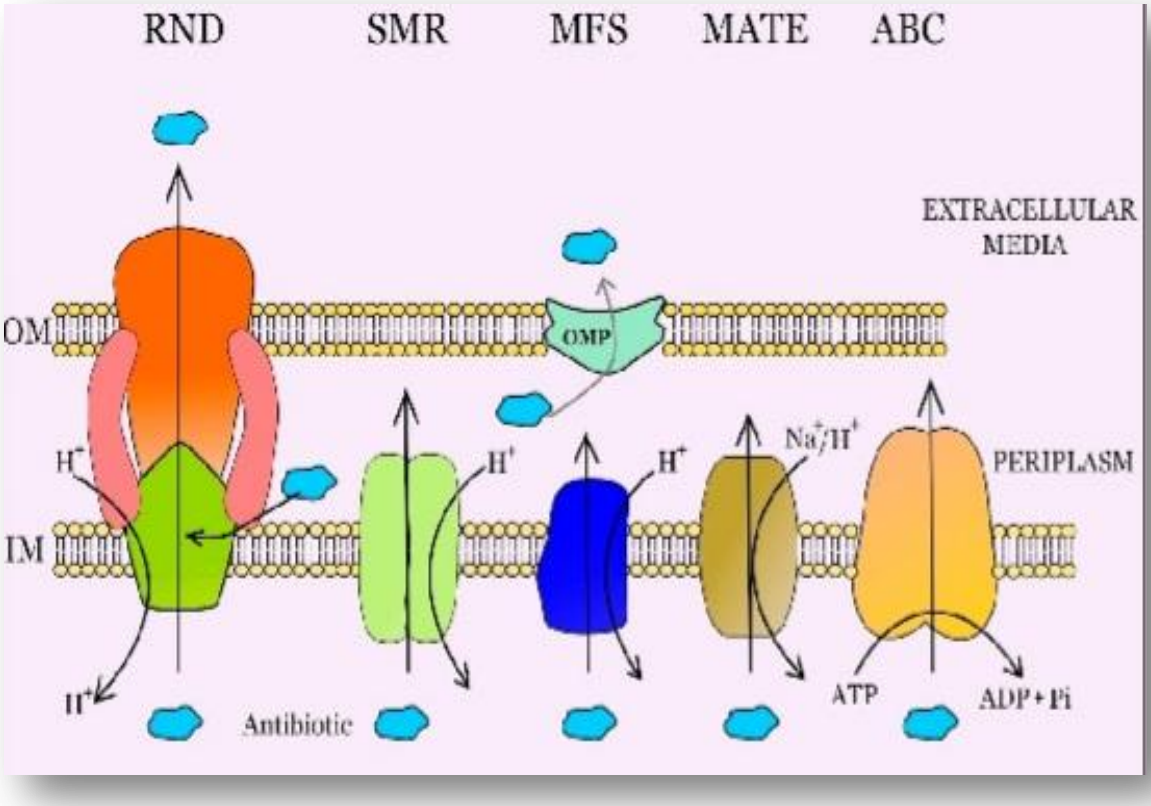


Figure1_3: Efflux pump types (RND), (SMR), (MFS), (MATE) and (ABC) (Blanco *etal.*,2016).

1.2.7.1.1 major facilitator superfamily(MFS)EffluxPumps

The major transporters of the facilitators form a superfamily consisting of a number of subfamilies; Among these subfamilies, polysaccharides and drug transporters are by far the most numerous. MFS transporters typically consist of about 400 amino acids hypothetically arranged into 12 transmembrane-spanning helices, with a large cytoplasmic loop between helices six and seven (Henderson *et al.,* 2021) The MFS family of drug

transporters consists of two domains centered around the central pore and two transposon domains Conformations from the cytoplasm to the peripheral side of the membrane in response to a Na+ or H+ ion gradient. MFS consists of membrane transport proteins present in bacteria to higher eukaryotes and involved in uniport, symport and antiport, from various substrates such as Krebs cycle intermediates, phosphate esters, oligosaccharides and antibiotics . into -12-helix and subfamilies 14-helix (eg, TetA(B) and TetA(K), class II and class K tetracycline transporters from *E. coli* and *S. aureus* respectively (Al-Dahmoshi *et al.*, 2022).

1.2.7.1.2 Small Multidrug Resistance (SMR) Efflux Pumps

Small multidrug resistance transporters (SMR) system provide to study the minimal requirements for active transport (Kermani *et al.*,2022). They are also the small multidrug transporters, with four trans-membrane helices and no significant extra membrane domain, although they function as dimers the minimum functional unit is a bundle of eight α-helices (Alav *et al.*, 2021) SMR transporter exports a broad class of polyaromatic cation substrates, thus Confer resistance to drug compounds matching this chemical description. Genes encoding SMR proteins (variously annotated emrE, sugE, smr, qac, ebr) are frequently found in mobile drug resistance gene arrays, and provide a broad selective advantage by conferring resistance to ubiquitous environmental pollutants with low-grade toxicity to microbes (Kermani *et al.*,2020) . MDR efflux pumps can contribute to the phenotype of antibiotic resistance at different levels, depending on the expression level. Intrinsic resistance: Some MDR efflux pumps, such as *P. aeruginosa* MexAB-OprN (Singh *et al.*,2020) or *E.coli* AcrAB-TolC .Acquired resistance: De-repression of the expression of the efflux pumps can be achieved by mutation at the regulatory proteins, rendering stable acquired resistance (Gil-Gil *et al.*, 2023) In addition , multidrug efflux pumps display different functions with

relevance to bacterial adaptation]n to different habitats. Some of these functions, such as resistance to biocides, heavy metals, resemble antibiotic resistance, since they are adaptive responses to avriety types of external injuries, others are related to interna detoxification of intermediate toxic bacterial metabolites. In add , some efflux pumps are include antimicrobial or even interkingdom signalling. Among the latter, it is important to mention that different efflux pumps are include in bacterial virulence, in both plant and animal hosts. Currently available information support the notion that, besides contribute to the antibiotic resistance, multidrug efflux pumps display a variety of functions with relevance to bacterial behaviour in different ecosystem (Scoffone *et al.,* 2021; Gaurav *et al.,* 2023; Pasqua *et al.,* 2021).

1.2.7.1.3 ATP-binding Efflux pumps(ABC)

ATP-binding cassette (ABC) transporters are present in all cells of all organisms and utilize the energy of ATP binding/hydrolysis to transport substrates across cell membranes (Jones, & George, (2023). Typically, they are specific for a given ligand that can be an inorganic ion, amino acid, sugar, polypeptide, or any one of a number of other classes of molecule.Mammalian P-glycoprotein (ABCB1) is one of the best characterized ABC transporters and overexpressed confer resistance of cancer cells to a variety of chemotherapeutic drugs such as doxorubicin, taxol (Liu, 2019). Two cytoplasmic, nucleotide-binding domains (NBDs) bind and hydrolyse ATP and share a common protein fold distinct from that of other ATP-binding proteins.Two transmembrane domains (TMDs) each consist of multiple (generally six) membrane spanning α-helices and form the pathway through which substrates cross the membrane (Srikant, 2020).

1.2.7.1.4 Resistance Nodulation Division Efflux Pumps (RND)

Transporters of the RND family are limited to Gram-negative bacteria and play central roles in multidrug export, Valencia further subdivided the HME-RND proteins into sub-groups, according to the substrate they transport: HME1 (Zn2+, Co2+, Cd2+), HME2 (Co2+, Ni2+), HME3a (divalent cations), HME3b (monovalent cations), HME4 (Cu+ ou Ag+) and HME5 (Ni2+)(Valencia *et al.*,2013).In Gram-negative bacteria, the resistance-nodulation-cell-division (RND) solute transporter superfamily consists of a broad periplasmic domain that assembles with periplasmic fusion proteins and an outer membrane pore to form a complete tripartite channel from the inner cytoplasm past the outer membrane (Alenazy, (2022); Sagar *et al.*, 2019).Since this system fully extrudes harmful substances from the bacterial cell, this family of membrane proteins is an efficient mechanism for membrane transport through proton antiport.

The AcrAB-TolC system of E.coli is the most well-known RND efflux pump(Sagar *et al.*, 2019) . RND type multidrug transporters also export the broadest range of compounds of any known multidrug efflux system, including a variety of antibiotics (for example, ciprofloxacin, chloramphenicol,carbenicillin, and tetracycline), dyes (such as ethidium bromide), detergents (such as sodium dodecylsulfate), disinfectants (such as triclosan), organic solvents (such as pxylene), toxic lipids, and metabolic inhibitors (Chopra, B., & Dhingra, A. K. 2021).

1.2.7.1.5 Multidrug and Toxic Extrusion (MATE) Efflux Pumps

Export of substrates and toxins by the cell is a fundamental life process and members of the MATE family represent the last class of multidrug resistance (MDR) transporters to be structurally characterized. MATE transporters involved a variety of important biological functions across all kingdoms of life (Upadhyay *et al.*, 2019). MATE transporters are very similar in size to the MFS transporters and are typically composed of approx. 450 amino acids which are putatively arranged into 12 helices however, they do not have any sequence similarity to members of the MFS transporters (Al-Dahmoshi *et al.*, 2022). Another MATE transporter, YdhE from *E. coli*, also has been characterized and shown confer resistance to the cationic antimicrobials (Kumawat et al. 2023) Latest investigation reveal that bacterial MATE transporters function as antiobiotic efflux pumps and confer resistance to tigecycline, a new glycylcycline class antibiotic developed to overcome methicillian-resistant and vancomycin-resistant *S. aureus* (Seukep *et al.*, 2022) also These proteins mediate resistance to hydrophilic compounds, dyes, aminoglycosides and fluoroquinolones. Norfloxacin accumulation experiments appeared that *Vibrio parahaemolyticus* consist an energy dependent efflux system designated (Sharma *et al.*, 2023).

1.2.8 Efflux pumpes types

1.2.8.1 AcrAB-TolC efflux pump

AcrAB-TolC, one of the efflux pumps, primarily expressed in Escherichia coli, consists of the outer membrane protein TolC, the inner membrane transporter AcrB, and the periplasmic adapter protein AcrA (Fanelli *et al.*, 2023).The efflux pump is an intrinsic mechanism of multidrug resistance in Gram-negative bacteria (Zwama, M., & Nishino, K. (2021) AcrA, a highly elongated protein, is thought to bring the outer and inner membranes closer.

It consists of a reactant breaker with monomeric AcrB, which was shown by in vitro reconstitution to be protonated (Compagne *et al.*, 2023). Purified TolC is in a closed state, but TolC is open in the purified AcrAB-TolC complex in the presence of antibiotics or inhibitors. In the absence of ligands, the TolC-locked complex can only be achieved using the disulfide-engineered AcrA-AcrB crosslinking pump . The AcrAB system is primarily expressed in *E. coli*, and is largely responsible for the property Autoimmune resistance of this organism to dyes, detergents and most lipophilic antibiotics (Roy *et al.*, 2023; Shi *et al.*,2019).

1.2.8.2 ACRD Efflux Pumps

AcrD is a component of the efflux pump that mediates the export of aminoglycosides and a few amphipathic compounds such as sodium dodecyl sulfate (SDS), deoxycholate and novobiocin, AcrA is a peripheral fusion protein that also jointly exports aminoglycosides with the cytoplasmic protein AcrD . The biofilm proteins encoded by csgBD, and the efflux pump AcrD have an effect on biofilm formation, and appear to play a special biological function, according to the findings (Guérin *et al.*, 2023; Al-Dahmoshi *et al.*, 2022).The copies showed significant changes supporting this theory. The transcripts of the acrD mutant were compared with that of the acrB mutant, in which a previously released AcrD is not a 'backup' efflux pump, but performs a physiological function in the cell, as evidenced by the fact that the effect was quite distinct (Buckner *et al.*, 2016).This comparison found 232 major changes in gene expression that were caused only by ACRD inactivation and not by acrB inactivation. Each of the acrB and acrD mutant transcripts contained 169 genes that were differentially expressed compared to the acrB mutant transcript. Experiments have shown that AcrB and AcrD efflux pumps have different substrate profiles when aminoglycoside antibiotics are indicated (Alav *et al.*, 2021; Wójcicki *et al.*, 2021).

1.2.8.3 EmrAB-TolC efflux pump

EmrAB components in Escherichia coli were first identified more than decade ago, resistance to hydrophobic toxins such as carbonyl cyanide m-chlorophenyl-hydrazone (CCCP) was discovered more than a decade ago (Al-Dahmoshi *et al.,* 2022). EmrB is a transmembrane protein with 14 proposed TM domains and homology to MFS vectors, according to preliminary sequence analyses, whereas EmrA has a broad soluble C-terminal domain with a single N-terminal TM domain and homology to MFP, HlyD. EmrAB, together with the outer membrane channel TolC, is thought to form a ternary efflux mechanism dependent on homology with the HlyBD TolC system (Henderson *et al.,* 2021), and these complexes efficiently pump material out of the cell . Other components of TolC-dependent ternary efflux systems are an endomembrane-associated transporter, such as the RND AcrB family transporter or the major superfamily (MFS) transporter EmrB, both catalyzed by H influx, or the ABCuperfamily MacB transporter, which is driven by ATP hydrolysis . a TolC-dependent efflux mechanism is responsible for the export of intracellular metabolites such as enterobactin, porphyrin, and excess cysteine, as well as the expulsion of toxic compounds (Guest, 2017).

1.2.8.4 MacBA-TolC efflux Pump

In Gram-negative bacteria , such as *Escherichia.coli*, a MacA-MacB-TolC pump has been detected (Okada, & Murakami, 2023) .The MacB endomembrane transporter is a non-inclusive member of the ATP-binding cassette (ABC) family (Okada, & Murakami, 2022). and biochemical analyzes revealed that it forms a homodimer. Only the 14- and 15-membered macrolide antibiotics can be transported by MacB (Varela *et al.,* 2021). Under normal laboratory conditions, it was difficult to detect their function in antibiotic resistance. In the AcrAB macrolide-sensitive *E. coli* strain, only overexpression of MacAB might increase resistance to macrolide antibiotics.

On the other hand, MacAB has been recently linked to the secretion of heat-stable enterotoxin E. s, expected to share structural similarities with AcrA (44%) (Okada, U., & Murakami, S. 2023).The periplasmic near-membrane domain of MacA is required for MacA interactions and this MacB. By modulating MacA transmembrane near-domain formation and disrupting proper assembly of the MacA-MacB complex, a single G353A substitution in this domain impairs MacAB-TolC function (Alav, et al ., 2022).

1.2.9 Efflux pump inhibitors (EPIs)

Efflux pump inhibitors can be divided into three categories according to its source of derivation: Plant derivatives, which are primary resources; chemical derivatives; and derivatives from microorganisms .

EPIs can also be defined as inhibiting drug efflux pump prevents build-up of antibiotics needed to kill bacteria and reduce MDR resistance and reversal (Konwar *et al.,* 2022). In AcrAB-TolC, the subrequired shifting is performed using the previously described functional rotation mechanism, which is required all protein subunits work together. Small molecule inhibitors for you proteins in the AcrB homotrimer lead to inactivation of the entire AcrAB-TolC pump (Alenazy, 2022).

Small molecules that stop these multidrug efflux systems from working may be able to do just that administered as an "adjuvant or chemosensitizer" in addition to existing antibiotic therapy to improve its effectiveness (Douafer *et al.,* 2019).

Research is currently underway to investigate this approach to fighting MDR is particularly problematic due to the lack of new antibiotics in the pipeline for Gram-negative bacterial infections . However, EPIs have not yet reached clinical application due to reasons such as high toxicity and poor pharmacology, no research was carried out.This makes finding the development of new EPIs that are safer and more effective is an important

priority . Efflux pump inhibitors are classified as compounds that inhibit the activity of efflux pumps in a non-competitive manner with substrates. There are a series of efflux pump inhibitors such as natural and synthetic efflux pump inhibitors (Sharma *et al.*, 2023). EPIs will increase the intracellular accumulation of many antibiotics, making bacteria more susceptible to antimicrobials in a general way (Blanco *et al.*, 2018) This increased sensitivity has two consequences: first, allowing re-sensitization of antibiotic-resistant organisms, and second, reducing the chances of selection for resistant mutations since, even such mutations are present in the population, the lower level of resistance that occurs due to the presence of the inhibitor would It hinders, at least in some cases, their choice.

It is also worth noting that, in addition to extruding toxic compounds, MDR efflux pumps are involved in many aspects of bacterial physiology, including intercellular communication and pathogenicity (Agreles *et al.*, 2021). Thus, inhibition of the efflux pump also affects the virulence of bacterial pathogens (Pun *et al.*, 2023) .

1.2.9.1. Inhibitors methods

Inhibition of the pump activity of Gram-negative bacteria may occur in a variety of ways:

- By modifying the chemical design of previous antibiotics for reduce the affinity of flow recognition and binding sites or to prevent flow transmission.
- By altering the regulatory steps that regulate the expression of efflux pumps
- By blocking the oute r membrane channel (TolC, OprM) with a stopper
- By generating a molecule that competitively or non-competitively inhibits the convergent sites of the efflux pump with an antibiotic.

- By disentangling the energy of flux, direct/specific via an anti-convective site or indirect/general by disentangling the energy mechanisms driven by the bacterial vector.
- By obstructing the functional assembly of the multi-component pump (Seukep *et al.*, 2022).

1.2.9.2 Carbonyl cyanide-m-chlorophenylhydrazone (CCCP)

Is perhaps the most famous EPI lab. It is an anion carrier that disrupts the proton motive force (PMF) by affecting both of its components, $\Delta\psi$ and $\Delta pH20$. This also renders the bacterial cells metabolically inactive which raises the debate as to whether they are synergistic (Ageyeva *et al.*, 2022).

The effect that CCCP shows with a combination of antibiotics is actually a result of inactivity of the efflux pump or metabolic inactivity of the cells as show in (Figure 1-5). CCCP has been reported to revive tetracycline activity in *Helicobacter pylori* and *Klebsiella* spp. Synergy between carbapenems and CCCP was also reported, which was independent of the efflux-inhibiting activity of CCCP, supporting the previous hypothesis that (Morais Oliveira-Tintino *et al.*,2023; Sharma *et al.*, 2019).

Carbonyl cyanide-m-chlorophenylhydrazone leads to metabolically inactive cells resulting in a synergistic effect with antibiotics. This combined with its cytotoxicity toward mammalian cells kept CCCP limited to in vitro use only (Xia *et al.*, 2021;Yarlagadda *et al.*, 2020).

The molecule was found to be highly effective in reversing resistance against fluoroquinolones in both recombinant Escherichia coli and clinical strains of *A. baumannii* overexpressing the MATE AbeM efflux pump.

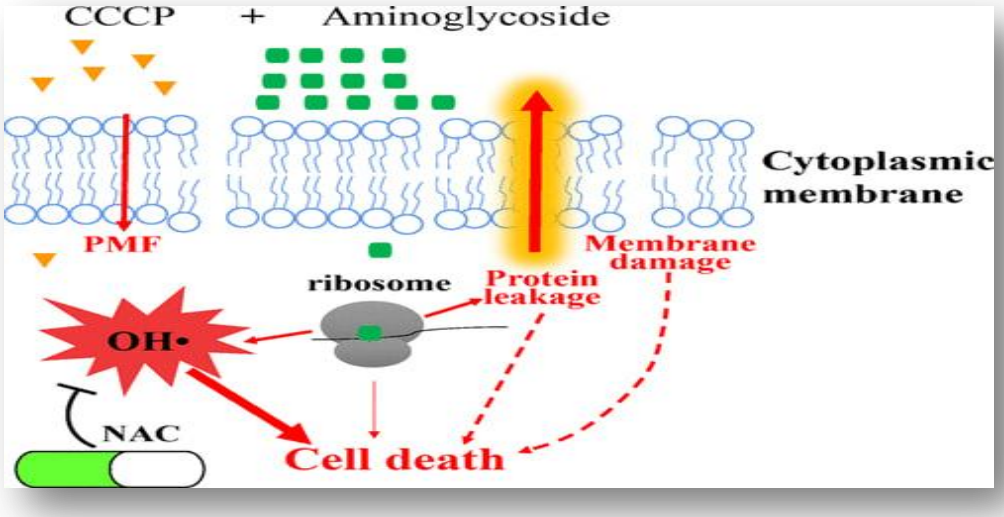


Figure 1-5:effect the efflux pump inhibiters CCCP and antibiotic on bacterial cell (Li etal., 2023)

1.2.10 Efflux pump inhibitors as new therapeutic agents

Considering the importance of efflux in mediating antibiotic resistance , it is worthwhile to expect that circumventing these determinants of resistance could potentiate the activity of substrate antibiotics. Abolishment the efflux could be achieved by different ways namely:

- downregulating the expression of efflux pump genes by interfering in genetic regulation
- redesigning antibiotics that are no longer recognized as substrates
- inhibiting the assembly of functional efflux pumps
- blocking the pump to avoid substrate binding to the active site
- collapsing the energy mechanism responsible for energizing these pumps.

A chemical entity would have to go through a stringent checklist to make it as a successful EPI. First, the molecule must not be antibacterial per se. An antibacterial molecule would ultimately lead to selection of mutants resistant to its action that will severely impact its utility as an EPI. Second, the molecule should be selective and not target any eukaryotic efflux pumps. Since efflux pumps are ubiquitous and their basic functional aspects tend to be

similar across the life forms, selective inhibition of bacterial efflux pumps becomes a difficult task. Third, it should possess ideal pharmacological features such as non-toxicity, high therapeutic and safety indices, good ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) profile and serum stability. Finally, to be successful at a commercial level, the production of the EPI must be economically feasible (Sharma *et al.*,2019).

1.2.11 OqxAB

Plasmid-borne genes conferring quinolone resistance have been increasingly recognized .Recently a plasmid-encoded efflux pump, OqxAB, conferring resistance to the quinoxaline-di-N-oxide olaquindox, which has been used as a growth promoter in pigs, was discovered in Escherichia coli isolates of porcine origin in Denmark and Sweden *oqx*AB was encoded by the genes *oqx*A and *oqx*B located on a 52-kb conjugative plasmid designated pOLA52 and conferred resistance to multiple agents, including fluoroquinolones .

Several studies have investigated the prevalence of this plasmid-encoded multidrug efflux pump in clinical isolates of Enterobacteriaceae and have for the first time identified an *oqx*AB-encoding plasmid in an *E. coli* isolate of human origin (Li *et al.*,2019; Monárrez *et al.*, 2019**;** Ni *et al.*, 2022).

The *oqxAB* gene generally locates on chromosome and/or plasmids flanked by IS26-like elements in clinical isolates of *Enterobacteriaceae* and *Klebsiella pneumoniae*, conferring low to intermediated resistance to quinoxalines, quinolones tigecycline, nitrofurantoin, several detergents and disinfectants(benzalkonium chloride, triclosan and SDS). It could co-spread with other antimicrobial resistance genes ($bla_{\text{CTX-M}}$, *rmtB* and *aac(6')-Ib* etc.), virulence genes and heavy metal resistance genes (*pco* and *sil* operons). Both RarA(activator) and *oqx*R (repressor) play important roles on regulation of the expression of *oqx*AB(Li, J *etal.*, 2019).

Chapter Two

Materials and Methods

2. Materials and Method

2.1 The Materials:

2.1.1 Equipment and Instruments:

The instruments and equipment which were used in the present study are listed in Table (2-1).

Table (2-1): Laboratory Equipment's and Instruments.

No.	Equipment /Instruments	Company	Origin
1	Autoclave	Metoree	China
2	Benchtop centrifuge	Memmert	Germany
3	Centrifuge	Wisd	Korea
4	DNA extraction tubes 100 µl, P.C.R tubes (50µl)	Capp	Germany
5	Disposable Loop	J.W.R	China
6	Eppendorf tubes	Eppendorf	Germany
7	Freezer	Kelon	China
8	Gel electrophoresis system	Bioneer	Korea
9	Incubator, oven	Memmert	Germany
10	Laboratory distillation unit	Cryste	Korea
11	Laminar flow cabinet	Capp	Germany
12	Micropipettes	Capp	Germany
13	Parafilm	Citotest LaB ware	Pakistan
14	PCR thermo cycler	Analytik Jena	Germany
15	Petri dishes (9 cm)	Afco-Dispo	Japan
16	Plain tube (10 ml or 15 ml)	Afco-Dispo	Japan
17	Platinum Wire Loop	Himedia	Indian
18	Polyethylene tube (15 ml)	Afco-Dispo	Japan
19	Refrigerator	Concord	Italy
20	Sensitive balance	Denver	USA
21	Sterile swab	Lab.service	Spain
22	Transport collection swabs	Citotest Lab ware	China
23	Vortex	Heidolph	Germany

24	Vitek 2 compact system	Biomerieux	Franch
25	UV-trans illuminator	Clever	USA

2.1.2 Chemical Materials:

The main stains and chemical materials used in the present study are listed in Table (2-2).

Table (2-2): Chemical Material.

No	Chemicals	Company / Origin
1	Alcohol (70%)	Fluka chemical/
2	Agarose	Condalab / Spain
3	DNA ladder (100 bp)	Promega / USA
4	Glucose (C6H12O6)	BDH/ England
5	Glycerol ($C_3H_8O_3$)	Switzerland
6	Loading dye (bromophenol blue)	Promega / USA
7	Nuclease free water	Bioneer (Korea)
8	Tris-EDTA buffer (TE)	Promega / USA
9	Tris-Borate-EDTA (TBE) buffer	

2.1.3 Biological Materials:

The main biological materials used throughout the present study are listed in Table (2-3).

Table (2-3): biological materials.

Media	Company / Origin
Brain Heart Infusion Broth	Promega/ USA
Eoasin methylene blue agar	Himedia/India
Inhibitor Carbonyl Cyanide m-Chlorophenyl-hydrazine (CCCP)	Solarbio/chian
Muller- Hinton agar	Himedia/India
MacConkey agar	
Nutrient agar	
Nutrient broth	

2.1.4 Antibiotics Disks:

Antibiotic disk diffusion was performed according to CLSI, (2023).

Table (2-4): Antibiotics used in the present this study.

No	Group	Antimicrobial Agent	Assembly	Content	Company/Origin
1	Aminoglycosides	Amikacin	AK	30 (mg)	Himedia/India
		Gentamicin	CN	10 (mg)	
		Kanamycin	K	30 (mg)	
		Streptomycin	S	300 (mg)	
2	Quinolons	Ciprofloxacin	CIP	5 (mg)	Himedia/India
		Gatifioxacin	GAT	5 (mg)	
		Levofloxacin	LE	5 (mg)	
		Norfloxacin	NX	10 (mg)	
3	Aminoglycosides	Amikacin	AK	30 (mg)	Liofilchem/Italy
		Gentamicin	CN	10 (mg)	Tm midia/India
		Kanamycin	K	30 (mg)	
		Streptomycin	S	25 (mg)	Bioanalyse/Turkey
4	Quinolons	Ciprofloxacin	CIP	5 (mg)	Tm midia/India
		Gatifioxacin	GAT	5 (mg)	
		Levofloxacin	LE	5 (mg)	
		Norfloxacin	NX	10 (mg)	Liofilchem/Italy
5	Aminoglycosides	Gentamicin	CN	40mg/ml (amp)	Menarini/ Italy
6	Quinolons	Ciprofloxacin	CIP	500 mg (cap)	Sanavity/Germany
		Levofloxacin	LE	500 mg (cap)	LDP/Spian

2.1.5. Primer Pairs

The Primer used in present study with condition are listed in table (2-5).

Table (2-5): Sequencing and PCR conditions designed in the present study.

Genes	Sequence 5-3	Bp	Conditions	Referenc
*oqx*A	*oqx*AF-5′-CTCGGCGCGATGATGCT-3′ *oqx*AR-5′-CCACTCTTCACGGGAGACGA-3′	392	Step 1: initial denaturation 94ºC, 5min. Step 2: denaturation 94ºC, 45 sec.by 34 cycles Step 3: annealing at 51ºC, 45 sec. Step 4: extension 68ºC, 1 min Step 5: final step of extension 72ºC, 10 min	(Kim *etal.*, 2009)
*oqx*B	*oqx*Bs-5′-TTCTCCCCCGGCGGGAAGTAC-3′ *oqx*Ba2-5′-CTCGGCCATTTTGGCGCGTA-3′	512	Step 1: initial denaturation 94ºC, 5 min. Step2:denaturation at 94ºC, 45 sec.by 32 cycles Step 3: annealing at 64ºC, 45 sec. Step 4: extensionat at72ºC, 1 min Step5: final step of extension at72ºC,10 min.	

2.1.6 The Kits used for diagnosis:

Table (2-6) includes a list of the molecular assay and diagnostic kits used in the current study.

Table (2-6): The materials and Diagnostic Kit Used in Molecular Study.

No.	Types of kits	Company/Country
1	Master mix	Promega/USA
2	DNase I enzyme kit DNase I enzyme 10x buffer Free nuclease water	

2.1.6.1Master Mix:

Table (2-7): Contents of Master Mix

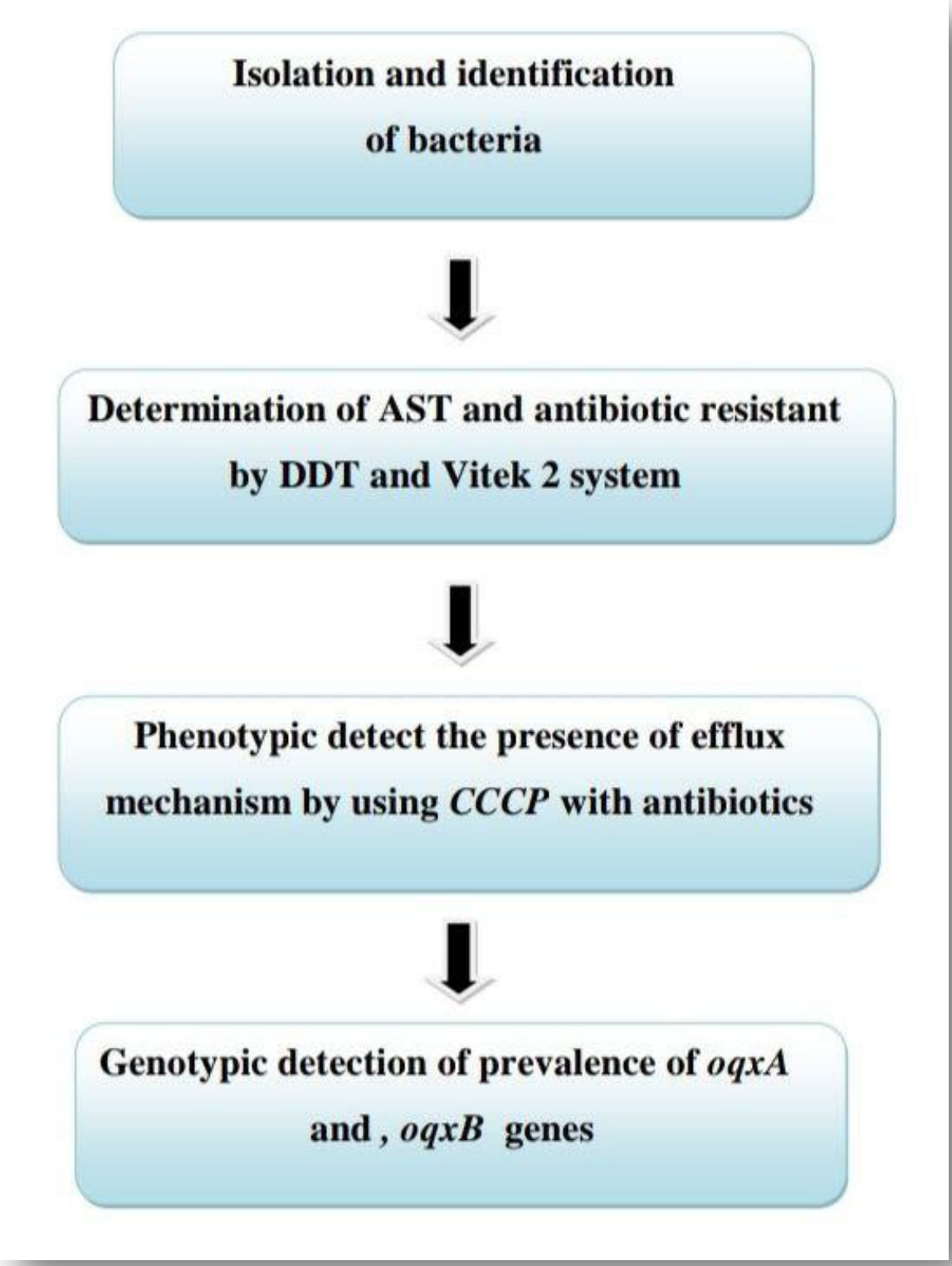
No.	Materials
1	DNA polymerase enzyme (Taq)
2	dNTPs (400µm dATP, 400µm d GTP, 400µm dCTP, 400µm
3	MgCl2 (3mM)
4	Reaction buffer (pH 8.3)

Table (2-8): Contents of PCR Reaction Mixture

Contents of reaction mixture	Volume
Master Mix	12 µl
Template DNA	2 µl
Forward primer (10 pmol/µl)	1 µl
Reverse primer (10 pmol/µl)	1 µl
Nuclease free water	9 µl
Total volume	25 µl

2.2 Methods

2.2.1 Study design: Cross-sectional study



2.2.2Ethical approval and consent

Verbal consent will be taken from each patient before sampling. This study was approved by the committee of publication ethics at college of medicine, University of Babylon, Iraq, No. 522 in 12 November 2022.

2.2.3 Sample Collection and Diagnosis

In the present stydy , a total of 300 samples were isolated from Marjan Teaching Hospital, Al-Mahaweel General Hospital, and Al-Mahaweel and Musayyib laboratories in Babylon, between December (2022) and March(2023)

Isolation was carried out at both public hospitals and laboratories , samples were labeled and transported to the University of Babylon Laboratory for processing and before starting this study.

All samples were cultured on MacConkey's and Eosin methylene blue and incubated at 37 C˚ for 24 hrs. confirmed by polymerase/chain reaction (PCR) using species-specific primer pairs *oqx*A, *oqx*B among MDR Enterobacteriaceae.

This / study was conducted at the University of Babylon / College of Medicine / Department of Medical Microbiology.

2.2.4 Preparation of Reagents and Solutions

2.2.4.1 Standard Solution No. 0.5 by McFarland:

McFarland turbidity standards are prepared by mixing various volumes of 1% sulfuric acid and 1% barium chloride to obtain solutions with specific optical densities. By adjusting the volume of these two chemical reagents.

McFarland standards of varying degrees of turbidity can be prepared which represent different bacterial density or cell count. 0.5 McFarland turbidity standard provides an optical density comparable to the density of a bacterial suspension with a 1.5 x 10^8 colony forming units (CFU/ml).

2.2.4.2 TBE (Tris-Borate-EDTA) Buffer

TBE was prepared and stored as a 10× stock solution. The 10× working solution was prepared by dissolving 108 g of Tris base, 55 g of boric acid, and 40 ml of 0.5 M EDTA in 1000 ml of D.W. However, final concentration of 1× TBE solution was prepared by adding 100 ml of 10× TBE buffer to 900 ml of sterile D.W.

2.2.5 Preparation of Culture Media

The ready culture media were prepared according to the manufactures instructions (Mueller –Hinton agar medium, MacConkey's agar, Eosien methylene blue agar , Luria broth and Blood agar) were prepared using the routine methods.

2.2.6 Preserving and maintaining bacterial isolates.

2.2.6.1 Short-term preservation

All tubes were planted on the nutrient agar denying medium in a planed and slanted manner. The bacterial isolates were incubated using the planning method, and the tubes were incubated at 37°C for 22 hours.

2.2.6.2 Long-term preservation

The tubes were inoculated on to 5 ml of brain heart infusion broth medium, and addition 15% glycerol with isolates, then these tubes were incubated with 37 °C for 22 hours, the entire tubes were kept at 21°C What is the use.

2.2.7. Antibiotic Susceptibility Test

2.2.7.1. Disc Diffusion Method

Using the disc diffusion method by Kirby-Bauer traditional susceptibility tests of Enterobacteriaceae have been done to detect antibiotic resistance on Muller Hinton agar. Antimicrobial susceptibility testing was done with 8 different antibiotics using the disk diffusion method, including, Gentamicin, kanamycin, Amikacin, Streptomycin, Ciprofloxacin, Levofloxacin , Gatifioxacin, Norfioxacin, after adjusting the inoculum to 0.5 Macfarland, as per the Clinical and Labrotary Standards Instituite 2023 (CLSI-2023), a table lists the antibiotics utilized in the style, diffusion of antibiotics.

The 134 isolates used in this test by culturing the bacterial suspension of the isolates using the streaking method, by sterile swab on to mueller hinton agar plate. The antibiotic discs were placed on the surface of the medium with flamed forceps at evenly spaced intervals, and incubation was normally done overnight at 37℃. Antibiotic inhibition zones were measured and compared with CLSI 2023 to find the susceptibility of organisms to each antibiotic (Kebede *et al.,* 2021).

2.2.7.2.Determination of Antibiotic Sensitivity test (AST) by Vitek

The isolated bacteria suspension is used to be diagnosed by the turbidity device Vetik 2 (Densichek), which the turbidity must equal (0.5) approximately 1.5×10^{8} CFU/ml. An aliquout of 145 µL was transferred. The first tube to the second for the antibiotic susceptibility test, The two test tube containing bacterial suspension was placed into a cassette the device cut off the transport tube then transferred into the incubator card to incubate at 37°C, and the result was reading and printed diagnostic report for each card existing within the reader with antibiotic susceptibility test according to company instruction (biomerieux).

2.2.7.3.Detection of Antibiotic Resistance Patterns (ARP)

Multidrug resistant (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antibiotic classes. Extensively drug resistant (XDR) was defined as non-susceptibility to at least one agent in all but one or two antibiotic classes. Pan drug resistant (PDR) was defined as non-susceptible to all-antibiotic classes (Magiorakos *et al.*, 2012). The susceptibility to 22 antibiotics with 8 antibiotic classes were tested to determine the ARP of the all Enterobacteriaceae isolates .

Table 2-10: Classes and types of antibiotics used to determine the Antibiotic Resistance Patterns for Enterobacteriaceae isolates.

NO.	Classes	types of antibiotics
1	Penicillins	Amoxicillin,Ampicillin,Piperacillin/Sulbactum ,Piperacillin/ Tazobactum,
2	Cephalosporens	Cefazolin , Cefotetan, Cefoxitin, Cefoperazone,
		Ceftazidime , Ceftizoxime , Ceftriaxone , Cefepime
3	Carpapenem	Ertapenem, Imipenem, Meropenem ,Panipenem
4	Aminoglygosiades	Amikacin ,Gentamicin ,Isepamicin,Topramycin
5	Fluoroquinolones	Ciprofloxacin, Levofloxacin, Sparfloxacin,
6	Glycylcyclines	Tigecycline
7	Sulfonamides	Trimethoprim/Sulfamethoxazole
8	Quinolons	Nitrofurantoin

2.2.7.4 MIC Determination by Agar Dilution Method

Minimum inhibitory concentrationtest assay was performed by agar dilution method, in which Mueller-Hinton agar plates and appropriate concentrations[0.5-128 µg/mL] of antimicrobial agent were prepared , inoculated with enterobacterial suspensions, and the plates were incubated at 37 °C in the same micro-atmosphere for 24 hours. The MICs for antimicrobials were scored as the lowest concentration of antimicrobial agent that completely inhibited the growth of Enterobacteriaceae on agar plates.

2.2.8 Phenotypic detection of efflux mechanism by using the Inhibitor [CCCP] with antibiotics

Levofloxacin ,Ciprofioxacin and Gentamycin minimal inhibitory concentrations (MIC) were measured in order to assess the effects of utilizing an efflux pump inhibitor [CCCP].

The previous experiment was repeated in the same way except adding the inhibitor.To confirm the mechanism of efflux pump, the efflux pump inhibitor (CCCP) was supplemented to each of the M-H agar plates containing 128 µg/mL Levofloxacin (LE) Ciprofioxacin (CIP) and Gentamycin (CN) .The final concentration of (CCCP) in the M-H agar was 25 µg/mL and recording the results.

2.2.9 Detection of Antibiotic Resistance Genes by Polymerase Chain Reaction (PCR)

A PCR assay was conducted as a confirmatory detection test. Enterobacteriaceae isolates resistance genes. This assay was accepted out in the manner designated by Weissensteiner *et al.*, (2003).

2.2.9.1. Genomic DNA Extraction

DNA template was prepared by the boiling method ,Briefly,

- 3-4 bacterial colonies were suspended in 500 µl TE buffer.
- Samples were incubated at 95°C for 15 minutes.
- They were centrifuged for 10 minutes at 4°C and 12000 rpm.
- The supernatants were stored in Eppendorf microtubes at −20°C.
- Which were used as DNA templates.

A simple and rapid method for preparing plasmids for restriction enzyme analysis has been developed. Bacteria are boiled for 15–40 s and an insoluble clot of genomic DNA and debris is removed by low-speed centrifugation. Plasmids are recovered from the supernatant by isopropanol precipitation and can be resuspended in buffer and immediately restricted. A 5-ml bacterial

culture yields enough plasmids for many restriction enzyme digestions, but a single colony on a petri dish or a 0.5-ml miniculture will suffice for a few experiments. In addition, the procedure can be readily adapted for the preparation of plasmids from liter cultures with quantitative yields.

2.2.9.2. Primers Preparation

The primers stock tube (100 pmol/µl) was prepared and then the working solution (10 pmol/µl) was prepared from primer stock tube according to the instruction provided by primer manufacturer (Macrogen/ Korea).

2.2.9.3 Technique of Polymerase Chain Reaction (PCR)

Using specified primer pairs, conventional PCR was performed to amplify the target DNA. It consisted of three processes that were repeated for a certain number of cycles in order to obtain a PCR result (amplicon) that could then be detected after agarose gel electrophoresis.

2.2.9.4 Agarose Gel Electrophoresis

After extraction, a concentration of agarose gel was 1.5 % that utilized to separate genomic DNA (5– 10 kb), whereas 1.2 gels were employed to achieve good resolution for small fragments of PCR product (0.2-1 kb). However, 100ml of 1xTBE buffer was added to the specified weight of agarose, which was then heated in the microwave until the solution became clear. After cooling the agarose to 50–55°C, 5 µl of Ethidium Bromide dye (10 mg/ml) were added to 100 ml of melting agarose gel to achieve the final concentration of 0.5 g/ml. The agarose was poured into the gel tray, sealed at the ends, and the comb was appropriately inserted before drying. The samples were loaded into their own wells on the gel, while the marker was loaded into one well.

2.2.10 Statistical Analysis

To analyze the data, a chi-squared test was run in SPSS version 16 (SPSS Inc., Chicago, IL, USA). P value <0.05 was considered statistically significant.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1 Clinical specimens and bacterial isolation

The results found out of 300 samples 280 (93%) culture-positive and 20 (6.6%) culture-negative clinical samples.

The results found that out of 280 clinical samples, only 134 (47.86%) samples were belonged to Enterobacteriaceae. The remaining 146 (52.14%) samples were belonged to other types of bacteria.

The reason for culture-negative samples may be due to the presence of fastidious of unculturable bacteria or fungi.

After observing the cultural and morphological characteristics of the bacterial isolates, were as follows: *E. coli* n=94 (70.1%) isolates, *Klebsiella. pneumoniae* n=24 (17.9%), *Proteus.spp* n=7 (5.2%) and *Enterobacter.spp* n=9 (6.7%).

The identification of all *E. coli, K. pneumoniae , Enterobacter* spp., and *Proteus* spp. isolates was confirmed by Vitek-2 automated identification system. However, no other Enterobacteriaceae species were recovered in this study.

3.2 Distribution of Enterobacteriaceae according to Infection type

The distribution of Enterobacteriaceae isolates among collected samples types illustrated in (Table 3-1). The results of isolation of Enterobacteriaceae revealed high percentage of *E.coil* 94 isolates distributed as urinary tract infections (UTIs) (43 isolates), lower respiratory tract infection (RTIs) (20 isolates), (17 isolates) for Bacteremia, and (14 isolates) for bacterial vaginitis.

The isolation rate of *K. pneumoniae* 24 isolates was as follows: from (UTIs) (4 isolates), (RTIs) (8 isolates), and (12 isolates) for Bacteremia.

Regarding the distribution of *Enterobacter* spp. among clinical samples, 7 isolates were recovered from (UTIs)-and (2 isolates) from Bacteremia. For

Proteus spp., 6 isolates were recovered from (UTIs) and 1 isolate from Bacteremia as shown in (Table 3-1).

Table 3-1: Distribution of Enterobacteriaceae isolates according to the types of samples

Infection type	Specimen	Sample n=134 (%)	*E.coil*	*K.pneumoniae*	*Enterobacter* spp.	*Proteus* Spp.
Urinary tract infections	Midstream Urine	60	43 (71.6%)	4 (4.4%)	7 (11.6%)	6 (16%)
Respiratory tract infections	Bronco alveolar Lavage	28	20 (71.4%)	8 (28.5%)	-	-
Bacteremia	Blood Stream	32	17 (53.1%)	12 (37.5%)	2 (6.25%)	1 (3.1%)
Vaginitis & cervical swabs	High Vaginal Swab	14	14 (100%)	-	-	-
Total		**134**	**94**	**24**	**9**	**7**

3.3. Distribution the type of infection according to sex

Table-2 showed that 61(64.8%) out of 94 patients were infected with *E. coli* among female group versus 33 (35.1%) were among male group. The most patients infected with *K.pneumonieae* infections were among females 14 (58.3%) versus 10 (41.6%) among males.

The results also showed that 4 (44.4%) out of 9 patients were infected *Enterobacter.spp* among femlae group versus 5 (55.5 %) were recorded among male group . and the most patients infected with *Proteus.spp* infections were recorded among female group 3(42.8%) versus 4 (57.1%) among male group, and statistically these differences were non-significant (P-value = 0.05).

These results indicated that there was a predominance of females (61.1%) than males (39%). This finding was compatible with the results obtained by several authors worldwide (Krishnan *et al*., 2023).

Table 3-2: Distribution the bacterial isolates according to patients sex

Sex	Study Groups				Total	P-value
	E.coli	***K.pneumoniae***	***Entero*.spp**	***Proteus*.spp**		
Male	33 (35.1%)	10 (41.6%)	5 (55.5%)	4 (57.1%)	52 (38.8%)	Pvalue =0.5 (N.S)
Female	61 (64.8%)	14 (58.3%)	4 (44.4%)	3 (42.8%)	82 (61.1%)	
Total	94 (100%)	24 (100%)	9 (100%)	7 (100%)	134 (100%)	

3.4 Distribution of Enterobacteriaceae isolates according to the age groups

The results found that most cases of Enterobacteriaceae infections were 40 (29.8%) out of 134 cases occurred among age groups (1-19) years while the frequencies and percentage of Enterobacteriaceae infections were decreased among the patients at the sixth to seventh decade of age 19 (14.1%).

Regarding *E. coli* infections, the results found that most cases 33 (35.1%) were among age group (1-19) years out of 94 cases, while most cases 8 (33.3%) of *K. pneumonia* infections were distributed among most the age groups of (20-39) years out of 24 cases. Regarding *Proteus* spp., infections the results found that most cases 3 (42.8%) were among age group (20-39) years out of 7 cases, while most cases 4 (44.4%) of infections with *Enterobacter* spp., were distributed among most the age groups of (20-39) years out of 9 cases, statistically these differences were non-significant (P value = 0.7), as shown in (Table 3-3).

Table 3-3: Distribution of Enterobacteriaceae according to categorial age groups

Study Groups	(1-19)	(20-39)	(40-59)	(60-75)	Total
E.coli	33 (35.1%)	27(28.7%)	23(24.4%)	11(11.7%)	94
K.pneumoniae	5 (20.8%)	8(33.3%)	5(20.8%)	6(25%)	24
Proteus spp.	1(14.2%)	3(42.8%)	2(28.5%)	1(14.2%)	7
Enterobacter spp.	1(11.11%)	4(44.4%)	3(33.3%)	1(11.11%)	9
Total	40(29.8%)	42(31.34%)	33(24.6%)	19(14.1%)	134

3.5 Antibiotic susceptibility patterns of the bacterial isolates

The antibiotic susceptibility (AST) of *E.coli* (n =94), *Enterobacter* spp. (n=9), *Proteus* spp (n =7) and *K. pneumoniae* (n= 24) isolates was performed by Vitek 2 system and disk diffusion test (using 8 antibiotics). The results of AST were interpreted based on CLSI guidelines (2023) (Figure 1, Appendix-1).

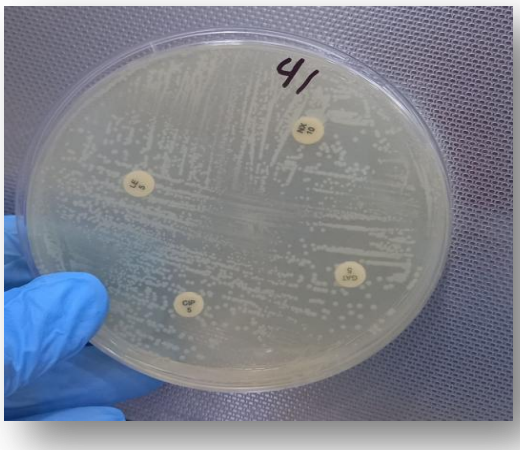


Figure 3-1 : Antibiotic susceptibility of, *E.coli* 41 isolate using DDT.

3.5.1 Antibiotic susceptibility of isolates to flouroquinoloines & aminoglycosides

The results of the present study regarding the susceptibility of Enterobacteriaceae isolates to flouroquinoloines found that *E.coli* isolates were resistant to ciprofloxacine, levofloxacine, Norfloxacin, Gatifioxacin with resistance rates 63%, 49.4%, 57.4% and 44.6% respectively.

K.pneumoniae isolates, they were resistant to ciprofloxacine, Levofloxacine, Norfloxacin, Gatifioxacin with resistance rates 79%, 87.%,75%, and 62.5% respectively.

Regarding *Enterobacter* spp., the isolates were resistant only to ciprofloxacine, levofloxacine , Norfloxacin, and Kanamycin with resistance rates 55.5%, 67%, 78% and 67% respectively.

Regarding *Proteus* spp., the isolates were highly sensitive to all members of flouroquinoloines, with sensitive rate 100%.

Regarding the susceptibility of Enterobacteriaceae isolates to aminoglycosides, *E.coli* isolates exhibited low resistance rate against Kanamycin, streptomycin with percentage of 29%, and 5% respectively, also *E.coli* isolates exhibited low resistance rates against Amikacin, Gentamicin with 1%, 34%, rates versus 66%, 67% resistance rates exhibited by *K. pneumoniae* isolates.

K.pneumoniae and *Proteus* spp. isolates were highly resistance to Kanamycin and Streptomycin antibiotics with 100%, 100% respectively. Also *Proteus* spp. isolates were resistant to Amikacin, Gentamycin with 71% and 86% respectively.

Regarding *Enterobacter* spp. isolates were resistance to Kanamycin with percentage of (66.6%). and isolates were sensitive to Amikacin, Gentamicin, and Streptomycin with rates (55.5%), (56%), and (55.5%) respectively as shown in (Table 3-4).

(Table 3-4): Antibiotic susceptibility of *E. coli* (n=94), *Enterobacter* spp. (n=9), *Proteus* spp. (n=7) and *K.pneumoniae* (n=24) isolates by DDT for fluoroquinolones and Aminoglygosides.

Antibiotic Class	Antibiotic	Suscept. status	Bacterial isolates				Total
			E. coli n= 94	*K.pneumoniae* n= 24	*Enterobacter* Spp. n=9	*Proteus* spp. n=7	
Fluoroquinolones	CIP	R	60 (63.8%)	19 (79.1%)	5 (55.5%)	0 (0.0%)	84 (62.6%)
		S	30 (32.2%)	0 (0.0%)	4 (44.4%)	7 (100 %)	41 (30.5%)
		I	4 (4.3%)	5 (20.8%)	0 (0.0%)	0 (0.0%)	9 (6.7 %)
	LE	R	46 (48.9%)	21 (87.5%)	6 (66.6%)	0 (0.0%)	73 (54.4%)
		S	34 (36.1%)	3 (12.5%)	3 (33.3%)	7 (100 %)	47 (35.0%)
		I	14 (14.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (14.8%)
	NX	R	54 (57.4%)	18 (75%)	7 (77.7%)	0 (0.0%)	79 (58.9%)
		S	34 (36.1%)	2 (8.3%)	2 (22.2%)	7 (100 %)	45 (33.5%)
		I	6 (6.3%)	4 (16.6%)	0 (0.0%)	0 (0.0%)	10 (7.4%)
	GAT	R	42 (44.6%)	15 (62.5 %)	0 (0.0%)	0 (0.0%)	57 (42.5%)
		S	40 (42.5%)	2 (8.3%)	4 (44.4%)	7 (100 %)	53 (39.5%)
		I	12 (12.9%)	7 (29.1%)	5 (55.5%)	0 (0.0%)	24 (17.9%)
Aminoglygosides	AK	R	4 (1.0%)	16 (66.6%)	0 (0.0%)	5 (71.4 %)	25 (18.6%)
		S	89 (94.6%)	1 (4.1%)	5 (55.5%)	0 (0.0%)	95 (70.8%)
		I	1 (1.0%)	7 (29.1%)	4 (44.4%)	2 (28.5%)	14 (10.4%)
	CN	R	32 (34.4%)	16 (66.6%)	0 (0.0%)	6 (85.7 %)	54 (40.2%)
		S	54 (57.4%)	2 (8.3%)	5 (55.5%)	0 (0.0%)	61 (45.5%)
		I	8 (8.6%)	6 (25%)	4 (44.4%)	1 (14.2%)	19 (14.17%)
	KAN	R	39 (41.4%)	24 (100 %)	6 (66.6%)	7 (100 %)	76 (56.7%)
		S	40 (42.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	38 (28.3%)
		I	15 (15.9%)	0 (0.0%)	3 (3.33%)	0 (0.0%)	18 (13.4%)
	HLS	R	5 (5.3%)	24 (100 %)	0 (0.0%)	0 (0.0%)	29 (21.6%)
		S	80 (86.0%)	0 (0.0%)	5 (55.5%)	6 (85.7 %)	91 (67.9%)
		I	9 (9.5%)	0 (0.0%)	4 (44.4%)	1 (14.2%)	14 (10.4%)

Abbreviations: CN; Gentamicin, K; Kanamycin, AK; Amikacin, S; Streptomycin, CIP; Ciprofloxacin, LE; Levofloxacin, NX ; Norfloxacin, GAT ; Gatiflioxacin.

The results of the current study regarding the resistance to ciprofloxacin were consistent with the results obtained by the Tajbakhsh *et al.*, (2016) who found that the rate of bacterial resistance to this antibiotic was 56.25%. It was shown that the rate of resistance to antibiotics, including this antibiotic, is higher in biofilm-producing bacterial isolates from non-forming bacterial isolates. The results were also compatible with by Rameriz-Castillo *et al*., (2018) who revealed that the rate of bacterial resistance to this antibiotic was 47.3%. It has been shown that the high resistance to ciprofloxacin is due to this group of antibiotics has become the first choice for treatment of urinary tract infections, which has led to using it excessively.

This study was also consistent with the results obtained by Malekzadegan *et al*., (2018), who showed that a percentage of (56.5%) of bacterial isolates were resistant to ciprofloxacin, in addition to showing that the high rate of resistance is higher in the bacterial isolates taken from urine, from than bacterial isolates taken from other sites of the urinary tract, such as the urea and kidneys. This antibiotic is the most common antibiotic from the group of quinolones used in treatment urinary tract infections, and its excessive use has led to a significant increase in the resistance rate over the latter years.

The results was also consistent with Abdu *et al.,* (2018) who found that the rate of bacterial resistance to this antibiotic was (46%), and it was shown that the bacteria were multi-resistant to antibiotics. However, the results of the current study did not consistent with the results obtained by Asadi (2018), as the rate of bacterial resistance to this antibiotic was (21.7 %).

One of the reasons for the resistance of *E. coli* bacteria to antibiotics belonging to the quinolones group, it is a change in the target site, a decrease in the permeability of the outer membrane of the bacteria and its possession of efflux systems which includes pumps (AcrAB-ToIC, MdfA, YhiV) (Paltansing, 2015), and Inhibiting DNA synthesis by inhibiting DNA gyrase enzyme or by genetic mutations in it (Zaman et al. 2017).

3.6 Antibiotic Resistance patterns

The Antibiotic Resistance patterns (ARP) of Enterobacteriaceae isolates was determined according to Magiorakos *et al*. (2012). The ARP of the isolates depended on the results of Vitek 2 system (Index-1) and were interpreted according to CLSI guidelines (CLSI, 2023).

The results of (Table 3-5) showed that ARP of Enterobacteriaceae isolates found that out of 134 isolates, 61 were sensitive and 73 showed different antibiotic resistance patterns as follows: **48 (35.8%)** were **MDR**, **17 (12.6%)** were **XDR**, and **8 (5.9%)** were **PDR**.

The antibiotics resistance pattern PDR was only detected in *Klebsiella* isolates, XDR was detected only in *E. coli* and *Klebsiella*. while MDR was detected in all Enterobacteriaceae isolates except *Proteus* spp. isolates (n=7) which did not showed any type of ARP and were all sensitive to all antibiotics tested.

Several studies worldwide reported that many isolates of enteric gram-negative rods are highly antibiotic resistant because of the production of β-lactamases and other drug-modifying enzymes. These organisms undergo conjugation frequently, at which time they acquire plasmids (R factors) that mediate multiple drug resistance. For example, plasmid-encoded New-Delhi metallo-β-lactamase causes resistance to penicillins,cephalosporins, monobactams, and carbapenems (Theuretzbacher, 2020)

The results of the present study also found that out of *E. coli* (n=44), 11 (25%) were XDR, and 33 isolates (75%) were MDR. However, no PDR patterns were found among *E. coli* isolates.

Regarding *K.pneumoniae* (n=24), 8 isolates (33%) were PDR, 6 (25%) XDR, and 10 isolates (42%) were MDR. However, no isolates from *K. pneumoniae* were found to be sensitive (susceptible) to all antibiotics tested.

Results also found that all of *Enterobacter* spp (n=5), were MDR. However, no PDR and XDR patterns were found among *Enterobacter* spp isolates.

Regarding the antibiotic resistance patterns among *Enterobacteriaceae* isolates, the results revealed that *K.pneumoniae* isolates were more resistant to the antibiotics than *E.coli*, and *Enterobacter* spp, because *K.pneumoniae* has PDR resistance pattern (34%) while isolates of *E.coli and Enterobacter.spp,* did not have this type of resistance.

In addition to that, the isolates of *E.coli* and *Enterobacter spp.* were the highest in possession of MDR (75%), and (100%) respectively, compared to in *K.pneumoniae* isolates (42%), which indicates the low efficiency of these isolates in the antibiotic resistance. Parajuli *et al.,* (2017) revealed that out of 739 *E.coli* isolates, (64.9%) were multidrug resistant (MDR) and (5%) were extensively drug resistant (XDR) from patients with UTIs, while Jain *et al.,* (2021) revealed that (98%) of *E. coli* isolates were MDR.

The emergence of MDR is considered as a major public health concern. Multi-Drug Resistance (MDR) Pathogenic organisms have mechanisms of resistance to combat the harmful effect of many classes of antimicrobials. MDR is defined as the resistance of pathogenic organisms to one or more antimicrobial agents. Based on this criterion, multi-resistant isolates were classified as MDR, XDR or PDR as in (Table 3-5).

The researcher Lee *et al.*, 2021 revealed that there was no PDR pattern among *E. coli* isolates recovered from patients with bacteremia. They also revealed that there was no XDR pattern among *K.pneumoniae* isolates recovered from the same patients.

Many health organizations such as the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC) have warned that MDR bacterial infections are becoming difficult and costly to treat using expensive medications, and require prolonged treatment regimens (Terreni *et al.*, 2021). As the criteria proposed by (Silva *et al.*, 2022) for a multiple antibiotic resistant organism, current study showed that all *E. coli* and *K. pneumoniae* isolates exhibited multiple antibiotic resistant (resistant to at least one antibiotic in three or more of the 5 antimicrobial classes tested in this study.

Table 3-5: Antibiotic resistance patterns of Enterobacteriaceae isolates based on Vitek 2 system results

***Enterobacteriaceae* isolates**	**Type of multidrug resistance pattern (N,%)**		
	MDR	**XDR**	**PDR**
***E. coli* (n=44)**	33 (75%)	11 (25%)	-
***K. pneumonia*(n=24)**	10 (42%)	6 (25%)	8 (33 %)
***Enterobacter* spp (n=5)**	5 (100%)	-	-
***Proteus* spp. (n=7)**	-	-	-
Total	48 (35 .8%)	17 (12.6%)	8 (5.9%)

Only 14 isolates were selected to determine their antibiotic susceptibility against two classes of antibiotics, fluoroquinolones and Aminoglygosides by disc diffusion test. The result showed that Enterobacteriaceae isolates had higher resistance to fluoroquinolones than Aminoglygosides, as shown in the (Table 3-6).

Table 3-6: Antibiotic susceptibility of selected Enterobacteriaceae isolates (n=14) using DDT for fluoroquinolones and amino glycoside

NO	Bacterial species	Fluoroquinolones				Aminoglygosides			
		CIP	LE	NX	GAT	AK	CN	K	HLS
1	***E.coli*-39**	R	R	R	R	S	R	I	S
2	***E.coli*-47**	R	R	R	R	S	R	R	S
3	***E.coli*-49**	R	R	R	R	S	R	R	S
4	***E.coli*-57**	R	R	R	R	S	R	R	S
5	***E.coli*-63**	R	R	R	R	S	R	R	S
6	***E.coli*-65**	R	R	R	R	S	R	R	S
7	***E.coli*-66**	R	R	R	R	S	R	R	I
8	***E.coli*-72**	R	R	R	R	S	R	R	S
9	***E.coli*-74**	R	R	R	R	S	R	R	S
10	***E.coli*-78**	R	R	R	R	S	R	R	S
11	***E.coli*-81**	R	R	R	R	S	R	R	S
12	***E.coli*-82**	R	R	R	R	S	S	R	R
13	***K.P*-3**	R	R	R	R	R	R	R	R
14	***Enterocter* spp.-5**	R	R	R	S	S	S	R	S

3.7 Molecular detection of *oqx*A, *oqx*B genes among Enterobactriecae isolates

In this study, *oqx*AB genes were detected in selected clinical isolates of (MDR), (XDR) and (PDR) Enterobacteriaceae. The selected isolates were as follows: 12 XDR *E. coli* one PDR *K. pneumonia* , and one MDR *Enterobacter* spp. isolate.

Since bacterial resistance to antibiotics is related to different agents, in this study, detection of efflux pump genes was performed by PCR on selected 14 isolates to determine whether their resistance to antibiotics, including ciprofloxacin, levofloxacin and gentamycin, depended on the presence of these genes.

Analysis of PCR results showed that 6 (42.8%) out of 14 isolates were positive for oqxA gene, and 8 (57.1%) of the isolates were negative for *oqx*A gene, Howerver, no isolates showed positive results for the *oqx*B gene.

One of the most important resistance mechanisms in Enterobacteriaceae, especially *K. pneumoniae* is the production of efflux pump proteins by encoding the pump genes, especially the species belonging to the family (RND), which are specialized in resistance to antibiotics within the classes Amioglycosid, fluoroquinolones, and β-lactams (Onishi, 2023). The resistance of the isolates without efflux pump may be affected by other factors, such as reduced antibiotic permeability, decreased accumulation of intracellular antibiotics, and inactivation of the drugs.

Moosavian *et al*. (2012) showed that *oqx*AB genes was 40% and 5.7% for *E. gergoviae* and *E. coli*, respectively. Yuan *et al*. (2012) showed that *oqx*A and *oqx*B genes were present in 6.6% of *E. coli* strains. Kao *et al*. (2016) reported *oqx*AB genes in 6.05% of *E. coli* isolates.

Kim *et al.* (2009) investigated the prevalence of plasmid-encoded multidrug efflux pump in clinical isolates of Enterobacteriaceae. In their survey, 0.4% of *E. coli* isolates and 4.6% of *E. cloacae* isolates were positive for both *oqx*A and *oqx*B genes. Because these studies were performed at different times, therefore, it could be the reason for different results. Zhao *et al*. (2010) reported the *oqx*A gene in 30.3% of *E. coli* isolates which were collected from farm- worker

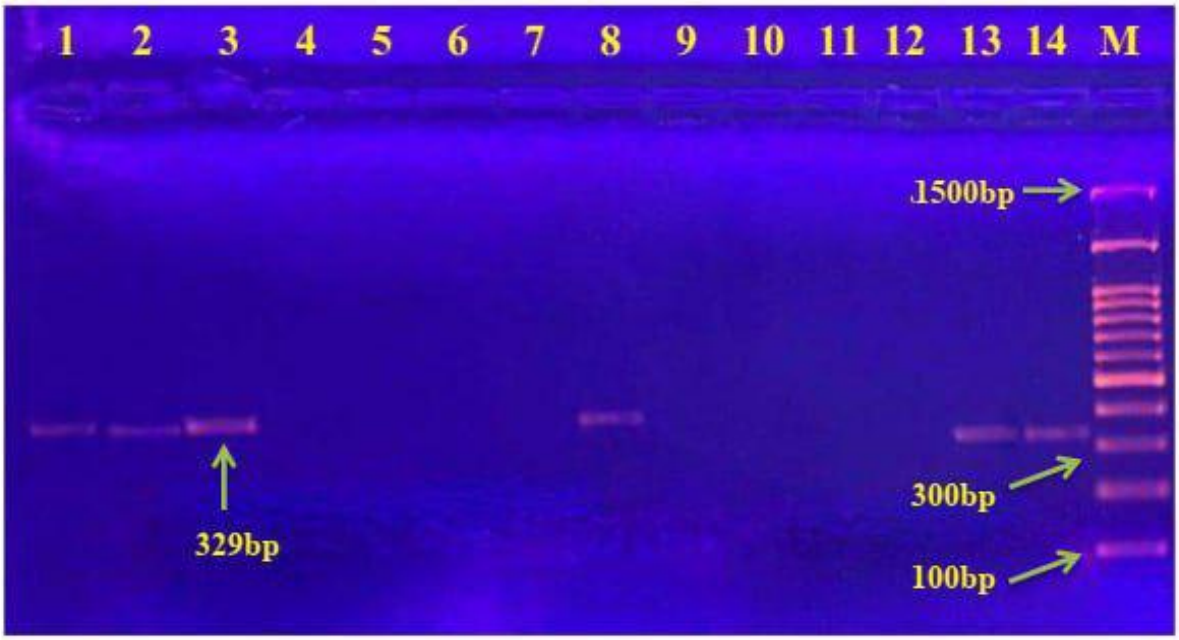


Figure 3-2. Agarose gel electrophoresis of PCR products of ***oqx*A** gene (329 bp), among *E. coli* (1,2,4,5,6,7,9,10,1,12,13,and14) *Enterobacter* spp.(8), and*K. pneumonia* (3) isolates. M=DNA molecular marker. Isolates**1,2,3,8**, are positives to the *oqx*A gene.
Step 1 : initial denaturation at 94ºC, 5 min. Step 2 :denaturation at 94ºC, 45 sec.by 34 cycles Step 3:annealing at 51ºC, 45 sec. Step 4: extension at 68ºC, 1 min Step 5:final step of extension at 72ºC, 10 min

In this study, all *oqx*A-positive Enterobacteriaceae strains were resistant to ciprofloxacin, and levofloxacin.

As some reports have confirmed that *oqx*A and *oqx*B efflux pumps confer resistance to multiple antimicrobial agents (Rodríguez-Martínez *et al*., 2013); Park *et al*., 2012), therefore, in this study also, the presence of *oqx*A efflux pumps in clinical isolates of *E. coli* (5 isolates) and 1 Kp isolate containing *oqx*A genes has conferred resistance to multiple antimicrobial agents (AMG, Qns, and β-lactams) as shown in (Figure 3-2).

3.8 Evaluating the Efflux Pump Inhibitor's Effect on Selected Isolates

In order to confirm the effective role of the efflux pump in our isolates, the MICs of antibiotics (levofloxacin, Ciprofloxacin, and Gentamycin) were compared with and without the efflux pump inhibitor (CCCP).

The following criteria were used to select the isolates for studying the effect of Efflux Pump Inhibitor's on the Isolates: the presence of *oqx*A gene and the high rate of MIC of the isolate (MIC ≥128 μg/ml).

The results of MIC for 14 Enterobactriecae isolates against three antibiotics (levofloxacin, Ciprofloxacin and Gentamycin), showed that all isolates had MIC ≥128 μg/mL in the absence of CCCP for Levofloxacin, Ciprofloxacin, and Gentamycin (Tables 3-8, 3-9, and 3-10).

In general, our findings regarding the utilization of the inhibitor, helped to clarify the important function that pumps conduct and how resistance develops in resistant isolates. The isolates that showed a decrease in MIC were consistent with the study conducted by several authors worldwide (El-Said *et al.*, 2012; Iman *et al.*, 2018; Honarmand Jahromy *et al.*, 2018; Baron and Rolain, 2018; Sanchez-Carbonel *et al*., 2021; El-Mahdy and Mashaly, 2022) who measured MICs for different MDR bacterial isolates in the presence and absence of the efflux pump inhibitor carbonyl cyanide 3-chlorophenylhydrazone and they showed the effect of CCCP was significantly greater on intrinsically antibiotic-resistant bacteria (i.e. *P. aeruginosa, Acinetobacter baumanii, E. coli, K. pneumonia, Proteus* spp., *Serratia marcescens, Morganella morganii* and *Providencia* spp.) than on other Enterobacteriaceae.

Table (3-7): Minimum inhibitory concentration of levofloxacin and Ciprofloxacin among PDR, XDR and MDR *Enterobactriecae* isolates with and without addition of the inhibitor (CCCP).

Isolates	Antibiotic resistance patterns	Efflux pumps genotype	MIC (µg/mL) of Levofloxacin and Ciprofloxacin Without CCCP	MIC (µg/mL) of Levofloxacin and Ciprofloxacin With CCCP
E. coli-**47**	**XDR**	***oqx*A**	**≥ 128**	**N.G***
E. coli-**49**	**XDR**	***oqx*A**	**≥ 128**	**N.G**
E. coil-**63**	**XDR**	***oqx*A**	**≥ 128**	**N.G**
E. coli-**66**	**XDR**	***oqx*A**	**≥ 128**	**N.G**
E. coli-**78**	**XDR**	***oqx*A**	**≥ 128**	**N.G**
K.pneumoniae-**3**	**PDR**	***oqx*A**	**≥ 128**	**N.G**

*NG:

These results in (Table 3-7) showed the effect of the inhibitor in reducing the MIC of levofloxacin among PDR, XDR and MDR Enterobactriecae isolates. The results showed the MIC of Levofloxacin was reduced for XDR *E.coli* (5 isolates) and PDR *K.pneumoniae* (one isolate) and inhibition of the growth in presence the inhibitor (CCCP). This results was compatible with El-Mahdy and Mashaly (2022) who revealed a decrease in MICs for different MDR *E.coli* and *K. pneumoniae* isolates in the presence and absence of the efflux pump inhibitor (CCCP).

Also, the results in the (Table 3-7) showed the effect of the inhibitor in reducing the MIC of Ciprofloxacin among PDR, XDR and MDR Enterobactriecae isolates. The results showed that the MIC of Ciprofloxacin was reduced for *E.coli* (5 XDR isolates) and one PDR *K.pneumoniae* isolate, and the results showed the disappearance of the bacterial growth in presence of the inhibitor (CCCP). The decrease in MIC of the isolates were consistent with the results obtained by El-Mahdy and Mashaly (2022) who measured the MICs for different MDR *E.coli* and *K. pneumonia* isolates in the presence and absence of the efflux pump inhibitor (CCCP).

The isolates that showed a decrease in MIC were consistent with the studies conducted by (El-Said *et al.*, 2012; Iman *et al*., 2018), who measured the MICs for different MDR *P. aeruginosa* isolates in the presence and absence of the efflux pump inhibitor. The result that showed a decrease in MIC are consistent with the studies by (Sanchez-Carbonel *et al.*, 2021) who measured the MICs in clinical strains of *Acinetobacter.baumannii* isolates in the presence and absence of the efflux pump inhibitor (CCCP) and they revealed that the isolates showed a decrease in MICs in the presence of the efflux pump inhibitor (CCCP).

Table 3-8: Minimum inhibitory concentration of Gentamicin among PDR, XDR and MDR Enterobactriecae isolates with and without addition of the inhibitor (CCCP).

Isolates	**Antibiotic resistance patterns**	**Efflux pumps genotypes**	**MIC (µg/mL) of Gentamycin Without** CCCP	**MIC (µg/mL) of Gentamycin With** CCCP
E.coli-**47**	XDR	***oqx*A**	≥ 128	≥ 128
E.coli-**49**	XDR	***oqx*A**	≥ 128	≥ 128
E. coil-**63**	XDR	***oqx*A**	≥ 128	≥ 128
E. coli-**66**	XDR	***oqx*A**	≥ 128	≥ 128
E. coli-**78**	XDR	***oqx*A**	≥ 128	≥ 128
K.pneumonia-**3**	PDR	***oqx*A**	≥ 128	≥ 128

Regarding the effect of the inhibitor in reducing the MIC of Gentamicin, the results of the present study found that there was no reduction in the MIC of isolates for Gentamicin (Table 3-8) and all PDR, XDR and MDR Entero-bacteriaceae isolates showed high MIC values (≥ 128 µg/ml) even in the presence of the CCCP which indicates no effect the inhibitor in reducing the MIC of the isolates for Gentamycin.

However, the results of the present study were inconsistent with the results obtained by Honarmand Jahromy *et al.,* (2018) who studied MIC of amikacin and gentamicin before and after treatment by efflux inhibitor CCCP and they found that lowest resistance to gentamycin (62%), and amikacin (74.5%).

Conclusions
&
Recommendations

Conclusions:

The present study concludes the following

1. High prevalence of efflux pumps gene (*oxq*A) was detected among MDR and XDR Enterobacteriaceae isolates.
2. No isolates showed positive results for the efflux pumps gene (*oxq*B)
3. The efflux pump inhibitor (CCCP) has a positive effect and improves the sensitivity of MDR isolates to Ciprofloxacin, levofloxacin but not gentamicin.
4. The antibiotics resistance pattern PDR was only detected in *Klebsiella* isolates, XDR was detected only in *E. coli* and *Klebsiella*. while MDR was detected in all Enterobacteriaceae isolates except *Proteus.*
5. Enterobacteriaceae isolates resistance to flouroquinoloines more than aminoglycosid,

Recommendations

The present study recommends the following

1. Further investigation of inhibitory effect of on (CCCP) all component of efflux pumps gene expression and on all outer membrane protein gene expression.
2. Study of the cytotoxicity and selectivity of the (CCCP)
3. Studying the inhibitory effect of natural products on efflux pumps gene expression.
4. Studying the effect of the inhibitor (CCCP) on other types of gram negativ bacteria.
5. Study the inhibitory effect of (CCCP) on susceptibility of other antibiotics.

References

References

Abalkhail, A., AlYami, A. S., Alrashedi, S. F., Almushayqih, K. M., Alslamah, T., Alsalamah, Y. A., & Elbehiry, A. (2022). The prevalence of multidrug-resistant Escherichia coli producing ESBL among male and female patients with urinary tract infections in Riyadh Region, Saudi Arabia. In *Healthcare* 10(9):1778.

Abdul-Mutakabbir, J. C., Kebriaei, R., Jorgensen, S. C., & Rybak, M. J. (2019). Teaching an old class new tricks: a novel semi-synthetic aminoglycoside, plazomicin. *Infectious Diseases and Therapy*, *8*, 155-170.

Agarwal, J., & Radera, S. (2019). Biofilm-Mediated Urinary Tract Infections. *Biofilms in Human Diseases: Treatment and Control*, 177-213.

Ageyeva, M., Veselov, A., Vodeneev, V., & Brilkina, A. (2022). Cell-Type-Specific Length and Cytosolic pH Response of Superficial Cells of Arabidopsis Root to Chronic Salinity. *Plants*, *11*(24), 3532.

Agreles, M. A. A., Cavalcanti, I. D. L., & Cavalcanti, I. M. F. (2021). The role of essential oils in the inhibition of efflux pumps and reversion of bacterial resistance to antimicrobials. *Current Microbiology*, *78*(10), 3609-3619.

Ait-Mimoune, N., Hassaine, H., & Boulanoir, M. (2022). Bacteriological profile of urinary tract infections and antibiotic susceptibility of Escherichia coli in Algeria. *Iranian journal of microbiology*, *14*(2), 156.

Alav, I., Bavro, V. N., & Blair, J. M. (2022). A role for the periplasmic adaptor protein AcrA in vetting substrate access to the RND efflux transporter AcrB. *Scientific Reports*, *12*(1), 4752.

Alav, I., Kobylka, J., Kuth, M. S., Pos, K. M., Picard, M., Blair, J. M., & Bavro, V. N. (2021). Structure, assembly, and function of tripartite efflux and type 1 secretion systems in gram-negative bacteria. *Chemical Reviews*, *121*(9), 5479-5596.

Al-Dahmoshi, H. O., Al-Khafaji, N. S., Al-Allak, M. H., Salman, W. K., & Alabbasi, A. H. (2020). A review on shigellosis: Pathogenesis and antibiotic resistance. *Drug Invention Today*, *14*(5).

Al-Dahmoshi, H., Ali, S. A., & Al-Khafaji, N. (2022). Efflux Pumps among Urinary E. coli and K. pneumoniae Local Isolates in Hilla City, Iraq. *The Global Antimicrobial Resistance Epidemic: Innovative Approaches and Cutting-Edge Solutions*, 239.

Alenazy, R. (2022). Drug efflux pump inhibitors: a promising approach to counter multidrug resistance in Gram-negative pathogens by targeting AcrB protein from AcrAB-TolC multidrug efflux pump from Escherichia coli. *Biology*, *11*(9), 1328.

Ali, S., Alam, M., Hasan, G. M., & Hassan, M. I. (2022). Potential therapeutic targets of Klebsiella pneumoniae: a multi-omics review perspective. *Briefings in functional Genomics*, *21*(2), 63-77.

Al-Khazaali H. M. K. (2021). The Influence of Interactive Classroom Management Strategies on Iraqi EFL Students' Academic Performance at Secondary School. International Journal of Early Childhood Special Education (INT-JECSE), 13 (2): 114-119. *International Journal of Early Childhood*, *13*(2), 114-119.

Al-Obaidi, H. M. J., & Mohammed, S. H. (2022). Isolation and Identification of Some Bacteria that Cause Urinary tract Infection in Pregnant Women in Kirkuk City. *HIV Nursing*, *22*(2), 2333-2339.

Arato, V., Raso, M. M., Gasperini, G., Berlanda Scorza, F., & Micoli, F. (2021). Prophylaxis and treatment against Klebsiella pneumoniae: current insights on this emerging anti-microbial resistant global threat. *International Journal of Molecular Sciences*, *22*(8), 4042.

Azargun, R., Sadeghi, M. R., Hossein, M., Barhaghi, S., Kafil, H. S., Yeganeh, F., & Ghotaslou, R. (2018). The prevalence of plasmid-mediated quinolone resistance and ESBL-production in Enterobacteriaceae

isolated from urinary tract infections. *Infection and drug resistance*, 1007-1014.

Bahjat, S. A., Al-Taee, M. F., Al-Hassani, A. M., & Al-Hassani, O. M. (2019). Molecular and Bacteriological Method for Identification of Lactose Fermenting Salmonella in Mosul Province. *Indian Journal of Public Health Research & Development*, *10*(12).

Baron, S. A., & Rolain, J. M. (2018). Efflux pump inhibitor CCCP to rescue colistin susceptibility in mcr-1 plasmid-mediated colistin-resistant strains and Gram-negative bacteria. *Journal of Antimicrobial Chemotherapy*, *73*(7), 1862-1871.

Bazaid, A. S., Punjabi, A. A., Aldarhami, A., Qanash, H., Alsaif, G., Gattan, H., & Alqadi, A. (2022). Bacterial infections among patients with chronic diseases at a tertiary care hospital in Saudi Arabia. *Microorganisms*, *10*(10), 1907.

Bhatnagar, K., & Wong, A. (2019). The mutational landscape of quinolone resistance in Escherichia coli. *PLoS One*, *14*(11), e0224650.

Bitar, I. (2016). Antimicrobial susceptibilities, drug resistance gene arrays and epidemic potential of Enterobacteriaceae and other Gram negative clinical isolates: novel diagnostic and therapeutic strategies.

Blanco, P., Hernando-Amado, S., Reales-Calderon, J. A., Corona, F., Lira, F., Alcalde-Rico, M., & Martinez, J. L. (2016). Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. *Microorganisms*, *4*(1), 14.

Blanco, P., Sanz-García, F., Hernando-Amado, S., Martínez, J. L., & Alcalde-Rico, M. (2018). The development of efflux pump inhibitors to treat Gram-negative infections. *Expert opinion on drug discovery*, *13*(10), 919-931.

Bolukaoto, J. Y., Kock, M. M., Strydom, K. A., Mbelle, N. M., & Ehlers, M. M. (2019). Molecular characteristics and genotypic diversity of

enterohaemorrhagic Escherichia coli O157: H7 isolates in Gauteng region, South Africa. *Science of the Total Environment*, *692*, 297-304.

Bonten, M., Johnson, J. R., van den Biggelaar, A. H., Georgalis, L., Geurtsen, J., de Palacios, P. I., & Poolman, J. T. (2021). Epidemiology of Escherichia coli bacteremia: a systematic literature review. *Clinical Infectious Diseases*, *72*(7), 1211-1219.

Bottalico, L., Charitos, I. A., Potenza, M. A., Montagnani, M., & Santacroce, L. (2022). The war against bacteria, from the past to present and beyond. *Expert Review of Anti-infective Therapy*, *20*(5), 681-706.

Broncano-Lavado, A., Senhaji-Kacha, A., Santamaría-Corral, G., Esteban, J., & García-Quintanilla, M. (2022). Alternatives to Antibiotics against Mycobacterium abscessus. *Antibiotics*, *11*(10), 1322.

Buckner, M. M., Blair, J. M., La Ragione, R. M., Newcombe, J., Dwyer, D. J., Ivens, A., & Piddock, L. J. (2016). Beyond antimicrobial resistance: evidence for a distinct role of the AcrD efflux pump in Salmonella biology. *MBio*, *7*(6), 10-1128.

Choi, Y. K., Kim, J. J., & Chang, Y. T. (2019). Holding-oriented versus gating-oriented live-cell distinction: highlighting the role of transporters in cell imaging probe development. *Accounts of Chemical Research*, *52*(11), 3097-3107.

Cholewińska, P., Moniuszko, H., Wojnarowski, K., Pokorny, P., Szeligowska, N., Dobicki, W., & Górniak, W. (2022). The occurrence of microplastics and the formation of biofilms by pathogenic and opportunistic bacteria as threats in aquaculture. *International Journal of Environmental Research and Public Health*, *19*(13), 8137.

Chopra, B., & Dhingra, A. K. (2021). Efflux Pump and its Inhibitors: Novel Targets to Combat Drug Resistance. *Anti-Infective Agents*, *19*(3), 231-251..

Cillóniz, C., Dominedò, C., & Torres, A. (2019). Multidrug resistant gram-negative bacteria in community-acquired pneumonia. *Annual Update in Intensive Care and Emergency Medicine 2019*, 459-475.

CLSI. M100Ed33. *Performance standards for antimicrobial susceptibility testing*. Wayne, PA: Clinical and Laboratory Standards Institute; **2023**

Cohen, K. A., Stott, K. E., Munsamy, V., Manson, A. L., Earl, A. M., & Pym, A. S. (2020). Evidence for expanding the role of streptomycin in the management of drug-resistant Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *64*(9), 10-1128.

Colclough, A. L., Alav, I., Whittle, E. E., Pugh, H. L., Darby, E. M., Legood, S. W., & Blair, J. M. (2020). RND efflux pumps in Gram-negative bacteria; regulation, structure and role in antibiotic resistance. *Future microbiology*, *15*(2), 143-157.

Compagne, N., Vieira Da Cruz, A., Müller, R. T., Hartkoorn, R. C., Flipo, M., & Pos, K. M. (2023). Update on the discovery of efflux pump inhibitors against critical priority gram-negative bacteria. *Antibiotics*, *12*(1), 180.

Dagur, P., Ghosh, M., & Patra, A. (2023). Aminoglycoside antibiotics. In *Medicinal Chemistry of Chemotherapeutic Agents* (pp. 135-155). Academic Press.

Dhanda, G., Acharya, Y., & Haldar, J. (2023). Antibiotic Adjuvants: A Versatile Approach to Combat Antibiotic Resistance. *ACS omega*, *8*(12), 10757-10783.

Dinev, T., Velichkova, K., Stoyanova, A., & Sirakov, I. (2023). Microbial Pathogens in Aquaponics Potentially Hazardous for Human Health. *Microorganisms*, *11*(12), 2824.

Douafer, H., Andrieu, V., Phanstiel IV, O., & Brunel, J. M. (2019). Antibiotic adjuvants: make antibiotics great again!. *Journal of medicinal chemistry*, *62*(19), 8665-8681.

Dunachie, S. J., Esmail, H., Corrigan, R., & Dudareva, M. (2022). Infectious Disease. In *Medicine for Finals and Beyond* (pp. 21-82). CRC Press.

Ehuwa, O., Jaiswal, A. K., & Jaiswal, S. (2021). Salmonella, food safety and food handling practices. *Foods*, *10*(5), 907.

Einstein, A., B. Podolsky, and N. Rosen, 1935, "Can quantum-mechanical description of physical reality be considered complete?", Phys. Rev. **47**, 777-780.

El-Tarabili, R. M., Ahmed, E. M., Alharbi, N. K., Alharbi, M. A., AlRokban, A. H., Naguib, D., & Mahmoud, A. E. (2022). Prevalence, antibiotic profile, virulence determinants, ESBLs, and non-β-lactam encoding genes of MDR Proteus spp. isolated from infected dogs. *Frontiers in Genetics*, *13*, 952689.

Fanelli, G., Pasqua, M., Prosseda, G., Grossi, M., & Colonna, B. (2023). AcrAB efflux pump impacts on the survival of adherent-invasive Escherichia coli strain LF82 inside macrophages. *Scientific Reports*, *13*(1), 2692.

Farhat, N., & Khan, A. U. (2022). Therapeutic approaches to combat the global antibiotic resistance challenge. *Future Microbiology*, *17*(18), 1515-1529.

Farmer, J. J. (2022). Antigenic structures. *Textbook of Diagnostic Microbiology-E-Book*, 432.

Farré-Maduell, E., & Casals-Pascual, C. (2019). The origins of gut microbiome research in Europe: From Escherich to Nissle. *Human Microbiome Journal*, *14*, 100065.

Fief, C. A., Hoang, K. G., Phipps, S. D., Wallace, J. L., & Deweese, J. E. (2019). Examining the impact of antimicrobial fluoroquinolones on human DNA topoisomerase IIα and IIβ. *ACS omega*, *4*(2), 4049-4055.

Gaurav, A., Bakht, P., Saini, M., Pandey, S., & Pathania, R. (2023). Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. *Microbiology*, *169*(5), 001333.

Gil-Gil, T., Laborda, P., Ochoa-Sánchez, L. E., Martínez, J. L., & Hernando-Amado, S. (2023). Efflux in Gram-negative bacteria: what are the latest opportunities for drug discovery?. *Expert Opinion on Drug Discovery*, 1-16.

Girlich, D., Bonnin, R. A., Dortet, L., & Naas, T. (2020). Genetics of acquired antibiotic resistance genes in *Proteus* spp. *Frontiers in microbiology*, *11*, 256.

Gourama, H. (2020). Foodborne pathogens. *Food safety engineering*, 25-49.

Gouveia, M. A. D. C., Lins, M. T. C., & Silva, G. A. P. D. (2020). Acute diarrhea with blood: diagnosis and drug treatment. *Jornal de pediatria*, *96*, 20-28.

Guérin, F., Gravey, F., Reissier, S., Penven, M., Michaux, C., Le Hello, S., & Cattoir, V. (2023). Temocillin Resistance in the Enterobacter cloacae Complex Is Conferred by a Single Point Mutation in BaeS, Leading to Overexpression of the AcrD Efflux Pump. *Antimicrobial Agents and Chemotherapy*, *67*(6), e00358-23.

Guest, R. L. (2017). Regulation of respiration by the Cpx response in enteropathogenic Escherichia coli.

Haidar-Ahmad, N., Manigat, F. O., Silué, N., Pontier, S. M., & Campbell-Valois, F. X. (2023). A Tale about Shigella: Evolution, Plasmid, and Virulence. *Microorganisms*, *11*(7), 1709.

Harahap, D. H., Marchira, C. R., Solikhah, E. N., & Moch, D. (2021). The role of aripiprazole in improvement of penile erection in schizophrenia patients with erectile dysfunction. *Med Glas (Zenica)*, *18*(2), 432-437.

Henderson, P. J., Maher, C., Elbourne, L. D., Eijkelkamp, B. A., Paulsen, I. T., & Hassan, K. A. (2021). Physiological functions of bacterial "multidrug" efflux pumps. *Chemical reviews*, *121*(9), 5417-5478.

Holland, M. S., Nobrega, D., Peirano, G., Naugler, C., Church, D. L., & Pitout, J. D. D. (2020). Molecular epidemiology of Escherichia coli causing bloodstream infections in a centralized Canadian region: a population-based surveillance study. *Clinical Microbiology and Infection*, *26*(11), 1554-e1.

Honarmand Jahromy, S., Fathi Rad, J., & Noorbakhsh, F. (2018). Effect of Inhibitor Carbonyl Cyanide 3-Chlorophenylhydrazone (CCCP) on the Minimum Inhibitory Concentration of Aminoglycoside and efflux pump activity determination in Acinetobacter baumannii Multiple-Drug Resistant isolates. *Iranian Journal of Biological Sciences*, *12*(4), 39-46.

Hu, X., Zhang, H., Wang, Y., Shiu, B. C., Lin, J. H., Zhang, S., ... & Li, T. T. (2022). Synergistic antibacterial strategy based on photodynamic therapy: Progress and perspectives. *Chemical Engineering Journal*, *450*, 138129.

Hussain, H., Ibraheem, N., Al-Rubaey, N., Radhi, M., Hindi, N., & AL-Jubori, R. (2022). A review of airborne contaminated microorganisms associated with human diseases. *Medical Journal of Babylon*, *19*(2).

Huynh, T. Q., Tran, V. N., Thai, V. C., Nguyen, H. A., Nguyen, N. T. G., Tran, M. K., & Nguyen, T. T. H. (2023). Genomic alterations involved in fluoroquinolone resistance development in Staphylococcus aureus. *Plos one*, *18*(7), e0287973.

Isaac, B. (2019). Unexplained fever in infectious diseases: section 2: commonly spirochetes, and parasites. *Unexplained Fever*, 327-354.

Jamshaid, M., Shahzad, A., Munir, H., Tayyab, M., Zafar, A. A., & Kamal, I. (2021). Invasive Klebsiella Pneumoniae Syndrome in Qatar. *Authorea Preprints*.

Jones, P. M., & George, A. M. (2023). The Switch and Reciprocating Models for the Function of ABC Multidrug Exporters: Perspectives on Recent Research. *International Journal of Molecular Sciences*, *24*(3), 2624.

Karampatakis, T., Tsergouli, K., & Behzadi, P. (2023). Carbapenem-resistant Klebsiella pneumoniae: Virulence factors, molecular epidemiology and latest updates in treatment options. *Antibiotics*, *12*(2), 234.

Kebede, T., Gadisa, E., & Tufa, A. (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLoS One*, *16*(3), e0249253.

Kermani, A. A., Burata, O. E., Koff, B. B., Koide, A., Koide, S., & Stockbridge, R. B. (2022). Crystal structures of bacterial small multidrug resistance transporter EmrE in complex with structurally diverse substrates. *Elife*, *11*, e76766.

Kermani, A. A., Macdonald, C. B., Burata, O. E., Ben Koff, B., Koide, A., Denbaum, E., & Stockbridge, R. B. (2020). The structural basis of promiscuity in small multidrug resistance transporters. *Nature Communications*, *11*(1), 6064.

Khalily, M. A., Anwar, H., Bashir, B., Farhat, A., Shafiq, M., & Tariq, S. (2022). Frequency And Antibiotic Sensitivity Of Most Common Organisms Causing Urinary Tract Infections In Children. *Pakistan Journal of Physiology*, *18*(1), 29-31.

Kim, H. B., Wang, M., Park, C. H., Kim, E. C., Jacoby, G. A., & Hooper, D. C. (2009). oqxAB encoding a multidrug efflux pump in human clinical isolates of Enterobacteriaceae. *Antimicrobial agents and chemotherapy*, *53*(8), 3582-3584

Kim, J., Cater, R. J., Choy, B. C., & Mancia, F. (2021). Structural insights into transporter-mediated drug resistance in infectious diseases. *Journal of molecular biology*, *433*(16), 167005.

Koch, N., Islam, N. F., Sonowal, S., Prasad, R., & Sarma, H. (2021). Environmental antibiotics and resistance genes as emerging contaminants: methods of detection and bioremediation. *Current research in microbial sciences*, *2*, 100027.

Konwar, A. N., Hazarika, S. N., Bharadwaj, P., & Thakur, D. (2022). Emerging Non-Traditional Approaches to Combat Antibiotic Resistance. *Current Microbiology*, *79*(11), 330.

Krishnan, D., Sabapathi, S. L., Raja, K. S., Gunaseelan, R., & Rohithraj, J. (2023). A Cross Sectional Study on Types, Clinical Assessment and Management of Inguinal Hernia. *Indian Journal of Pharmacy Practice*, *16*(4).

Kumar, P., & Kumar, M. (2023). Pancolitis in Enteric Fever: A Rare Occurrence. *Journal of Global Infectious Diseases*, *15*(2), 86-87.

Kumawat, M., Nabi, B., Daswani, M., Viquar, I., Pal, N., Sharma, P., ... & Kumar, M. (2023). Role of bacterial efflux pump proteins in antibiotic resistance across microbial species. *Microbial Pathogenesis*, 106182.

Laxson, L. C. (2022). 13 Chapter Osmotic Agents. *Havener's Ocular Pharmacology*, 139.

Li, J., Zhang, H., Ning, J., Sajid, A., Cheng, G., Yuan, Z., & Hao, H. (2019). The nature and epidemiology of OqxAB, a multidrug efflux pump. *Antimicrobial Resistance & Infection Control*, *8*(1), 1-13.

Li, Z., Wu, L., Huang, Z., Lv, B., Fu, Y., Zhou, L., & Fu, X. (2023). CCCP Facilitates Aminoglycoside to Kill Late Stationary-Phase Escherichia coli by Elevating Hydroxyl Radical. *ACS Infectious Diseases*, *9*(4), 801-814.

Liu, X. (2019). ABC family transporters. *Drug transporters in drug disposition, effects and toxicity*, 13-100.

Liu, Y., Li, R., Xiao, X., & Wang, Z. (2019). Antibiotic adjuvants: an alternative approach to overcome multi-drug resistant Gram-negative bacteria. *Critical reviews in microbiology*, *45*(3), 301-314.

Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.;Giske, C.G; Harbarth, S.; Kahlmeter, G.; Olsson-Liljequist, B. and Monnet D.L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiology Infection, 18: 268281

Malekzadegan, Y., Khashei, R., Sedigh Ebrahim-Saraie, H., & Jahanabadi, Z. (2018). Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic Escherichia coli isolates from Iranian patients. *BMC infectious diseases*, *18*(1), 1-9.

Martin-Loeches, I., Rodriguez, A. H., & Torres, A. (2018). New guidelines for hospital-acquired pneumonia/ventilator-associated pneumonia: USA vs. Europe. *Current opinion in critical care*, *24*(5), 347-352.

Meka, Y. (2023). *Pharmacology of Disulfiram as an Antibacterial Agent* (Doctoral dissertation, Marshall University).

Meng, M., Li, Y., & Yao, H. (2022). Plasmid-mediated transfer of antibiotic resistance genes in soil. *Antibiotics*, *11*(4), 525.

Mills, E. G., Martin, M. J., Luo, T. L., Ong, A. C., Maybank, R., Corey, B. W., ... & Lebreton, F. (2022). A one-year genomic investigation of Escherichia coli epidemiology and nosocomial spread at a large US healthcare network. *Genome medicine*, *14*(1), 1-13.

Mitiku, A., Aklilu, A., Tsalla, T., Woldemariam, M., Manilal, A., & Biru, M. (2022). Magnitude and antimicrobial susceptibility profiles of Gram-Negative bacterial isolates among patients suspected of urinary tract infections in Arba Minch General Hospital, southern Ethiopia. *PLoS One*, *17*(12), e0279887.

Moftian, N., Rezaei-Hachesu, P., Arab-Zozani, M., Samad-Soltani, T., Esfandiari, A., Tabib, M. S., & Mirnia, K. (2023). Prevalence of gram-negative bacteria and their antibiotic resistance in neonatal sepsis in Iran: a systematic review and meta-analysis. *BMC Infectious Diseases*, *23*(1), 1-15.

Morais Oliveira-Tintino, C. D., Muniz, D. F., dos Santos Barbosa, C. R., Silva Pereira, R. L., Begnini, I. M., Rebelo, R. A., & da Silva, T. G. (2023). NorA, Tet (K), MepA, and MsrA Efflux Pumps in Staphylococcus aureus, Their Inhibitors and 1, 8-Naphthyridine Sulfonamides. Current Pharmaceutical Design, 29(5), 323-355.

Monárrez, R., Braun, M., Coburn-Flynn, O., Botelho, J., Odetoyin, B. W., Otero-Vera, J. I., ... & Okeke, I. N. (2019). A large self-transmissible resistance plasmid from Nigeria contains genes that ameliorate a carrying cost. *Scientific Reports*, *9*(1), 19624.

Mone, N. S., Bhagwat, S. A., Sharma, D., Chaskar, M., Patil, R. H., Zamboni, P., ... & Satpute, S. K. (2021). Naphthoquinones and their derivatives: emerging trends in combating microbial pathogens. *Coatings*, *11*(4), 434.

Moosavian, M., Khoshkholgh Sima, M., Ahmad Khosravi, N., & Abbasi Montazeri, E. (2021). Detection of oqxab efflux pumps, a multidrug-resistant agent in bacterial infection in patients referring to teaching hospitals in ahvaz, southwest of Iran. *International Journal of Microbiology*, *2021*.

Muzembo, B. A., Kitahara, K., Mitra, D., Ohno, A., Khatiwada, J., Dutta, S., & Miyoshi, S. I. (2023). Burden of Shigella in South Asia: a systematic review and meta-analysis. *Journal of Travel Medicine*, *30*(1), taac132.

Myers, E. M. (2022). *Improving Modeling and Monitoring of Waterborne Sewage Contamination: Particle Association and Water Transparency*

Impacts on Fecal Pollution Persistence (Doctoral dissertation, Columbia University).

Nasiru, A., Yunusa, S., Kazeem,I., Auwal, M., & Abubakar, A. (2022). Isolation and Identification of Gastrointestinal Bacteria in Drinking Water Sources in Gwallameji, Bauchi. Bosák, J., Hrala, M., Micenková, L., & Šmajs, D. (2021). Non-antibiotic antibacterial peptides and proteins of Escherichia coli: efficacy and potency of bacteriocins. *Expert Review of Anti-infective Therapy*, *19*(3), 309-322.

Ndlovu, T., Kgosietsile, L., Motshwarakgole, P., & Ndlovu, S. I. (2023). Evaluation of Potential Factors Influencing the Dissemination of Multidrug-Resistant Klebsiella pneumoniae and Alternative Treatment Strategies. *Tropical Medicine and Infectious Disease*, *8*(8), 381.

Nye, T. M., Tükenmez, H., Singh, P., Flores-Mireles, A. L., Obernuefemann, C. L., Pinkner, J. S., & Hultgren, S. J. (2022). Ring-fused 2-pyridones effective against multidrug-resistant Gram-positive pathogens and synergistic with standard-of-care antibiotics. *Proceedings of the National Academy of Sciences*, *119*(43), e2210912119.

Okada, U., & Murakami, S. (2022). Structural and functional characteristics of the tripartite ABC transporter. *Microbiology*, *168*(11), 001257.

Okada, U., & Murakami, S. (2023). Structural and Functional Aspects of the Macrolide Efflux Transporter, MacB—A Tripartite ABC Transporter. In *Therapeutic Protein Targets for Drug Discovery and Clinical Evaluation: Bio-Crystallography and Drug Design* (pp. 1-23).

Oliveira, H. G. (2017, September). A survey on intelligent poetry generation: Languages, features, techniques, reutilisation and evaluation. In *Proceedings of the 10th international conference on natural language generation* (pp. 11-20).

Onishi, R., Shigemura, K., Osawa, K., Yang, Y. M., Maeda, K., Tanimoto, H., ... & Fujisawa, M. (2023). Molecular characteristics and genetic

analysis of the genes related to carbapenemase, efflux pump, and outer membrane proteins in carbapenem-resistant Klebsiella pneumoniae. *Letters in Applied Microbiology*, *76*(2), ovac069.

Orelle, C., Mathieu, K., & Jault, J. M. (2019). Multidrug ABC transporters in bacteria. *Research in Microbiology*, *170*(8), 381-391.

Ourama, H. (2020). Foodborne pathogens. *Food safety engineering*, 25-49.

Öztürk, R., & Murt, A. (2020). Epidemiology of urological infections: a global burden. *World journal of urology*, *38*, 2669-2679.

Pakbin, B., Brück, W. M., & Rossen, J. W. (2021). Virulence factors of enteric pathogenic Escherichia coli: A review. *International journal of molecular sciences*, *22*(18), 9922.

Paracini, N., Schneck, E., Imberty, A., & Micciulla, S. (2022). Lipopolysaccharides at solid and liquid interfaces: models for biophysical studies of the gram-negative bacterial outer membrane. *Advances in Colloid and interface Science*, *301*, 102603.

Park, K. S., Kim, M. H., Park, T. S., Nam, Y. S., Lee, H. J., & Suh, J. T. (2012). Prevalence of the plasmid-mediated quinolone resistance genes, aac (6′)-Ib-cr, qepA, and oqxAB in clinical isolates of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae in Korea. *Annals of Clinical & Laboratory Science*, *42*(2), 191-197.

Pasqua, M., Bonaccorsi di Patti, M. C., Fanelli, G., Utsumi, R., Eguchi, Y., Trirocco, R., & Colonna, B. (2021). Host-bacterial pathogen communication: the wily role of the multidrug efflux pumps of the MFS family. *Frontiers in Molecular Biosciences*, *8*, 723274.

Priyanka, A., Akshatha, K., Deekshit, V. K., Prarthana, J., & Akhila, D. S. (2020). *Klebsiella pneumoniae* infections and antimicrobial drug resistance. *Model organisms for microbial pathogenesis, biofilm formation and antimicrobial drug discovery*, 195-225.

Pun, M., Khazanov, N., Galsurker, O., Kerem, Z., Senderowitz, H., & Yedidia, I. (2023). Inhibition of AcrAB-TolC enhances antimicrobial activity of phytochemicals in Pectobacterium brasiliense. *Frontiers in Plant Science*, *14*, 1161702.

Raj, S., Sharma, T., Pradhan, D., Tyagi, S., Gautam, H., Singh, H., ... & Mohapatra, S. (2022). Comparative Analysis of Clinical and Genomic Characteristics of Hypervirulent Klebsiella pneumoniae from Hospital and Community Settings: Experience from a Tertiary Healthcare Center in India. *Microbiology Spectrum*, *10*(5), e00376-22.

Rippon, M. G., Rogers, A. A., & Ousey, K. (2021). Antimicrobial stewardship strategies in wound care: evidence to support the use of dialkylcarbamoyl chloride (DACC)-coated wound dressings. *Journal of wound care*, *30*(4), 284-296.

Rodríguez-Martínez, J. M., Díaz de Alba, P., Briales, A., Machuca, J., Lossa, M., Fernández-Cuenca, F., ... & Pascual, Á. (2013). Contribution of OqxAB efflux pumps to quinolone resistance in extended-spectrum-β-lactamase-producing *Klebsiella pneumoniae*. *Journal of Antimicrobial Chemotherapy*, *68*(1), 68-73.

Roy, S. (2023). *A New Strategy for Persister Control* (Doctoral dissertation, Syracuse University).

Roy, S., Hasan, I., & Guo, B. (2023). Recent advances in nanoparticle-mediated antibacterial applications. *Coordination Chemistry Reviews*, *482*, 215075.

Rubio, A., Garland, G. D., Sfakianos, A., Harvey, R. F., & Willis, A. E. (2022, November). Aberrant protein synthesis and cancer development: The role of canonical eukaryotic initiation, elongation and termination factors in tumorigenesis. In *Seminars in Cancer Biology* (Vol. 86, pp. 151-165). Academic Press.

Russo, A., Fusco, P., Morrone, H. L., Trecarichi, E. M., & Torti, C. (2023). New advances in management and treatment of multidrug-resistant Klebsiella pneumoniae. *Expert Review of Anti-infective Therapy*, *21*(1), 41-55.

Sader, J. E., Huertas-Cerdeira, C., & Gharib, M. (2016). Stability of slender inverted flags and rods in uniform steady flow. *Journal of Fluid Mechanics*, *809*, 873-894.

Saeed, A. Y., Noomi, B. S., Al-Janabi, A. S., & Saeed, A. Y. (2022). Evaluation of the Immunogenicity of Salmonella typhimurium Flagellar (H) Antigens and their Effect on the Tissues of White Male Rats. *HIV Nursing*, *22*(2), 945-954.

Sagar, S., Kaistha, S., Das, A. J., Kumar, R., Sagar, S., Kaistha, S., ... & Kumar, R. (2019). Intrinsic Antibiotic Resistance Mechanism in Bacteria. *Antibiotic Resistant Bacteria: A Challenge to Modern Medicine*, 69-85

Saleh, T. H., Hashim, S. T., Malik, S. N., & AL-Rubaii, B. A. L. (2019). Down-regulation of flil gene expression by Ag nanoparticles and TiO 2 nanoparticles in pragmatic clinical isolates of Proteus mirabilis and Proteus vulgaris from urinary tract infection. *Nano Biomedicine and Engineering*, *11*(4), 321-332..

Sanchez-Carbonel, A., Mondragón, B., López-Chegne, N., Peña-Tuesta, I., Huayan-Dávila, G., Blitchtein, D., and del Valle-Mendoza, J. (2021). The effect of the efflux pump inhibitor Carbonyl Cyanide m-Chlorophenylhydrazone (CCCP) on the susceptibility to imipenem and cefepime in clinical strains of Acinetobacter baumannii. *PLoS One*, *16*(12), e0259915.

Sanz-García, F., Gil-Gil, T., Laborda, P., Ochoa-Sánchez, L. E., Martínez, J. L., & Hernando-Amado, S. (2021). Coming from the wild: multidrug resistant opportunistic pathogens presenting a primary, not

human-linked, environmental habitat. *International Journal of Molecular Sciences*, *22*(15), 8080.

Sarshar, S., Mirnejad, R., & Babapour, E. (2021). Frequency of blaCTX-M and blaTEM Virulence Genes and Antibiotic Resistance Profiles among Klebsiella pneumoniae Isolates in Urinary Tract Infection (UTI) Samples from Hashtgerd, Iran. *Reports of Biochemistry & Molecular Biology*, *10*(3), 412.

Schwaber, M. J., Temkin, E., Lobl, R., Schechner, V., Nutman, A., & Carmeli, Y. (2022). Hospital-acquired bacterial infections in coronavirus disease 2019 (COVID-19) patients in Israel. *Infection Control & Hospital Epidemiology*, 1-6.

Scoffone, V. C., Trespidi, G., Barbieri, G., Irudal, S., Perrin, E., & Buroni, S. (2021). Role of RND efflux pumps in drug resistance of cystic fibrosis pathogens. *Antibiotics*, *10*(7), 863.

Seukep, A. J., Mbuntcha, H. G., Kuete, V., Chu, Y., Fan, E., & Guo, M. Q. (2022). What Approaches to Thwart Bacterial Efflux Pumps-Mediated Resistance?. *Antibiotics*, *11*(10), 1287.

Sharma, A., Gupta, V. K., & Pathania, R. (2019). Efflux pump inhibitors for bacterial pathogens: From bench to bedside. *The Indian journal of medical research*, *149*(2), 129.

Sharma, S., Kaushik, V., Kulshrestha, M., & Tiwari, V. (2023). Different Efflux Pump Systems in Acinetobacter baumannii and Their Role in Multidrug Resistance. *Advances in Microbiology, Infectious Diseases and Public Health: Volume 17*, 155-168.

Shi, X., Chen, M., Yu, Z., Bell, J. M., Wang, H., Forrester, I., ... & Wang, Z. (2019). In situ structure and assembly of the multidrug efflux pump AcrAB-TolC. *Nature communications*, *10*(1), 2635.

Shoeb, M., Islam, R., & Parvin, N. (2023). Antibiotic Resistance: A Global Threat to Humanity. In *Transcending Humanitarian Engineering Strategies for Sustainable Futures* (pp. 82-105). IGI Global.

Silva, R. D. C. B., Andrade, J., Dias, V. C., Sequeto, J. C., Santos, N. F., da Silva, V. L., & Diniz, C. G. (2022). Physiological Characteristics of Putative Enterobacteria Associated with Meat and Fish Available in Southern Brazilian Retail Markets: Antimicrobial Susceptibility, Toxic Metal Tolerance and Expression of Efflux Pumps. *Antibiotics*, *11*(12), 1677.

Singh, T., Dar, S. A., Das, S., & Haque, S. (2020). Importance of efflux pumps in subjugating antibiotic resistance. In *Drug Discovery Targeting Drug-Resistant Bacteria* (pp. 273-299). Academic Press.

Sionov, R. V., & Steinberg, D. (2022). Targeting the holy triangle of quorum sensing, biofilm formation, and antibiotic resistance in pathogenic bacteria. *Microorganisms*, *10*(6), 1239.

Smithers, L., Olatunji, S., & Caffrey, M. (2021). Bacterial lipoprotein posttranslational modifications. new insights and opportunities for antibiotic and vaccine development. *Frontiers in Microbiology*, *12*, 788445

Song, M., Liu, Y., Huang, X., Ding, S., Wang, Y., Shen, J., & Zhu, K. (2020). A broad-spectrum antibiotic adjuvant reverses multidrug-resistant Gram-negative pathogens. *Nature Microbiology*, *5*(8), 1040-1050.

Srikant, S. (2020). Evolutionary history of ATP-binding cassette proteins. *FEBS letters*, *594*(23), 3882-3897.

Sundaresan, S., & Rathinavelan, T. (2023). Species Serotyping Using the Sequences of O-Antigen Biosynthesis Proteins and H-Antigen Filament Proteins.

Tajbakhsh, E.; Ahmadi, P. Abedpour-Dehkordi, E.; Arbab-Soleimani, N. andKhamesipour, F. (2016). Biofilm Formation, Antimicrobial Susceptibility, Serogroups and Virulence Genes of Uropathogenic *E. coli*

Isolated from ClinicalSamples in Iran. Antimicrob Resist Infect Control. 5(11): 1-8.

Terreni, M., Taccani, M., & Pregnolato, M. (2021). New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. *Molecules*, *26*(9), 2671.

Theuretzbacher, U., Bush, K., Harbarth, S., Paul, M., Rex, J. H., Tacconelli, E., & Thwaites, G. E. (2020). Critical analysis of antibacterial agents in clinical development. *Nature Reviews Microbiology*, *18*(5), 286-298.

Upadhyay, N., Kar, D., Deepak Mahajan, B., Nanda, S., Rahiman, R., Panchakshari, N., & Datta, S. (2019). The multitasking abilities of MATE transporters in plants. *Journal of experimental botany*, *70*(18), 4643-4656.

Van Duin, D., & Paterson, D. L. (2016). Multidrug-resistant bacteria in the community: trends and lessons learned. *Infectious disease clinics*, *30*(2), 377-390.

Varela, M. F., Stephen, J., Lekshmi, M., Ojha, M., Wenzel, N., Sanford, L. M., ... & Kumar, S. H. (2021). Bacterial resistance to antimicrobial agents. *Antibiotics*, *10*(5), 593.

Vergalli, J., Bodrenko, I. V., Masi, M., Moynié, L., Acosta-Gutierrez, S., Naismith, J. H., ... & Pagès, J. M. (2020). Porins and small-molecule translocation across the outer membrane of Gram-negative bacteria. *Nature Reviews Microbiology*, *18*(3), 164-176.

Verma, H., Patra, R. K., Sethi, D., & Pattanayak, S. K. (2022). Isolation and characterization of native Rhizobium from root nodules of raikia french bean growing area of Odisha. *Indian Journal of Biochemistry and Biophysics (IJBB)*, *59*(9), 918-926.

Walia, K. (2017). *Targeted low cost strategies to combat Salmonella spp. in finisher pigs and in the slaughterhouse* (Doctoral dissertation, Waterford Institute of Technology).

Wang, W., Li, H., Li, M., Dong, Y., Bai, Y., Li, F., & Xiao, J. (2022). Molecular Evolution and Genomic Insights into Community-Acquired Methicillin-Resistant Staphylococcus aureus Sequence Type 88. *Microbiology Spectrum*, *10*(4), e00342-22.

Wójcicki, M., Świder, O., Daniluk, K. J., Średnicka, P., Akimowicz, M., Roszko, M. Ł., & Juszczuk-Kubiak, E. (2021). Transcriptional regulation of the multiple resistance mechanisms in salmonella—a review. *Pathogens*, *10*(7), 801.

Xia, Y., Cebrián, R., Xu, C., Jong, A. D., Wu, W., & Kuipers, O. P. (2021). Elucidating the mechanism by which synthetic helper peptides sensitize Pseudomonas aeruginosa to multiple antibiotics. *PLoS pathogens*, *17*(9), e1009909.

Yarlagadda, V., Medina, R., & Wright, G. D. (2020). Venturicidin A, a membrane-active natural product inhibitor of ATP synthase potentiates aminoglycoside antibiotics. *Scientific Reports*, *10*(1), 8134.

Yuan, J., Xu, X., Guo, Q., Zhao, X., Ye, X., Guo, Y., & Wang, M. (2012). Prevalence of the oqxAB gene complex in Klebsiella pneumoniae and Escherichia coli clinical isolates. *Journal of Antimicrobial Chemotherapy*, *67*(7), 1655-1659.

Zaman, S. B., Hussain, M. A., Nye, R., Mehta, V., Mamun, K. T., & Hossain, N. (2017). A review on antibiotic resistance: alarm bells are ringing. *Cureus*, *9*(6).

Zhang, Q. Y., Yan, Z. B., Meng, Y. M., Hong, X. Y., Shao, G., Ma, J. J., & Fu, C. Y. (2021). Antimicrobial peptides: mechanism of action, activity and clinical potential. *Military Medical Research*, *8*, 1-25.

Zhao, J., Chen, Z., Chen, S., Deng, Y., Liu, Y., Tian, W., & Liu, J. H. (2010). Prevalence and dissemination of oqxAB in Escherichia coli isolates from animals, farmworkers, and the environment. *Antimicrobial agents and chemotherapy*, *54*(10), 4219-4224

Zhu, L. T., Huang, H. N., Avellán-Llaguno, R. D., Qin, Y., An, X. L., Su, J. Q., & Zhu, Y. G. (2022). Diverse functional genes harboured in extracellular vesicles from environmental and human microbiota. *Journal of Extracellular Vesicles*, *11*(12), 12292.

Zwama, M., & Nishino, K. (2021). Ever-adapting RND efflux pumps in Gram-negative multidrug-resistant pathogens: a race against time. *Antibiotics*, *10*(7), 774.

Appendices (I)

Vitek (2) Reports:

bioMérieux Customer:

Microbiology Chart Report

Printed July 3, 2023 1:32:32 PM CDT

Patient Name: qasem 1, hadeel
Location:
Lab ID: 17620231

Patient ID: 17620231
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source:

Collected:

Comments:	

Susceptibility Information	**Analysis Time:** 8.73 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Imipenem	>= 16	R
+Amoxicillin		R	+Meropenem		
Ampicillin	>= 32	R	+Panipenem		
+Piperacillin/Sulbactam		R	Amikacin	<= 2	S
Piperacillin/Tazobactam	>= 128	R	Gentamicin	>= 16	R
Cefazolin	>= 64	R	+Isepamicin		S
+Cefotetan			+Tobramycin		
Cefoxitin	>= 64	R	Ciprofloxacin	>= 4	R
+Cefoperazone			Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	+Sparfloxacin		R
+Ceftizoxime			Tigecycline	<= 0.5	S
Ceftriaxone	>= 64	R	Nitrofurantoin	<= 16	S
Cefepime	>= 64	R	Trimethoprim/ Sulfamethoxazole	>= 320	R

AES Findings	
Confidence:	Consistent

bioMérieux Customer:

Microbiology Chart Report

Printed July 5, 2023 8:37:29 AM CDT

Patient Name: qasem 12, hadeel
Location:
Lab ID: 4720232

Patient ID: 4720232
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Enterobacter aerogenes

Source:

Collected:

Comments:	

Susceptibility Information	**Analysis Time:** 9.47 hours	**Status:** Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL			Imipenem	<= 0.25	S
+Amoxicillin		R	+Meropenem		S
Ampicillin			+Panipenem		
+Piperacillin/Sulbactam			Amikacin	<= 2	S
Piperacillin/Tazobactam	>= 128	R	Gentamicin	>= 16	R
Cefazolin	>= 64	R	+Isepamicin		S
+Cefotetan		R	+Tobramycin		
Cefoxitin	8	*R	Ciprofloxacin	>= 4	R
+Cefoperazone			Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	+Sparfloxacin		R
+Ceftizoxime			Tigecycline	<= 0.5	S
Ceftriaxone	>= 64	R	Nitrofurantoin	<= 16	S
Cefepime	>= 64	R	Trimethoprim/ Sulfamethoxazole	>= 320	R
Ertapenem	<= 0.5	S			

***= AES modified **= User modified**

AES Findings	
Confidence:	Consistent

Appendices (2)

Antibiotic susceptibility profile of Enterobacteriaceae isolates to flouroquinoloines and Aminoglygosides. using DDT results

NO	Name	Flouroquinoloines				Aminoglygosides				ASP*
		CIP	LE	NX	GAT	AK	CN	K	HLS	
1	**E.coli-1**	S	S	S	S	S	S	I	S	S
2	**E.coli-2**	S	S	S	S	S	R	S	R	S
3	**E.coli-3**	S	S	S	S	S	S	S	S	S
4	**E.coli-4**	S	S	S	S	S	S	R	S	S
5	**E.coli-5**	S	S	S	S	S	R	R	R	MDR
6	**E.coli-6**	S	S	S	S	S	S	I	S	S
7	**E.coli-7**	S	S	S	S	S	S	R	S	S
8	**E.coli-8**	R	I	R	S	S	R	R	S	MDR
9	**E.coli-9**	S	S	S	S	S	S	R	S	S
10	**E.coli-10**	I	S	S	S	S	S	R	S	S
11	**E.coli-11**	R	I	R	S	S	S	I	S	S
12	**E.coli-12**	S	S	S	S	S	S	S	S	S
13	**E.coli-13**	S	S	S	S	S	S	S	S	S
14	**E.coli-14**	R	I	R	I	S	S	R	S	MDR
15	**E.coli-15**	S	S	S	S	S	S	S	S	S
16	**E.coli-16**	R	R	R	R	S	S	R	S	MDR
17	**E.coli-17**	S	S	S	S	S	S	S	S	S
18	**E.coli-18**	I	S	S	S	S	S	S	S	S
19	**E.coli-19**	R	R	R	R	S	R	S	S	MDR
20	**E.coli-20**	I	S	S	S	S	S	S	S	S
21	**E.coli-21**	R	I	R	R	S	R	S	S	MDR
22	**E.coli-22**	S	S	S	S	S	S	S	I	S
23	**E.coli-23**	R	I	R	I	S	R	R	S	MDR
24	**E.coli-24**	R	I	R	S	S	R	R	I	MDR
25	**E.coli-25**	R	S	R	S	S	S	R	R	MDR
26	**E.coli-26**	S	S	S	S	S	I	R	I	S
27	**E.coli-27**	R	R	R	R	S	R	S	S	MDR
28	**E.coli-28**	R	R	R	R	S	I	S	S	MDR
29	**E.coli-29**	S	R	S	S	S	S	S	S	S
30	**E.coli-30**	R	S	S	S	S	S	R	S	S
31	**E.coli-31**	S	R	S	S	S	S	S	S	S
32	**E.coli-32**	R	S	R	R	S	I	R	S	MDR
33	**E.coli-33**	R	R	R	R	S	R	I	S	MDR
34	**E.coli-34**	R	R	R	R	S	R	I	S	MDR
35	**E.coli-35**	R	R	R	R	S	R	I	S	MDR
36	**E.coli-36**	S	I	S	S	S	S	S	S	S
37	**E.coli-37**	S	S	S	S	S	R	I	S	S

38	**E.coli-38**	R	R	R	R	S	S	R	S	MDR
39	**E.coli-39**	R	R	R	R	S	R	I	S	XDR
40	**E.coli-40**	R	I	S	S	S	S	I	S	S
41	**E.coli-41**	S	S	R	S	S	S	R	S	S
42	**E.coli-42**	R	R	R	R	S	I	S	S	MDR
43	**E.coli-43**	R	R	R	R	S	S	S	S	MDR
44	**E.coli-44**	R	R	R	R	S	S	R	S	MDR
45	**E.coli-45**	S	S	S	S	S	R	S	S	S
46	**E.coli-46**	S	S	S	R	S	S	S	S	S
47	**E.coli-47**	R	R	R	R	S	R	R	S	XDR
48	**E.coli-48**	R	R	R	R	S	S	I	S	MDR
49	**E.coli-49**	R	R	R	R	S	R	R	S	XDR
50	**E.coli-50**	R	R	R	R	S	S	R	S	MDR
51	**E.coli-51**	S	S	S	S	S	S	S	S	S
52	**E.coli-52**	R	R	R	R	S	S	S	S	MDR
53	**E.coli-53**	R	R	R	R	S	S	I	S	MDR
54	**E.coli-54**	R	R	R	R	S	I	R	S	MDR
55	**E.coli-55**	R	R	R	R	S	R	R	S	MDR
56	**E.coli-56**	S	S	S	S	S	S	I	S	S
57	**E.coli-57**	R	R	R	R	S	R	R	S	XDR
58	**E.coli-58**	S	S	S	S	S	S	S	S	S
59	**E.coli-59**	R	R	R	I	S	S	S	S	S
60	**E.coli-60**	R	R	R	R	S	S	S	S	S
61	**E.coli-61**	R	R	R	R	S	R	R	S	MDR
62	**E.coli-62**	R	R	R	I	S	S	S	S	S
63	**E.coli-63**	R	R	R	R	S	R	R	S	XDR
64	**E.coli-64**	S	S	S	S	S	S	S	S	S
65	**E.coli-65**	R	R	R	R	S	R	R	S	XDR
66	**E.coli-66**	R	R	R	R	S	R	R	I	XDR
67	**E.coli-67**	R	R	R	I	S	S	S	S	S
68	**E.coli-68**	R	R	R	R	S	S	S	S	S
69	**E.coli-69**	R	R	I	R	S	S	S	S	S
70	**E.coli-70**	R	R	R	R	S	S	S	S	MDR
71	**E.coli-71**	R	R	I	R	S	S	S	S	S
72	**E.coli-72**	R	R	R	R	S	R	R	S	XDR
73	**E.coli-73**	R	R	I	I	S	S	I	S	S
74	**E.coli-74**	R	R	R	R	S	R	R	S	XDR
75	**E.coli-75**	R	R	I	I	S	S	S	S	S
76	**E.coli-76**	R	R	I	R	S	I	S	S	S
77	**E.coli-77**	R	R	R	R	S	I	S	S	MDR
78	**E.coli-78**	R	R	R	R	S	R	R	S	XDR
79	**E.coli-79**	S	S	S	S	S	R	I	S	S
80	**E.coli-80**	R	R	R	I	I	R	R	S	MDR

81	**E.coli-81**	R	R	R	R	S	R	R	S	XDR
82	**E.coli-82**	R	R	R	R	S	S	R	R	XDR
83	**E.coli-83**	S	I	S	S	S	S	S	S	S
84	**E.coli-84**	R	I	R	S	S	R	R	S	MDR
85	**E.coli-85**	R	S	R	I	S	S	R	S	S
86	**E.coli-86**	R	S	S	S	S	S	R	R	S
87	**E.coli-87**	R	R	R	R	S	S	S	S	MDR
88	**E.coli-88**	R	I	R	I	S	S	I	S	S
89	**E.coli-89**	R	I	R	I	R	R	R	I	MDR
90	**E.coli-90**	S	S	S	S	R	S	R	S	S
91	**E.coli-91**	R	I	R	I	R	R	R	S	MDR
92	**E.coli-92**	I	S	I	S	R	R	S	S	S
93	**E.coli-93**	S	S	S	S	S	S	S	I	S
94	**E.coli-94**	S	S	R	S	S	S	S	I	S
95	**K.P-1**	R	R	R	R	R	R	R	R	PDR
96	**K.P-2**	R	R	R	R	R	R	R	R	PDR
97	**K.P-3**	R	R	R	R	R	R	R	R	PDR
98	**K.P-4**	R	S	R	R	R	I	R	R	XDR
99	**K.P-5**	R	R	I	S	R	R	R	R	XDR
100	**K.P-6**	R	R	R	R	R	R	R	R	PDR
101	**K.P-7**	R	R	I	R	I	R	R	R	XDR
102	**K.P-8**	I	R	R	I	R	I	R	R	MDR
103	**K.P-9**	I	R	R	S	I	R	R	R	MDR
104	**K.P-10**	R	S	I	I	R	R	R	R	MDR
105	**K.P-11**	R	R	R	R	R	R	R	R	PDR
106	**K.P-12**	R	R	I	R	R	R	R	R	XDR
107	**K.P-13**	R	R	R	R	R	R	R	R	PDR
108	**K.P-14**	R	R	R	R	I	I	R	R	XDR
109	**K.P-15**	R	R	S	R	S	R	R	R	MDR
110	**K.P-16**	R	S	R	I	I	R	R	R	MDR
111	**K.P-17**	I	R	S	R	R	I	R	R	MDR
112	**K.P-18**	R	R	R	I	I	R	R	R	MDR
113	**K.P-19**	I	R	R	R	I	I	R	R	MDR
114	**K.P-20**	R	R	R	R	R	R	R	R	PDR
115	**K.P-21**	R	R	R	I	I	S	R	R	MDR
116	**K.P-22**	I	R	R	I	R	S	R	R	MDR
117	**K.P-23**	R	R	R	R	R	R	R	R	PDR
118	**K.P-24**	R	R	R	I	R	I	R	R	XDR
119	**Entero.spp -1**	S	S	R	S	S	S	S	S	S
120	**Entero.spp -2**	R	R	R	S	S	S	R	S	MDR
121	**Entero.spp -3**	S	S	R	S	S	S	S	S	S

122	**Entero.spp -4**	R	R	R	S	S	S	R	S	MDR
123	**Entero.spp -5**	R	R	R	I	S	S	R	I	MDR
124	**Entero.spp -6**	S	S	S	I	I	I	S	I	S
125	**Entero.spp -7**	R	R	R	I	I	I	R	I	MDR
126	**Entero.spp -8**	R	R	R	I	I	I	R	I	MDR
127	**Entero.spp -9**	S	R	S	I	I	I	R	I	S
128	**Proteus.sp p-1**	S	S	S	S	R	S	R	I	S
129	**Proteus.sp p-2**	S	S	S	S	R	S	R	S	S
130	**Proteus.sp p-3**	S	S	S	S	R	I	R	S	S
131	**Proteus.sp p-4**	S	S	S	S	R	S	R	S	S
132	**Proteus.sp p-5**	S	S	S	S	R	S	R	S	S
133	**Proteus.sp p-6**	S	S	S	S	R	I	R	S	S
134	**Proteus.sp p-7**	S	S	S	S	R	I	R	S	S
Total (134)		**MDR (48) , XDR (17), PDR (8) , S (62)**								

الخلاصة

تعد البكتيريا المعوية المقاومة للأدوية المتعددة (MDR) مصدر قلق كبير للصحة العامة وتتزايد المعدلات المبلغ عنها للبكتيريا المعوية المقاومة للأدوية المتعددة، ولم تعد العدوى بهذه الكائنات تقتصر على تلك المرتبطة بالرعاية الصحية في المرضى المصابين بأمراض خطيرة أو العجزة. يتم الآن وصف العدوى المكتسبة من المجتمع. وكان الغرض من هذه الدراسة هو دراسة دور نظام التدفق النشط لمقاومة الأمينوغليكوزيد والكينولون في العزلات السريرية للبكتيريا المعوية باستخدام مثبط مضخة التدفق CCCP.

حيث تم جمع ٣٠٠ عينة سريرية مختلفة من المرضى (الإناث ١٧٢:٥٧,٣%، الذكور ١٢٨:٤٢,٦%) المصابين بالتهاب المسالك البولية (البول)، الجهاز التناسلي (مسحات)، المرضى الذين يعانون من تجرثم الدم (الدم) وعدوى الجهاز التنفسي السفلي (البلغم) في مستشفى المحاويل العام ومستشفى مرجان التعليمي والمختبرات الخاصة خلال الفترة من نوفمبر ٢٠٢٢ إلى مارس ٢٠٢٣.

وجدت النتائج أنه من بين ٣٠٠ عينة، ٢٨٠ (٩٣٪) كانت موجبة و ٢٠ (٦,٦٪) عينة سريرية سالبة .وجدت النتائج أنه من بين ٢٨٠ عينة سريرية، كانت ١٣٤ عينة فقط (٤٧,٨٥%) تنتمي إلى البكتيريا المعوية. أما العينات الـ ١٤٦ المتبقية (٥٢,١٤٪) فكانت تنتمي إلى أنواع أخرى من البكتيريا.

بعد ملاحظة الخصائص المظهري للعزلات البكتيرية كانت العزلات البكتيرية على النحو التالي: تم الحصول على *E.coli* n=94 (70%) و n=24(17.9%)عزلة تنتمي إلى *Klebsiella* الرئوية ، *Proteus* spp (5.2%) n=7 و*Enterobacter* 6.7% (n=9 spp).تم تأكيد الفحص على جميع عزلات البكتيريا المعوية بواسطة نظام التعريف الآلي Vitek-2.أظهرت نتائج عزلات البكتريا المعوية وجود نسبة عالية من *E.coil* 94 عزلة توزعت على شكل التهابات المسالك البولية (٤٣عزلة)، التهابات الجهاز التنفسي السفلي (٢٠عزلة)، (١٧ عزلة) لبكتيريا الدم، (١٤ عزلة) لالتهاب المهبل البكتيري. و أظهرت نتائج ٢٤عزلة من *k.pneumoniae* (٤ عزلات) من مرضى التهابات المسالك البولية (٨عزلات) من مرضى التهابات الجهاز التنفسي السفلي ، (١٢ عزلة) لتجرثم الدم. *Enterobacter* spp. ٤ عزلات من مرضى التهابات المسالك البولية (٧ عزلات) و(٢ عزلة) لتجرثم الدم. *Proteus* spp. ٧ عزلات من التهابات المسالك البولية و (عزلة واحدة) لبكتيريا الدم.

وأظهرت النتائج أن ٦١ من أصل ٩٤ مريضا أصيبوا ببكتيريا الإشريكية القولونية بين الإناث مقابل ٣٣ من الذكور. أكثر المرضى المصابين بعدوى *K.pneumoniae* كانوا بين الإناث ١٤ مقابل ١٠ من الذكوركما أظهرت النتائج أن ٤ من أصل ٩ مرضى أصيبوا ببكتيريا *Enterobacter* spp. بين الإناث مقابل ٥ بين الذكور. وسجلت معظم المرضى المصابين بعدوى *Proteus* spp. بين الإناث ٣ مقابل ٤ بين الذكور, تم إجراء حساسية المضادات الحيوية (AST) لجميع عزلات البكتيريا المعوية بواسطة نظام Vitek 2 وكذلك اختبار انتشار القرص (باستخدام ٨ مضادات حيوية). أظهرت نتائج الدراسة الحالية أن جميع عزلات البكتيريا المعوية بشكل عام كانت مقاومة للفلوروكينولونات أكثر من الأمينوجليكوزيدات.

تم تحديد أنماط مقاومة المضادات الحيوية (ARP) لعزلات البكتيريا المعوية. واعتمدت أنماط مقاومة المضادات الحيوية (ARP) للعزلات على نتائج نظام(Vitek 2 Index-1) وأظهرت النتائج أن أنماط مقاومة المضادات الحيوية لعزلات البكتيريا المعوية وجدت أنه من بين ١٣٤ عزلة، كانت ٦١ عزلة حساسة و ٧٣ عزلة أظهرت أنماط مختلفة لمقاومة المضادات الحيوية على النحو التالي: ٤٨ كانت مقاومة للأدوية المتعددة، ١٧ كانت مقاومة واسعة النطاق للأدوية، و ٨ كانت مقاومة للأدوية.

في هذه الدراسة، تم الكشف عن جينات *oqx*AB في عزلات سريرية مختارة من البكتيريا المعوية المقاومة للأدوية المتعددة والمقاومة الشاملة للأدوية والبكتيريا المعوية المقاومة للأدوية. وكانت العزلات المختارة على النحو التالي: ١٢ عزلة من بكتيريا *E.coli* ، وعزلة واحدة من *K. pneumoniae*، المقاومة الشاملة للأدوية وعزلة واحدة من بكتيريا *Enterobacter* spp. المقاومة للأدوية المتعددة .أظهر تحليل نتائج PCR أن ٦ (٤٢,٨%) من أصل ١٤ عزلة كانت إيجابية لجين oqxA، و ٨ (٥٧,١%) من العزلات كانت سلبية لجين oqxA، لكن، لم تظهر أي عزلات حاملة لجين oqxB.

من أجل تأكيد الدور الفعال لمضخة التدفق في عزلاتنا، تمت مقارنة أقل تركيز مثبط لنمو البكتريا لثلاثة مضادات حيوية مع وبدون مثبط مضخة التدفق (CCCP). أظهرت النتائج لـ ١٤ عزلة من البكتيريا المعوية ضد هذه المضادات الحيوية الثلاثة أن جميع العزلات لديها MIC ≥128 ميكروغرام / مل في غياب CCCP لليفوفلوكساسين والسيبروفلوكساسين والجنتاميسين.كما أظهرت النتائج انخفاض الحد الأدنى من تركيز الليفوفلوكساسين والسيبروفلوكساسين في العزلات، و تم تثبيط نمو البكتيريا في وجود المثبط (CCCP).

مع ذلك، أظهرت جميع عزلات البكتيريا المعوية قيم MIC عالية (≤ ١٢٨) حتى في وجود CCCP مما يشير إلى عدم وجود تأثير للمثبط في تقليل MIC للعزلات للجنتاميسين.

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة بابل
كلية الطب
قسم الأحياء المجهرية الطبية

دراسة دور Carbonyl Cyanide 3-Chlorophenyl- hydrazine كمثبط لمضخة الدفق بين عزلات البكتريا المعوية في بابل ، العراق

رسالة مقدمة الى مجلس كلية الطب /جامعة بابل
وهي جزء من متطلبات نيل درجة الماجستيرفي العلوم / الأحياء المجهرية الطبية

تقدمت بها
هديل قاسم جبر ادريس العيساوي
بكالوريوس علوم الحياة/ جامعة بابل (2020)

إشراف
الاستاذ الدكتور
علاء هاني الجرّاخ

٢٠٢٣ م ١٤٤٥ هـ