Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Kerbala /College of Science

Department of Biology

Molecular characterization of some antibiotics resistance determinants in coliform bacteria isolated from urinary tract infections(UTI) in children and AL-Hussainiya water river.

A thesis

Submitted to the Council of the College of Science, University of Karbala, in Partial Fulfilment of the Requirements for the Degree of Master in Biology
By

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2025A.D 1446A.H

﴿قَالُواْ سُبۡحَٰنَكَ لَا عِلۡمَ لَنَآ إِلَّا مَا عَلَّمۡتَنَآۖ إِنَّكَ أَنتَ ٱلۡعَلِيمُ ٱلۡحَكِيمُ ٣٢﴾

صَدَقَ اللهُ العَليّ العَظيمْ

سورة البقرة الآية ٣٢

Supervisors Certification

We certify that this M.Sc. thesis titled:(**Molecular characterization of some antibiotics resistance determinants in coliform bacteria isolated from urinary tract infections(UTI) in children and AL-Hussainiya water river.)**

Was carried out under our supervision at the College of Science /University of Kerbala, as a partial fulfillment of the requirement for the Degree of Master in Biology sciences.

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DEDICATION

This thesis is dedicated to:

The sake of Allah My Creator and My Master,

My great teacher and messenger, Mohammed (May Allah bless and grant him) who taught us the purpose of life,

The higher example Ahl al-Bayt (peace be upon them) My supervisors who supported me all the time,

My father and mother May God have mercy on them,

My husbands who supported and helped me since the beginning of my study,

Those beautiful flowers around me my children and my brother,

All my friends and colleagues,

Finally, this thesis is dedicated to all those who believe in the benefits and richness of learning.

Acknowledgments

Praise and thanks be to God, Lord of the Worlds, and prayers and peace be upon the Seal of the Prophets, our Master Muhammad, and his pure and good family.

Firstly, thanks to Allah, the gracious the merciful for this entire blessing during the pursuit of my academic study and for the strength He gave me in completing my project.

I would like to thank the Dean of the College of Science at the University of Kerbala Dr. Hassan Jamel al fatlawi ,Thanks to all my professors in the College of Science, from Head of the Department, Dr. Muayad Naeem Karim, and the faculty members in the College

I would like to express my gratitude and sincere thanks to my honourable supervisor **Dr. Ali Atia AI-Hasnawi**.

My thanks and appreciation to all the staff at the Children's Teaching Hospital in Karbala, especially the Microbiology Laboratory Unit and Immunity unit, for the assistance and facilities they provided**.**

God Bless you all

Reasercher(2025).

Summary

Urinary tract infections (UTIs) in children are common diseases. The misuse of antibiotics leads to the emergence of resistance by the bacteria causing the disease. One hundred and fifty samples were collected from children in age between 1-10 years who suffered from UTIs and referred to the Children's Teaching Hospital in Karbala- Iraq. The present study lasted from the late July2023 until February2024. Samples were collected in sterile bottles. In addition, Water samples were collected in a sterile bottle with capacity of 100 ml from three position located at Al-Hussainiya river distributed as (Al-Atishi, Al-Baron hotel and the white arch) which were 15 samples for each season. Most probable number method MPN was applied for the detection of coliform bacteria. The water samples were collected in August and December 2023 for summer and winter seasons, respectively.

All urine and water samples were cultured on proper media and pure colonies were obtained after incubation time. Phenotypic and biochemical tests were performed for diagnosis these colonies.

Further, Vitek 2 system was used for testing the antibiotic resistance activity for these isolates against around 23 types of common antibiotics. Further more,polymerase chain reaction using a specific primers was performed for the amplification of (*CTX-M, TEM*bla, *SHV*bla, *qnr-A* and *Aac(6)Ib* antibiotic resistance genes.

Finally, some isolates which revealed antibiotics resistances were selected for investigating their ability to produce biofilm by tube method.

Twenty isolates of coliform bacteria from urine samples were obtained (14 isolates of *E. coli*, 5 of *Klebsiella pneumonia*, and one isolate of *Enterobacter* spp. Thirty isolates of coliform bacteria from water samples were obtained (5 of *E.coli*,5 *Klebsiella pneumonia* and 5 *Enterobacter* spp*)* for each season.

Results showed that a significant difference was found between two seasons in each station and between stations regarding the number of coliform bacteria. These numbers were significantly higher in Al-Atishi and Al-Baron hotel stations during summer season in comparison to the numbers during winter seasons at the same stations ($P \leq 0.05$).

that *E. coli* were more resistant to the used were sensitive to antibiotics, as they were the only microbe resistant to the antibiotics, including a ceftriaxone, cefuroxime, cefuroxime-Axetil, Ertapenem, Amoxicillin acid , nitrofurantoin, and fosfomycin. On the other hand, all the tested bacteria revealed variable resistance to ceftazidim, cefepime, ciprofloxacin, Imipenem, piperacillin, meropenem, Ampicillin, cefazolin, cefeazidime, ceftazidime/Avibactam, Imipenem,Amikacin, Gentamycin and cefazolin, trimethoprim and sulfamethoxazole.

All bacterial coliform isolates during summer were not exhibit antibiotic-resistance, where as three isolates of *E. coli* isolated during winter season revealed antibiotics resistance to cefotaxime, ciprofloxacin and trimethoprim.

PCR results of urine isolates demonstrated that 5 isolates (100%) carried genes including *CTX-M*, bla*TEM* and bla*SHV*bla.three isolates of *Klebsiella pneumonia* had *CTX-M* gene and tow isolates had bla *SHV* and bla*TEM* genes and one isolate of *Enterobacter* spp. carried *CTX-M* gene only. PCR results of water isolates demonstrated that 3 isolates of *E. coli* out of 5 appeared to have the tested genes (*CTX-M*, bla*TEM*, bla*SHV*, *qnr-A* and *Aac*(6)Ib) during winter season only.

The ability of bacterial coliforms isolated from urine to produce biofilm showed that all the isolates of *E. coli* were as strong and moderate biofilm former, whereas all *Klebsiella pneumonia* were strong biofilm former. All coliform bacteria isolates revealed biofilm activity during both season .

It can be concluded that coliform bacteria are the causative agents of UTI in particular *E. coli* with a range of antibiotic resistance and biofilm formation. Isolation of coliform bacteria from water indicated that river water is contaminated with coliform bacteria, which could be transmitted to people in some way.

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List of Abbrevitions:

Abbreviation	meaning
AMR	Antimicrobial Resistance
ARB	antibiotic-resistant bacterial
ARGs	Antibiotic resistance genes
cnf1	cytotoxic-necrotizing-factor
DHF	dihydrofolate
EHEC	enterohemorrhagic *E. coli*
EIEC	enteroinvasive *E. coli*
EMB	Eosin methylene blue
EPEC	enteropathogenic *E. coli*
FQ	fluoroquinolones
GNB	Gram-negative bacteria
GPB	Gram-positive bacteria
HGT	horizontal gene transfer
hly	hemolysins
LMICs	low and middle income countries
MDR	multidrug-resistant
MPN	Most probable number
NAG	N-acetylglucosamine
NAM	Nacetylmuramic acid
PABA	para-aminobenzoic acid
pap	P fimbriae

PBPs	Penicillin-binding proteins
PCR	Polymerase chain reaction
PMQR	plasmid-mediated quinolone resistance
R	Resistance
rRNA	ribosomal RNA
S	Sensitive
SDWF	Safe Drinking Water Foundation
sfa	S-fimbriae
THF	tetrahydrofolic-acid
TMP/SMX	trimethoprim/sulfamethoxazole
UTI	Urinary tract infection
WHO	World Health Organization

Chapter: One

Introduction

1. Introduction

A serious public health concern, urinary tract infections (UTIs) are frequent bacterial infections. Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumonia), Proteus mirabilis (P. mirabilis), Enterococcus faecalis (E. faecalis), and Staphylococcus saprophyticus (S. saprophyticus) are frequently responsible for them. The most frequent cause of both simple and complex UTIs is uropathogenic E. coli (UPEC)(Zhou *et al* 2023) In children, urinary tract infections (UTIs) are frequent and linked to substantial short- and long-term morbidity. Both anatomical and functional problems are linked to them, and their recurrence rate is considerable. The age of the child and risk factors determine whether to test for UTI. Urinalysis helps with decision-making. Urine cultures are required for a conclusive diagnosis of UTI and to assist in determining when to begin antibiotics.

The presence of a large number of microbial infections in the urinary tract is referred to as a UTI. Any pathogen that colonizes in the urinary system, including viruses, fungi, and parasites, can cause a UTI; however, enteric bacteria are the most common culprits (Ismael *et al.*, 2022). The most prevalent pathogenic bacteria linked to UTIs, accounting for 60–80% of all UTIs, is *E. Coli* (Belete and Saravanan, 2020).

Other pathogens that are relevant as hospital-acquired and catheter-associated infectious agents include *Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus saprophyticus, Enterococcus faecalis*, Group B *Streptococcus* (GBS), *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Candida spp*. (Flores-Mireles *et al.*, 2015; Chakrabarty *et al.*, 2022).

Enterobacteriaceae and some other gram-negative pathogens became highly Extended Spectrum Beta-Lactamase(EsßL) producing bacteria. ESßL encoding genes are located on bacterial chromosome DNA, plasmids or transposons that can transfer easily between two or more bacteria such as *E. coli, K. pneumonia, Enterobacter*. In addition, Gram bacteria -

finally to water sources (Karkman *et al.*, 2018). Virulence negative bacteria display the broadest range of resistance, with bacterial species expressing extended-spectrum-ßlactamases (ESßLs), AmpC, and carbapenemases (Niumsup *et al.*, 2018; Moghaddam *et al.*, 2014). Carbapenem antibiotics are the choice for treating infections caused by ESBL or AmpC-producing bacteria (Vardakas *et al.*, 2012). Carbapenem resistance is caused mainly by carbapenemase enzymes. However, the misuse of antimicrobial substances are the major cause of emergence of antibiotic resistance in pathogenic bacteria (Singh *et al.*, 2018; Meng *et al.*, 2020).

The virulence factors such as adhesive subunit of type 1 fimbriae (fimH), usp, AIM and IMP could also play an essential role in AMR(Antimicrobial resistance) mechanisms among different bacteria (Ahmed *et al.*, 2020). The pathogenicity of Enterobacteriaceae in urinary tract infections increases with the presence of virulence factors. Indeed, Enterobacteriaceae strains harbor several virulence genes associated with serious or recurrent urinary tract infections (Naboka *et al.*, 2021). Among these genes, P fimbriae (*pap*), S-fimbriae (*sfa*), hemolysins (*hly*), cytotoxic-necrotizing-factor (*cnf1*) and Aerobactin (*iucD*) are the most relevant (Sarowska *et al.*, 2021).

Rod-shaped, spore-less coliform bacteria are Gram-negative bacteria. These organisms can be motile or non-motile, ferment lactose, and create gas and acid. When found in food or drink products, coliform bacteria pose a health danger even though they are common and generally harmless. Environmental causes are usually to blame when coliform contamination is detected because *(E. coli*) is a subgroup of coliforms that are commonly found in soil and plants (Bird *et al.*, 2021). The environment also contains E. coli and coliforms in water sources including groundwater and rivers. Groundwater is primarily a clean water source that is commonly utilized by the population in rural regions due to its accessibility and lack of processing requirements (Kairunnisa *et al.*, 2021; Invik *et al.*, 2021).

Human sources of drinking water can get contaminated by resistant bacteria. 98–100% ampicillin resistance was found in Enterobacteriaceae isolated from drinking water. Several antibiotic-resistant waterborne *Shigella spp.* (15%), *Salmonella spp.* (22.7%), and Escherichia coli (35%) were detected in the drinking water (Chalachew *et al.*, 2020). Any water source for human consumption must follow national water quality regulations. To address the hazards to human health posed by environmental exposures to antimicrobial-resistant bacteria, particularly those resulting from exposures to water and human excreta, it is necessary to identify the types of these bacteria in the environment (Sobsey, 2014).

Aim of the Study:

The present study was conducted to seek for any connection between the antibiotics resistance emergency in coliform bacteria isolated from either children with UTI and or AL-Hussainya river water and ability of biofilm formation. This aim was achieved by the following objectives.

Objective

1-Collecting Urine and AL-Hussainya river samples.

2-Isolation of coliform bacteria from these samples.

3- identification of coliform using general and selective media and biochemical tests.

4- applying Vitek2 system for the assessment of antibiotic resistance for all coliform bacteria from the two sources.

5-Molecular detection of the genes (bla*CTX-M*,bla*TEM*,bla*SHV*,*qnr-A*, *Aac(6)Ib*)encoded antibiotic resistance using PCR technique.

6-Phynotype investigation of biofilm formation using biochemical methods.

Chapter: Two

Literature review

2-**Literature review:**

2.1. Coliform:

According to Reza *et al.* (2014), water should be free of indicator bacteria and dangerous microorganisms that could be a sign of fecal pollution. A serious public health issue is drinking water tainted by feces (Fatahi-Bafghi 2017). Tap water is frequently contaminated with coliform bacteria. Therefore, identifying them as signs of human fecal contamination is essential for protecting the public's health (Cabral 2010). The majority of coliforms are discovered in fecal wastes because they are abundant in the gut flora of humans and other warm-blooded animals (Isfahani *et al.* 2017).

"In the context of international law, organic micropollutant contamination of water bodies is a persistent area of interest and investigation. Safe drinking water has been one of the most successful public health initiatives in human history. Lack of knowledge about the dangers and poor management and inadequate staff training of drinking water systems are the main causes of water contamination in underdeveloped nations. In many areas, outbreaks of preventable waterborne diseases can be caused by this lack of knowledge or training. The discharge of household waste in lakes is causing unintended alterations to the physiochemical and biological characteristics of these bodies of water (Singh *et al.* 2010). The most prevalent cause of disease in aquatic environments nowadays is microorganisms, including bacteria, viruses, and protozoa. Water-borne illnesses include viral hepatitis A and E, poliomyelitis, cholera, and other diarrheal illnesses. Sewage and feces pollution allows these germs to reach water sources. In natural ecosystems, microorganisms have very specific roles in material recycling and water purification, while bacteria and fungi dominate in aquatic systems. According to Pannerselvam and Arumugam (2012), *Salmonella, Acinetobacter, Chromobacterium, Alcaligens, Flavobacterium*,

Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium botulinum, Vibrio cholerae, and *Escherichia coli* are the main human pathogens that contaminate water.

2.1.1.*Escherichia coli*:

A facultative anaerobe that is not sporogenous, *Escherichia coli* is a Gram-negative bacteria that is a member of the Enterobacterales order. It is shaped like a rod, about 0.4 µm in diameter and 2-3 µm long. It is usually mobile because of the peritric flagella and may or may not be supplied with a capsule. It can withstand prolonged exposure to the environment and thrives in a broad range of temperatures (15–45 ◦C) (Poli *et al.*, 2005). The predominant facultative anaerobe in the gut flora of warm-blooded animals and humans is *Escherichia coli*, which is often non-pathogenic. The commensal microbial population of the intestine includes the majority of *E. coli* strains discovered since 1895. These strains are commonly found in feces in amounts ranging from 10 to 109 CFU/g and are typically the most common bacterium in coproculture of all mammals. However, according to Gyles *et al.* (2010) and Gomes *et al.* (2016), certain strains have evolved pathogenetic processes that can cause a variety of diseases, including severe ones, in both humans and animals.

Antimicrobial resistance can be detected in *E. Coli*. In addition to the "newer β-lactamases," this organism possesses consolidated resistance features that first emerged many years ago, such as *TEM*-1 β-lactamase. According to a WHO assessment from 2014, *E. Coli* is one of the top nine pathogens of international concern that cause the most common infections in hospitals, the population, and food chains (WHO, 2020).

Resistance to cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole, which are frequently used to treat hospital and community *E. coli* infections, causes delays in proper medication, which in turn increases morbidity and mortality (Tumbarello *et al.*, 2007). Since the environment and animals that produce food have a significant part in this issue, a Health strategy is definitely required (Pérez-Etayo *et al.*, 2022).

Regretfully, *E. coli's* synthesis of β-lactamase continues to be the primary mediator of β-lactam resistance. Bacterial enzymes called β-lactamases hydrolyze β-lactam antibiotics from 2021, 10, 1355, 15 or 25, rendering them ineffective (Livermore and Woodford, 2006). Clavulanic acid, sulbactam, and tazobactam are classic β-lactamase inhibitors that block these enzymes, which can hydrolyze penicillins, cephalosporins, and monobactams but not cephamycins or carbapenems (Paterson and Bonomo, 2005). Since the mid-2000s, *CTX-M* β-lactamases have been found in many Enterobacteriaceae family members, particularly in *E. coli*, and have emerged as the most prevalent form of extended-spectrum β-lactamases (ESBL) (Pitout & Laupland, 2008).

E. coli and coliforms can also be found in the environment in groundwater and waterways. Due to its ease of access and lack of processing requirements, groundwater is primarily a clean water source that is extensively utilized by the population in rural regions (Kairunnisa *et al.*, 2021 ; Invik *et al.*, 2017).Groundwater extraction will affect the amount and quality of the water supply since the growing population will increase demand for clean water (Kairunnisa *et al.*, 2021)

2.1.2.Klebsiella pneumoniae

Klebsiella pneumoniae is a commensal inhabitant of the mammalian nasopharynx and a member of the Enterobacteriaceae family ,One of the most significant opportunistic pathogens, the gastrointestinal tract causes nosocomial and community infections, especially in immunocompromised people, and is more common in hospital settings. The bacteria possesses virulence features such as adhesion, lipopolysaccharide, serum resistance, biofilm formation, and capsule (Abd Elhameed *et al*.,2023). Klebsiella pneumoniae (KPC) strains that produce carbapenemase are becoming more and more common worldwide.1 These enzymes are typically produced by bacteria that are only susceptible to a small number of remaining antibiotics and are linked to a high death rate,

particularly in patients. via infections of the bloodstream.2 Because the KPC genes are found in transposons like Tn4401, which are carried on transferable plasmids, KPC carbapenemases are not only resistant to all available -lactams but also have a high transmission (Falco Restrepo *et al*., (2017). The World Health Organisation listed this organism as one of the top three serious worldwide infections that need to be givenpriority for investigating, finding, and creating novel antibiotics(Kim,*et.al*,2023).

2.1.3.Enterobacter Spp.

Gram-negative, rod-shaped, facultatively anaerobic bacteria belonging to the Enterobacteriaceae family make up the genus Enterobacter. It is also said to be lactose fermenting, urease positive, flagella-containing, and non-spore-forming. Numerous factors influence this bacterium's virulence. The bacteria attach to host cells using adhesins, just like other gram-negative enteric bacilli. The bacteria may be able to evade opsonophagocytosis if they have a lipopolysaccharide (LPS) capsule. Sepsis may result from the LPS capsule's ability to start a chain reaction of inflammation in the host cell (Ramirz, *et. Al*, 2023).

The genus *Enterobacter*spp. contains 22 species. These species have been identified as opportunistic infections in humans, animals, and plants and are characterised in the environment. Because so little research has been done in this area, it is still uncertain how dangerous or virulent this bacterium is. On the other hand, a lot of research has been done on its resistance to antibacterial drugs. Via a variety of local and global regulator genes and the modification of the expression of various proteins, such as enzymes (β-lactamases, etc.) or membrane transporters (porins and efflux pumps), it can control numerous mechanisms of resistance in the face of antibiotic therapy. The Enterobacter aerogenes and E. cloacae combination displayed a multidrug-resistant phenotype throughout many hospital outbreaks(Davin, *et. Al,* 2019).

2.2. Antibiotics:

Among the most important medical innovations of the 20th century, antibiotics are the most important class of medications. Without a doubt, antibiotics have saved millions of lives and helped human society battle bacteria (da Cunha *et al.*, 2019). Nonetheless, the threat of incurable illnesses has been there since the turn of the twenty-first century, and the frequency of infections brought on by multidrug-resistant (MDR) bacteria is rising globally (Gajdács and Albericio, 2019). Antimicrobial resistance (AMR) poses a serious threat to all healthcare systems across the globe, despite the fact that antibiotics have made advances in a number of medical specialties possible, such as the successful completion of numerous surgical procedures, immunosuppressive treatments that depend on antibiotic prophylaxis, and the ability to control infectious complications (Dodds, 2017). To put it literally, antibiotics are "against life." In everyday speech, however, the term is used to describe a class of chemicals that inhibit or eradicate bacterial development. The most important secondary metabolites that bacteria create are antibiotics, which are commercially extracted and utilized in a wide range of applications. It is believed that British scientist Alexander Fleming was the first to recognize that another creature might inhibit the growth of bacteria in 1928. On his plate, he found a mold (fungus) that was inhibiting the growth of *Staphylococcus aureus* bacteria. The mold, known as Penicillin notatum, was named after the antibiotic that was discovered soon after (Adeoye *et al.*, 2020). Many bacteria have the capacity to produce antibiotics. For instance, there are *lactobacillus* species that produce antibiotics like nisin, which is produced by *Lactobacillus lactis,* and bacillus species that produce antibiotics like bacitracin, pumulin, and gramicidin that are effective against Gram-positive bacteria like *Staphylococci*, *Streptococci*, *Corynebacter*, and *Streptomyces* species that produce antibiotics like tetracycline, cloramphenicol, vancomycin, and gentamycin that are effective against Gram-negative bacteria (Waites *et al.*, 2008). The importance of antibiotics as weapons or shields in microbial interactions in soil environments is often

questioned because of the underlying evidence's indirectness and the apparent limitations on antibiotic synthesis in natural settings. Actually, the majority of antibiotics' negative effects on other microbes have been found using carefully monitored in vitro tests (Allen *et al.*, 2010).

In 2019, the World Health Organization (WHO) reported that AMR(antimicrobial resistance) killed 700,000 people; by 2050, that number is expected to have increased to 20 million, at a cost of more than $2.9 trillion (Watkins and Bonomo, 2016). Because of this, it has grown to be a significant issue that threatens both our economy and our lives. The rapid evolution of AMR (antimicrobial resistance) has led to reduced investment returns for the pharmaceutical R&D business, in addition to the high cost of antibiotic research and development. Numerous pharmaceutical firms have already stopped researching and developing new antibiotics (Mohr Kathrin, 2016). Even while the situation is dire, a number of emerging technologies have the power to improve things. Numerous scientific developments may also help in the development and investigation of novel antibiotics. In addition to genetic, functional genomic, and metagenomics research on bacteria, mammals, and even aquatic invertebrates, synthetic biology techniques seek to uncover the world's natural products more quickly than the discovery of antibiotics (Fortman and Mukhopadhyay, 2016). Various therapeutic and preventive methods, such as monoclonal antibodies (DiGiandomenico and Sellman, 2015), bacteriophages (Golkar *et al.*, 2014), and vaccines (Thanawastien *et al.*, 2015), are employed in place of or in addition to antibiotics to treat bacterial infections. Alongside these contemporary approaches, orthodox synthetic chemical and pharmaceutical chemistry continue to be crucial substances in the battle against antimicrobial resistance (Czaplewski *et al.*, 2016). According to Fortman and Mukhopadhyay (2016), the post-antibiotic era could be avoided with the advancement of regulatory activities.

2.2.1 Mode of action of antibiotics:

2.2.1.1 Inhibition of cell wall synthesis by anticbiotics:

In both Gram-positive and Gram-negative bacteria, peptididoglycan is an essential part of the cell wall that provides mechanical support. But in Gram-positive bacteria, peptidoglycan has ten to forty layers, but in Gram-negative bacteria, it has one or two layers (Mai-Prochnow *et al.*, 2016). cell wall of bacteria. The peptidoglycan backbone gives the bacterial cell wall its stiffness. Pentapeptides (l-Ala–d-Glu–l-Lys–d-Ala–d-Ala) that dangle from the layers cross-link the repeating units of N-acetylglucosamine (NAG) that are connected to N-acetylmuramic acid (NAM, β 1 → 4 glycosidic connections). Gram-positive bacteria (GPB) typically have l-Ala–d-Glu–l-Lys–d-Ala–d-Ala as the pentapeptide of their cell walls, but Gram-negative bacteria (GNB) typically substitute diaminopimelic acid (DAP) for the third amino acid (l-lysine). Nonetheless, species vary in the stem peptide sequence, peptide crosslink type, and secondary modification presence in glycan chains and peptides(Sarkar et al .,2017)

2.2.1.2 Inhibitoon of 30S subunit by antibiotics:

According to protein sedimentation rates, which are represented as "Svedberg" units, the 70S ribosome of bacteria is made up of 30S and 50S subunits. The 30S (aminoglycosides and tetracyclines) or 50S (chloramphenicol, macrolides, and oxazolidinones) subunits are the targets of antibiotics, which prevent the production of proteins (Krause *et al.*, 2016).Aminoglycosides, including streptomycin, neomycin, and gentamicin, are known to inhibit protein synthesis by firmly attaching themselves to the A-site on the 16S ribosomal RNA (rRNA) or 30S rRNA. Therefore, when the aminoacyl transfer RNA is administered, the antibiotics make it easier for codons to be misread. Erroneous protein synthesis results from this, enabling the wrong amino acids to combine to form apolypeptide, which is subsequently released and damages the cell membrane (Krause *et al.*, 2016). By preventing

tRNA from attaching to the mRNA-ribosome complex, tetracycline antibiotics also impede protein synthesis by passively diffusing via porin channels in the bacterial cell membrane and reversibly binding to the 30S ribosomal subunit (Grossman, 2016).

2.2.1.3 Inhibition of 50S subunit by antibiotics:

Azithromycin and other macrolides are known to attach to the 23S rRNA of the 50S ribosomal subunits of bacteria. According to Parnham *et al.* (2014), they hinder bacterial protein production by blocking the transpeptidation or translocation phase of protein synthesis, which leads to the early separation of incomplete peptide chains.

Chloramphenicol, on the other hand, is lipid-soluble, exhibits good absorption characteristics in vivo, and works via the membranes of bacteria. The L16 protein of the 50S subunit of bacterial ribosomes is then reversibly bound by it, preventing amino acid transfer to expand peptide chains and, as a result, inhibiting the formation of peptide bonds and protein synthesis (Dinos *et al.*, 2016). This suppresses peptidyl transferase activity. By preventing the translation of bacterial proteins, a class of oxazolidinones, including tedizolid and linezolid, function as antibacterial agents in the same way. By binding to a location on the 50S subunit of the bacterial 23S ribosomal RNA, they stop the formation of a functional 70S initiation complex, which is necessary for bacterial replication, and inhibit bacterial growth (Papich MG, 2016).

2.2.1.4. Antibiotics inhibiting nucleic acid synthesis:

According to Bhattacharjee (2016), several antibacterial medications, such as rifamycin and fluoroquinolones, work by blocking RNA and DNA, respectively.

Rifamycin directly blocks the elongating RNA by binding firmly to the polymerase subunit deep inside the DNA/RNA pathway, inhibiting bacterial DNA-dependent RNA polymerase. Because of their structural differences from eukaryotic RNA polymerase enzymes, bacterial RNA polymerase enzymes can be selectively harmful to bacterial cells (Bhattacharjee, 2016; Saito *et al.*, 2017). On the contrary, quinolones inhibit DNA gyrase and topoisomerase IV, two fundamental type II topoisomerases, which prevent DNA synthesis. A single double-stranded DNA molecule passes through both targets, relegating the original strand in the process (Nainu *et al.*, 2021).

2.2.1.5 Inhibition of metabolic pathways:

In order to manage bacterial infections, certain synthetic antibiotics work as anti-metabolites, or competitive inhibitors of bacterial metabolic enzymes. Trimethoprim and sulfonamides block distinct stages of the folic acid metabolic pathway (Fernández-Villa *et al.*, 2019). By competing with para-aminobenzoic acid (PABA) for binding to di-hydrofolate synthetase, a stage in the synthesis of tetrahydrofolic acid (THF), sulfonamides prevent pteridine and PABA from being converted to dihydropteroic acid by the enzyme. According to Akter *et al.* (2020), there is widespread belief that THF is essential for the synthesis of purines and dTMP, and that its inhibition limits the proliferation of bacteria. One of the primary enzymes involved in the conversion of dihydrofolate (DHF) to THF, dihydrofolate reductase, is invertedly inhibited by trimethoprim. Inhibiting THF's synthesis has bactericidal effects since it is essential for the synthesis of bacterial proteins and nucleic acids as well as for bacterial survival (Fernández-Villa *et al.*, 2019; Wróbel *et al.*, 2020). Sometimes, trimethoprim is used in conjunction with sulfamethoxazole (sulfonamide) to

stop the phase that precedes the creation of bacterial proteins. When combined, trimethoprim and sulfamethoxazole block two phases of bacterial protein and nucleic acid manufacturing. According to Wróbel *et al.* (2020), trimethoprim is bacteriostatic on its own but is thought to be bactericidal when coupled with sulfamethoxazole.

2.3.Antimicrobial resistances:

Antimicrobial resistance (AMR) is without a doubt a major global public health concern. Long thought to be solely a human health concern, antibiotic misuse in a variety of fields (agricultural, food animals, aquaculture, and the environment) has recently come to be recognized as a significant contributor to resistance. Nevertheless, we know very little about the size and scope of each sector's contribution to the total burden of AMR. In the cattle and aquaculture industries, where antibiotics are frequently utilized for medicinal, preventative, or growth-promoting objectives, intensive animal production methods are especially concerning. The majority of aquaculture output occurs in low- and middle-income nations, making it a fast expanding food sector. (LMICs) (FAO, 2016).

The number of diseases brought on by different bacteria, fungi, viruses, and parasites is the most significant of the many difficulties this industry faces. Antibiotics can be beneficial if used properly, that is, based on a correct diagnosis. However, this does not appear to occur in the majority of farms because farmers typically have limited access to diagnostic tools. The use of antibiotics on farms is also frequently impacted by recommendations from private veterinarians, feed firms, medicine suppliers, and other farmers in the area. AMR's debut in aquaculture and its extension to other industries are known to be facilitated by numerous variables.. This includes the use of a variety of chemicals, the nutrient-rich environment in the ponds, the release of untreated water and waste into the local ecosystem, high stocking densities that cause increased stress and illnesses in shrimp, and more (Thornber *et al.*, 2020). According to Watts *et al.* (2017), there have been reports of

antibiotic-resistant bacteria from a variety of aquaculture environments, including zoonotic and human infections.

Because of their significant capacity to develop resistance to a variety of antibiotics and spread widely, members of the Enterobacteriaceae family are particularly concerning among them. Globally, Enterobacteriaceae multidrug resistance is becoming a more serious issue in both healthcare and community settings. The widely varied and quickly changing class of beta-lactamases, including carbapenemases and extended-spectrum beta-lactamases (ESBLs), is largely to blame for this. Most often found in *Pseudomonas aeruginosa* and Enterobacteriaceae, ESBLs are a class of enzymes that confer resistance to aztreonam, penicillins, and first-, second-, and third-generation cephalosporins. They are typically inhibited by beta-lactamase inhibitors like clavulanic acid (Paterson and Bonomo, 2005). According to Patterson and Bonomo (2005), class A (TEM, SHV, CTX-M, GES, and VEB families) and class D (OXA family) beta-lactamases are the primary enzyme families having ESBL phenotype. The majority of ESBLs that were initially widely used were TEM or SHV variants with amino acid changes that altered their substrate profile to accommodate extended-spectrum cephalosporins. Although not all of them are ESBLs, there are currently 178 different forms of SHV enzymes and 183 different types of TEM enzymes (Bush and Bradford, 2020). On the other hand, the chromosomal bla genes of the harmless rhizosphere bacterium *Kluyvera spp*. were mobilized to produce the CTX-M type ESBLs (D'Andrea *et al.*, 2013). Since 2000, *Escherichia coli* has been the main pathogen using CTX-M type enzymes, which have become more popular than other ESBLs and spread globally, causing a "CTX-M pandemic" (Cantón *et al.*, 2012). ATB-resistant "priority pathogens" were listed by the World Health Organization in 2017. Among them, multidrug-resistant (MDR) bacteria are the most dangerous because they are especially dangerous in hospitals, assisted living facilities, and among people who need devices like blood catheters and ventilators. These include *Pseudomonas*, Acinetobacter, and several

Enterobacteriaceae that produce carbapenemases or extended spectrum beta-lactamases (ESBL), such as Klebsiella, E. coli, Serratia, and Proteus (WHO, 2017). Resistance to β-lactam ATBs with an oxyimino group (such as ceftazidime, cefotaxime, and aztreonam) and other non-penicillin ATB classes, such as aminoglycosides, trimethoprim/sulfa-methoxazole, ß-lactam/ß-lactamase inhibitor combinations, and fluoroquinolones (FQ), is caused by bacteria that produce ESBL. These bacteria cause morbidity, mortality, longer hospital stays, and higher treatment expenses (Pilmis *et al.*, 2018).

Human intestinal carriage of *E. coli* that produces ESBL is common in both hospital and community settings, according to recent investigations (Hocquet *et al.*, 2016). During a five-year period (2006–2011), the rate of healthy people with ESBL-producing *E. coli* fecal carriage increased tenfold in Paris (Pilmis *et al.*, 2018). In French hospitals, the frequency of Enterobacteriaceae that produce ESBLs quadrupled between 2002 and 2013. The percentage of *E. coli* strains that produce ESBL rose from 19 to 59 percent throughout this decade across the whole Enterobacteriaceae family (Hocquet *et al.*, 2016). Regardless of prior antibiotic exposures, intrinsic antibiotic resistance is a feature present in the bacterial species' genome (Cox and Wright, 2013) and is unrelated to horizontal gene transfer (HGT) (Reygaert, 2018). The intrinsic resistance mechanisms are often chromosome-encoded and are fixed in the microorganism's fundamental genetic makeup (Peterson and Kaur, 2018). Among the most prevalent are non-specific active efflux pumps, which might have actively developed in reaction to environmental pollutants (Peterson and Kaur, 2018). Additionally, there is restricted permeability of outer membranes, such the vancomycin resistance in *E. coli* (Arzanlou *et al.*, 2017). According to studies, this characteristic seems to be influenced by other genes and genetic loci (Cox and Wright, 2013). Compared to Gram-positive bacteria (GPB), Gram-negative bacteria (GNB) are known to possess greater inherent resistance. The presence of an outer membrane permeability barrier to antibiotic influx or to multiple MDR efflux pumps that lower the

intracellular concentration of medicines makes treating MDR-GNB difficult (Peterson and Kaur, 2018).

2.3.1. Phenotypic Resistance:

According to Olivares *et al.* (2013), phenotypic resistance occurs when vulnerable bacteria establish persistence, proliferate in biofilms, or adapt to swarming, which results in a temporary resistance. Persisters are a subset of bacteria that go into a stationary growth phase, cease to grow actively, and enter a dormant condition (Arzanlou *et al.*, 2017). According to Reygaert (2018), persistent cells may be the primary cause of chronic infections, although they are not the result of genetic alterations because of the two subpopulations' varying responses (Brauner *et al.*, 2016). According to Olivares *et al.* (2013), biofilm is a complex structure made up of bacterial colonies wrapped in a polymer matrix comprising proteins, extracellular DNA, and polysaccharides. bacteria in biofilms have up to 1000 times the antibiotic resistance of planktonic (free-swimming) bacteria. According to Arzanlou *et al.* (2017), the biofilm mode is the primary cause of chronic and device-related infections, while the planktonic form is indicative of acute infections.

2.3.2 Acquired Resistance:

The primary causes of acquired antibiotic resistance are bacterial chromosomal DNA alterations and (horiyontal gene transferase)HGT. Both temporary and permanent genetic material acquisition are possible (Reygaert, 2018). While bacteriophage-mediated transmission is thought to be uncommon, the plasmid-encoded specialized efflux pump is the most prevalent way to acquire resistance because it promotes the expression and spread of external genetic resistance determinants (Peterson and Kaur, 2018).

The evolution of antibiotic-resistant *Streptococcus* species was influenced by transformation (Johnston *et al.*, 2014). According to Haber *et al.* (2017), transduction might

have a role in the development of resistance in *Staphylococcus aureus*.Transduction and transformation processes are hard to find outside of lab conditions, and it's unknown how they contribute to resistance (Peterson and Kaur, 2018). The high density of bacteria, phages, and plasmids in these habitats, however, makes some hotspot environments for genetic exchange—like sewage and wastewater treatment plants—ideal sites for exchange events (Von Wintersdorff *et al.*, 2016).According to Volkova *et al.* (2014), the most common way for resistant genes to propagate across several antibiotic classes is by plasmid-mediated conjugation. The plasmid has the ability to spread genes to the host over great genomic distances and multiply on its own (Ramirez and Tolmasky, 2010). Conjugation-based gene transfer aids in the worldwide dissemination of resistance determinants in both the community and the hospital setting (Carattoli, 2013). High-density environments like the human or animal gut, biofilms, and co-infection situations are where this mechanism takes place (Huddleston, 2014). According to Carattoli (2013), conjugation is the mechanism that has been researched the most by PCR-based and DNA sequencing techniques.

2.4 The common genes encoded for antibiotics resistance :

2.4.1 ESBL

There are other forms of ESBL enzymes, however the most prevalent ones in *E. coli* are CTX-M, SHV, and TEM (Palmeira & Ferreira, 2020). The genes bla and the phenotypic name of the enzyme are responsible for encoding these β-lactamases. The narrow spectrum β-lactamases SHV-1, TEM-1, and TEM-2 are produced by modifications to the TEM and SHV variants (Khosravi *et al.*, 2013; Rahman *et al.*, 2018). These genes have been found in humans, animals, and the environment, which emphasizes the necessity of controlling the issue holistically (Dorado-García *et al.*, 2018; Rabello *et al.*, 2020). According to Fuga

et al. (2022), states like São Paulo and Paraná have already detected resistant E. coli strains circulating at the human-animal-food-environment interface.

2.4.1.1 *CTX-M , TEM , SHV, qnr* and *Aac* gene:

According to Bevan *et al.* (2017), CTX-M-type enzymes are currently the most common ESBLs in Enterobacteriaceae globally. They come in over 230 varieties, which are grouped into five main clusters: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 (Ramadan *et al.*, 2019). In contrast to the TEMs and SHVs, which were derived from plasmid-encoded penicillinases, the CTX-M enzymes are associated with chromosomal β-lactamases from various Kluyvera species (Cantón *et al.*, 2012). According to Branger *et al.* (2018), this implies that blaklu genes are mobilized into conjugative plasmids by insertion sequences and, to a lesser extent, bacteriophages. Other class A ESBLs encoded by plasmids are the enzymes SFO (Serratia fonticola β-lactamase), TLE (TEM-like enzyme), PER (Pseudomonas extended resistant β-lactamase), BES (Brazil extended-spectrum β-lactamase), and GES (Guiana extended-spectrum β-lactamase) that are found in Enterobacteriaceae (Zhou *et al.*, 2017 ; Jin *et al.*, 2015 ; Ur Rahman *et al.*, 2018). In the 1980s, the first reports of CTX-M-type β-lactamases were also published. Their inclusion in the list of ESBLs first raised some eyebrows, but since 2000, they have proliferated globally and are currently the most prevalent kind (Peirano and Pitout, 2019). The CTX-M-type enzymes can be phylogenetically separated into five different clusters, where members of the group share >94% identity (the members of the other groups share ≤90% identity) (Bonnet, 2004).

According to Drawz *et al.* (2014), all of the commercially available β-lactamase inhibitors, including the most recent additions, avibactam and vaborbactam, easily inhibit CTX-M-type enzymes. According to Canton *et al.* (2012), genes thought to be precursors of CTX-M-type enzymes have been found in the chromosomes of Kluyvera georgiana

(CTX-M-8, CTX-M-25, and CTX-M-9), Kluyvera ascorbata (CTX-M-2 group), and Kluyvera cryocrescens (CTX-M-1 group). According to Poirel *et al.* (2012), the blaCTX-M genes are housed in plasmids and conjugative genetic elements such ISEcp1-like, ISCR1, and class 1 integrons, which also aid in its mobilization. Although E. coli and K. pneumoniae are frequently found to have CTX-M β-lactamases, other Enterobacteriaceae species, such as typhoidal and nontyphoidal Salmonella spp. Shigella spp. C. freundii, Enterobacter spp., and S. marcescens, as well as a number of nonfermenter species, also have these enzymes (Peirano and Pitout, 2019, Canton *et al.*, 2012).

The two primary forms were TEM and sulphydryl variable SHV. However, the CTX-M type is more prevalent in some nations (Gupta *et.al.*, 2003). They are produced by Gram-negative bacteria and are rapidly evolving. They can hydrolyze all cephalosporins, aztreonam, and related oxyimino-beta lactams, as well as older penicillins, though clavulanic acid can inhibit this (Elnagar *et al.*, 2010). Genes for aminoglycosides, trimethoprim, sulfonamides, and tetracycline are also present in the plasmids that carry ESBL genes. Accordingly, the majority of Gram-negative bacilli that carry these plasmids are(antidruge resistance)MDR bacteria (Rozwandwicz *et al.*, 2018).

Worldwide, there have been numerous reports of infection epidemics caused by microbes that produce ESBL. Some hospitals have experienced endemicity of the strains that produce ESBLs, replacing early infection epidemics (Paterson and Bonomo, 2005). Controlling the first breakout of ESBL-producing organisms in a hospital or specialized unit of a hospital is crucial since this could result in an increase in patient mortality (Pishtiwan and Khadija, 2019). Globally, there are over 400 distinct varieties of ESBLs, with *TEM* and SHV being the most common. Mutations in the genes encoding these enzymes result in the formation of ESBLs with a wider substrate profile (Latipour *et al.*, 2016).

Both TEM and SHV types are developed from penicillinase, which is able to hydrolyze extended spectrum cephalosporins due to a number of single amino acid alterations (Mohammed and Abass, 2019). TEM-1 is the most prevalent β-lactamase found in bacteria that are Gram-negative. This enzyme produces up to 90% of the ampicillin resistance in E. coli and is also in charge of the ampicillin and penicillin resistance observed in H. influenzae and N. gonorrhoeae (Mawlood *et al.*, 2018).

Drug inactivation brought on by aminoglycoside-modifying enzymes (AMEs) expressed inside mobile genetic elements is thought to be the primary mechanism of staphylococci's resistance to aminoglycosides. Encoded by the gene *Aac (6')*, the bifunctional enzyme AAC (6')/APH (2') is the most common AME. The *aph (2')* Ia gene encodes the aph (3') IIIa enzyme, the ant (4')-I enzyme is encoded by the *ant (4')* Ia gene, and so on (Ramirez and Tolmasky, 2010).

Genes encoding the enzymes that acetylate fluoroquinolones, tobramycin, and amikacin, such as Aac (60)-Ib3 or Aac (60)-33 aminoglycoside acetyl transferases, Ant (20)-Ia aminoglycoside nucleotidyltransferase, or the Aac (60)-Ib-cr, were frequently found next to genes encoding β-lactamases.According to previous research on *E. coli*, the *aac (60)* and *ant(2'')*-Ia genes are located next to genes that encode β-lactamase (Bodendoerfer *et al.*, 2020).

The capacity of bacteria to reduce drug accumulation by efflux is also a major factor in fluoroquinolone resistance, albeit the role of various efflux mechanisms varies depending on the fluoroquinolone. Ciprofloxacin-resistant *E. coli*, for example, has 26% more AcrB expression than susceptible isolates (Sato *et al.*, 2013).

Despite this, fluoroquinolone resistance is unlikely to be primarily caused by this method. Plasmid-mediated quinolone resistance (PMQR) genes, such as *qnrA*, *qnrB*, *qnrC*, *qnrS*, *qnrD, qnrE, and qnrVC*, or specific quinolone efflux pump genes *qepA* and *oqxAB*, are

plasmid-encoded and can confer resistance in E. coli even in the absence of chromosomal alterations (Sato *et al.*, 2013). *Qnr* genes were found to be present in 47.74%, 47.10%, and 2.58% of strains of ESBL-producing E. coli, according to a study that examined the distribution of these genes. The ESBL genes *blaCTX-M*, *blaTEM*, and *blaSHV* were strongly linked to these genes (Salah *et al.*, 2019).

Twenty-one out of forty-three isolates had *qnrS*, six out of forty-five had *qnrB*, and two out of forty-three had *qnrA*, according to another study (Shetty *et al.*, 2019). They spread in combination with gentamicin, tetracycline resistance genes, and other β-lactams and are highly transmissible (Kuo *et al.*, 2020). The most often documented PMQR mechanism is the AAC(6′)-Ib-cr enzyme, which provides aminoglycoside resistance and dual inactivates ciprofloxacin. The subgroup sequence type ST-131, an antibiotic-resistant clone that has been reported to be quickly spreading and causing high rates of bloodstream infections and UTIs, has been discovered to contain it in more than 70% of strains (Phan *et al.*, 2022). Although the enzyme by itself raises the ciprofloxacin minimum inhibitory concentration (MIC) in *E. coli*, it increases resistance when paired with chromosomal changes linked to resistance (Machuca *et al.*, 2016).

2.5 Aminoglycosides:

Since aminoglycosides and beta-lactam or glycopeptides work in concert, they are frequently used in conjunction, particularly to treat complex staphylococcal infections (Davies, 2006).The primary source of staphylococci's resistance to aminoglycosides is thought to be drug inactivation brought on by aminoglycoside-modifying enzymes (AMEs) encoded in mobile genetic elements. The bi-functional enzymes AAC (6')/APH (2'), which is encoded by the gene *aac (6') Ie aph (2') Ia, APH (3') III*, which is expressed by the gene *aph (3') IIIa, and ANT (4')-I*, which is encoded by the gene ant (4') Ia, are the most common AMEs (Ramirez and Tolmasky, 2010).

Genes that encode the enzymes that acetylate fluoroquinolones, tobramycin, and amikacin, such as Aac (60)-Ib3 or Aac (60)-33 aminoglycoside acetyl transferases, Ant (20)-Ia aminoglycoside nucleotidyltransferase, or the Aac (60)-Ib-cr, were frequently found next to genes that encode β-lactamases. The discovery that the genes for β-lactamase are next to the *aac* (60) and *ant*(2")-Ia genes is in line with previous research on *E. coli* (Bodendoerfer *et al.*, 2020).

2.6 Fluoroquinolones:

According to Chao and Farrah (2019), fluoroquinolones such as ciprofloxacin, levofloxacin, norfloxacin, ofloxacin, and gatifloxacin are used to treat urinary tract infections (UTIs) and can be effective against both Gram-positive and Gram-negative bacteria. The EAU advises using ciprofloxacin for severe UTIs when the patient is contraindicated for third-generation cephalosporins or an aminoglycoside and the local resistance rate is less than 10% (Bonkat *et al.*,2023). However, it does not suggest it for uncomplicated UTIs. Resistance to fluroquinolones causes chromosomal changes to these targets, usually GryA. According to a study by Esmaeel *et al.* (2020) on fluroquinolone-resistant E. coli, 76.7% of the strains had mutations in the GyrA subunit, and those with a dual Ser-83 and Asp-87 mutation in that subunit exhibited the highest levels of resistance.

The capacity of bacteria to reduce drug accumulation by efflux is also a major factor in fluoroquinolone resistance, albeit the role of various efflux mechanisms varies depending on the fluoroquinolone. Ciprofloxacin-resistant *E. coli*, for example, has 26% more AcrB expression than susceptible isolates (Sato *et al.*, 2013).

Despite this, fluoroquinolone resistance is unlikely to be primarily caused by this method. Plasmid-mediated quinolone resistance (PMQR) genes, such as *qnrA, qnrB, qnrC, qnrS, qnrD, qnrE,* and *qnrVC,* or specific quinolone efflux pump genes *qepA* and *oqxAB,* are plasmid-encoded and can confer resistance in *E. coli* even in the absence of chromosomal

alterations (Sato *et al.*, 2013). QnrB, QnrS, and QnrA were found to be present in 47.74%, 47.10%, and 2.58% of strains of ESBL-producing *E. coli*, respectively, according to a study that examined the distribution of qnr genes among these bacteria. The ESBL genes *blaCTX-M*, *blaTEM*, and *blaSHV* were strongly linked to these genes (Salah *et al.*, 2019).

Another study discovered that 21 out of 43 isolates had qnrS, 6 out of 45 isolates had qnrB, and 2 out of 43 isolates had qnrA (Shetty *et al.*, 2019). They spread in combination with gentamicin, tetracycline resistance genes, and other β-lactams and are highly transmissible (Kuo *et al.*, 2020). The most often documented PMQR mechanism is the AAC(6′)-Ib-cr enzyme, which provides aminoglycoside resistance and dual inactivates ciprofloxacin. It was discovered to be present in more than 70% of strains from the subgroup sequence type ST-131, an antibiotic-resistant clone that has been linked to high incidence of bloodstream infections and urinary tract infections and has been believed to be spreading quickly (Phan *et al.*, 2022). Even though the enzyme by itself raises the ciprofloxacin minimum inhibitory concentration (MIC) in *E. coli*, it increases resistance when paired with chromosomal changes linked to resistance (Machuca *et al.*, 2016).

2.7 Waste water:

2.7.1.Water bacterial pollution:

Water contamination is a serious environmental problem that poses serious risks to ecosystems and human health. When harmful substances like chemicals, pollutants, and poisons are introduced into bodies of water, the water supply becomes contaminated (Bashir *et al.*, 2020). This is known as water pollution. Pathogenic bacteria contained the ability to cause infections or diseases to humans through ways such as releasing toxic substances which could damage human tissues, act as parasites inside human cells, or form colonies in the human body that could disrupt normal human functions. Many types of pathogenic bacteria could be found in water, including *Vibrio cholerae, Escherichia coli,*

Salmonella typhi, etc., which could cause various kinds of waterborne diseases, such as diarrhea, cholera, typhoid, etc. (Al-Abdan *et al.*, 2021).

Numerous nations have water sources that contain harmful microbes. Under a microscope, the gram-negative bacterium Escherichia had a rod-like shape with a tiny tail, and it was found in many different places in nature. According to Rossolini *et al.* (2017), gram-negative bacteria were naturally resistant to antibiotics. Consequently, it was more difficult to treat Escherichia-caused illnesses such diarrhea and gastroenteritis with antibiotics. As an example, Escherichia coli, Escherichia albertii, Escherichia fergusonii, Escherichia hermannii, and others are species of Escherichia. *Escherichia coli* (E. coli) was the most prevalent species of Escherichia in drinking water (Haasdijik & Ingen, 2018).

It may be frequently detected in the human stomach. *E. Coli* Exisit in a variety of forms, some of which were innocuous and others could infect people and cause illnesses. Enterotoxigenic *E. coli* (ETEC, also called O148), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC, also called O157), and enteroinvasive *E. coli* (EIEC, also called O124) were among the worst varieties of *E. coli*. Human and livestock excrement may contain ETEC (Bako *et al.*, 2017). Therefore, the water resources would be contaminated by ETEC when the feces were released into water sources. As a result, ETEC may spread through tap water and infect people if water is not properly treated. Cattle farming was the primary economic activity in the majority of developing nations, where residents lacked access to clean water and had inadequate sanitation because of a lack of funding (Bako *et al.*, 2017).

Water contamination is associated with the spread and transmission of pathogenic bacteria that cause infectious gastrointestinal disorders when water contaminated by human and animal waste is consumed (Cabral, 2010).According to Jang *et al.* (2017), Escherichia coli is one of the primary pathogens that indicates fecal contamination in water supplies; a level

of zero per 100 milliliters of water is deemed acceptable for human consumption (WHO, 2011).

Wastewater contains a variety of antimicrobial agents, including detergents, biocides, heavy metals, and partially metabolized antibiotics. Together, these substances can decrease susceptible populations, so applying selective pressure on the growth of antibiotic-resistant bacteria (ARB) (Martinez and Baquero, 2017). Given the abundance of nutrients in the wastewater distribution system, some environmental bacteria may find it easy to proliferate there (Pilmis *et al.*, 2020). The distribution system's high microbial load may be the perfect environment for ARB to proliferate via the(Horizontal gene transferase)HGT of(Antiresistance genes)ARGs pathway. Additionally, one of the fundamental processes that enables bacteria to thrive is the production of biofilms, whichcan be produced by many bacterial groups in sewerage networks for protection themselves from environmental challenges (Auguet *et al.*, 2017).).

Hospitals, assisted living facilities, and other healthcare facilities may be major point sources of ARB and ARG contamination of municipal wastewater systems (Khan *et al.*, 2019; Perry *et al.*, 2021). Comparing healthcare facilities to populations, more antibiotics of various classes and in greater quantities are being utilized (ECDC, 2020). Thus, sewage from medical facilities is a better indicator of clinical patients than the general population. Additionally, the sewage from medical facilities may contain a lot of leftover and partially metabolized medications that are expelled through clinical patients' urine and feces, which increases the selective pressure for ARB in sewer networks (Rodriguez-mozaz *et al.*, 2014).

2.7.1.1.Indicators for water bacterial pollution:

Human and animal intestines, food, and the environment are all habitats for *E. coli*. This is an extremely diverse and huge group of bacteria. Although the majority of *E. coli* strains are safe, they aid in the synthesis of essential vitamins and inhibit the growth of dangerous

bacterial species (SDWF (Safe Drinking Water Foundation), 2017). But although some strains can induce gastrointestinal disorders if consumed by healthy individuals, others might be opportunistic. Opportunistic pathogens are microorganisms that infect individuals with weakened immune systems but typically reside in the host without harming them (SDWF, 2017). Children and the elderly may become seriously ill or even die from the recently identified *E. coli* 0157: H7A strain (USGS, 2018).

Chapter Three:

Materials and Methods:

3. Materials and Methods

3.1:Materials:

3.1.1:Laboratory equipment and instruments

The equipment and instruments used in the present study are listed in Table 3-1.

Table 3-1: Equipment and Instruments used in the current study

Equipment and Instrument	**Manufacturer/Origin**
Autoclave	Hirayama (Japan)
Bench centrifuge	Memmert(Germany)
Benzes burner	Amal(Turkey)
Compound light microscope	Zeiss (Germany)
Digital camera	Sony(Japan)
Disposable and glassware	Cito (China)
Distillator	GFL (Germany)
Electric sensitive balance	Denver (USA)
elccterophoresis	Cleaver/ gemany
Eppendorf centrifuge	Hettich (Germany)
Hot plate with magnetic stirrer	Heidolph (Germany)
Laminar flow hood	Cryste (Korea)

Micropipette	Capp (Denmark)
Thermocycler	Biobase /china
pH-meter	WTW (Germany)
Refrigretor	Concord(lebanon)
UV transmillator	Biobase/China
Vortex	Gemmy(Taiwan)
Memmert(Germany)	

3.1.1 :Biological and chemical materials

Biological and chemical materials used in this study are listed in Table 3-2.

Table 3-2 Biological and Chemical Materials:

Biological and Chemical Materials	**Manufacturer(Origin)**
DNA Loading buffer Blue	Eurx (Poland)
Agarose, TBE buffer	Condalab (spain)
Barium chloride, Crystal violet, Glucose, Iodine, gram stain	CDH (India)
Glycerol ($C_3H_8O_3$)	Merck(England)
McFarland's standard solution	Biomerieux(France)
Nuclease Free Water	Bioneer (Korea)
Phosphate buffer tablet	Himedia (India)
Primers	Bioner(Korea)

3.1.2 : Kits

Kits used in the present study are illustrated in Table 3-3.

Table 3-3 kits used in the present study:

Type of Kit	Company(Origin)
DNA extraction Kit	Genaid (Taiwan)
DNA ladder	Bioner (Korea)
Green master mix	Bioner (Korea)

3.1.1 :Culture media

Culture media used in the present study are listed in Table 3-4. All media were prepared according to the manufacturer's (Himedia India, Conda/ Spain, Promega/ USA)

Table 3-4 Cultures media and Purpose of Using

NO.	Type of media	Company/ Origin	The purpose use and references
1	Brain heart infusion broth	Himedia/ India	It used for activation the bacterial isolates (Forbes *et al.*,2007)
2	Eosin methylene blue	Himedia/ India	**Selective media for E.coli**
3	MacConkey agar	Himedia/ India	Use to differentiate lactose fermenters from non-lactose fermenters (Winn *et al.*, 2006)
4	MR-VP broth	Himedia/ India	used to detect the partial an complete hydrolysis of glucos (MacFaddin, 2000).
5	Nutrient agar	Himedia/ India	Nutrient Agar is used as a genera purpose medium for the cultivatio

			of less fastidious microorganisms can be enriched with blood or othe biological fluids
6	Simmon citrate agar	Diffco-Michigan/ USA	used for determining the ability of bacteria to utilize citrate as the sole carbon source (MacFaddin, 2000)**.**

3-1-2 Reagents and solutions

3- 1- 2-1- : Methyl red reagent

The reagent was made by dissolving 0.1g of methyl red in 300ml of 99% ethanol, and then adding distilled water to get the amount up to 500ml. To determine the whole extent of glucose hydrolysis, this reagent was employed (MacFaddin, 2000).

3- 1- 2-2- : Kovac´s reagent

25 milliliters of concentrated HCl were added after 5 grams of P-dimethylaminebenzaldehyde had been dissolved in 75 milliliters of amyl alcohol. (MacFaddin, 2000) The reagent was used to detect indole.

3- 1- 2-3- : Gram stain solution

Gram Stain solution was supplied from Syrbio company. The solution was used to study Gram positive and Gram negative , morphology , bacterial cells ,and their arranged (Forbes *et al.*, 2007).

3.2 Methods

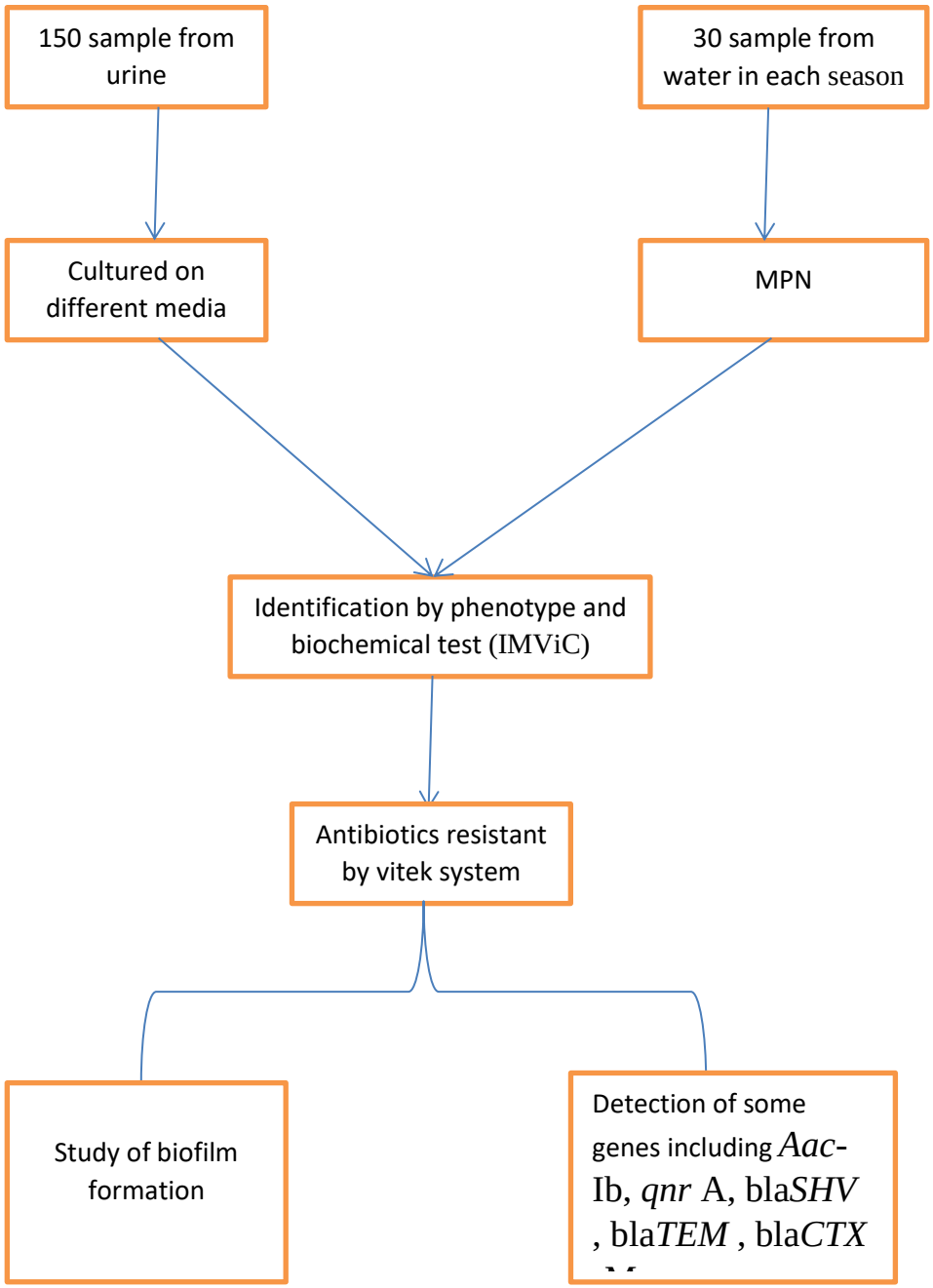

Figure (1) study design

3.2.1. Specimens or sample

During the period from July 2023 to February 2024, 150 urine samples were collected from the patients children with age between (1-10) year in order to isolate coliform bacteria the causative agents of urinary tract infections (UTIs). In addition, 30 water samples were collected from three different places along Al-Hussainiya river including (Baron hotel , Al-Atishi point and the white arch) distributed as 15 samples in each season (summer and winter). All obtained isolates were diagnosed and identified using combination between biochemical tests and Vitek 2 system. The antibiotic resistances activity for all the identified isolates were evaluated followed by study the encoding genes for this activity.

3-2-2 Preparation of solutions :

The following solutions were used in the present study. Solutions were sterilized by autoclaving at 121°C and 15 psi for 15-20 min. Heat-sensitive solutions such as antibiotics (sterile) and sugars (glucose) were sterilized by filtration (Millipore filters, 0.22 μm). The pH of the solutions was adjusted using either 1M NaOH or 1M HCl.

3-2-2-1 Normal Saline

Ready to use, sterile normal saline (NS) was used for the preparation of culture suspension.

3.2.2-2.Phosphate Buffer Saline (PBS).

One tablet of ready to use phosphate buffer saline (PBS) was dissolved in 100 ml of D.W. The PBS was sterilized by autoclave.

3-2-2-3. No.0.5 McFarland's turbidity stsndard.

The 0.5 McFarland's standard tube (1.5×108 CFU/ml) was made by mixing 1% sulfuric acid (H2SO4: H2O (v/v, 1 ml: 99 ml)) with 0.5 ml of 1.175% barium chloride (BaCl2.2H2O: H2O (w/v, 1.175g: 98.825 ml)) to create a barium sulfate precipitate. A visual comparison between the turbidity of a bacterial suspension and the 0.5 McFarland's

standard was made using the solution. To stop evaporation, the McFarland's standard tubes were wrapped in parafilm and kept at room temperature for up to six months in a dark location. The accuracy of a prepared 0.5 McFarland's standard was checked by using a spectrophotometer. The optical density was measured at 625 nm that should be between 0.08 and 0.1 (CLSI,2019).

3-2-2-4.Coliform bacterial isolation and identification

Urine samples were cultured on MaCconky agar media for 24 h at 37 ◦C. After that selected colonies were re-cultured on the same media many times in order to obtained pure colonies.

3-2-2-5.Most probable number method .

MPN was performed in three stages as Presumptive test, Confirmed test and Completed test.

With a presumptive test, the presence of gas in the lactose fermentation broth serves as the key indicator that Gram-negative coliform bacteria are present in the samples. Each sample being analyzed requires 15 sets of tubes containing lactose fermentation broth for the presumptive test process. 10 ml of fermentation broth and the water sample were inoculated in each test tube in the following order: 10 ml in five of each 2X lactose fermentation broth, 1 ml in five of each 1X lactose fermentation broth, and finally, 0.1 ml in five of each 10 ml 1X lactose fermentation broth.

One Derhum tube was reversely placed in each test tubes for detection of gas formation by coliform bacteria. Test tubes were incubated with half circled screw caps at 37 ◦C for 24 hours. This procedure was followed (Ahmed *et al.*, 2013). The numbers of positive tubes in each set were compared with standard tables and the results were expressed as MPN index or presumptive coliform count per 100 ml of water.

3-2-3- Completed test.

To confirm the positive lactose fermentation, a loopful of suspension was inoculated into a Durham tube containing Brilliant broth 1X medium. Following a 24-hour incubation

period at 44.5 °C, the tubes were checked for gas production. After 24 hours, tubes that tested negative for gas production were incubated further and reexamined at 48 hours (Hong *et al.*, 2021).

For the full E. coli test, the bright broth tubes that displayed positive gassing were used.

3-2-4- Confirmed test.

Positive samples that produce gas in the brilliant broth were chosen for the verification test methods to identify Escherichia coli as the indicator bacteria. By producing a green metallic sheen in the Eosin Methylene Blue MB media, other Gram-negative coliform bacteria were distinguished from *E. coli*. According to Hong *et al.* (2021), the presence of the indicator bacteria E. coli was verified by the green metallic sheen in the EMB.

These colonies' morphological characteristics namely colony size, form, color and pigment type, translucency, edge, elevation, and texture were followed for their identification. Following that, colonies were stained with Gram stain in order to detect particular intracellular chemicals, aggregation, response types, and shapes (Winn *et al.*, 2006).

3-2-5. Diagnostic tests

When the period of incubation was completed . Colonies with differing characteristic were subjected to various test , and it were identified according to (Macfaddin, 2000& Forbes *et al.*, 2007). single colony was inoculated in nutrient broth then incubated for 24 h at 37 temperature

3-2-5-1. Microscopic examination

Loop full of these colonies was separately transferred on slides and stained with Gram stain (Gram positive or Gram negative) and to show the form of bacteria.

3-2-5-2.Biochemical tests

3-2-5-2-1-: Indole test

In order to conduct this test, peptone water medium was inoculated with bacterial growth by the loop and incubated for 24 to 48 hours at 37 oC. Six to eight drops of Kovac's reagent

(p-dimethyl amino benzaldehyde in amyl alcohol) were added to conduct the indole test. The development of a red ring at the broth's top indicated a good reaction. (MacFaddin, 2000).

3-2-5-2-2-: Methyl –red test

Each bacterial colony was added to the tubes of MR-VP broth, which were then incubated for 24 to 48 hours at 37 oC. Following the incubation period, five drops of methyl red reagent were applied to this tube. The appearance and observation of red color means indicated for a positive result and a complete hydrolysis of glucose (MacFaddin, 2000).

3-2-5-2-3-: Vogues –proskauer test

Each bacterial colony was added to the tubes of MR-VP broth, which were then incubated for 24 hours at 37 oC. After that, 0.6 ml of alpha nephthol (reagent A) and 0.2 ml of 40% KOH solutions (reagent B) were added to read the result. The partial hydrolysis of glucose, which yields acetone or acetyl-methyl-carbinol, is the cause of the red color that appears after 15 minutes (MacFaddin, 2000).

3-2-5-2-4 Citrate utilization test

The bacterial colonies were inoculated and incubated for 24 hours at 37 oC following the autoclaving of Simmon's citrate medium. A positive outcome is shown by the media's color changing from green to blue with growth streaks, whereas a negative result is indicated by the medium remaining green with no growth (Winn *et al.*, 2006).

3-2-5-2-5 Vitek 2 system

In accordance with the manufacturer's recommendations, the Vitek 2 system was utilized to verify the biochemical test and investigate antibiotic sensitivity testing using specific coliform isolates.

Card filler mechanism, card cassette, bar code reader, cassette loading processing mechanism, card sealer cassette carousel, and incubator are among the several internal

components that make up this system, which includes a personal computer and reader incubator. Transmittance optics, electronics, waste processing, and firmware are all controlled by instruments. The system was outfitted with an expanded identification database for all standard identification tests, which improves microbiological diagnostic efficiency and eliminates the need for any extra tests, increasing test and user safety.

The following procedures were all prepared in accordance with the guidelines provided by the manufacturer. Using a loop full of a single colony, three milliliters of regular saline were added to a test tube. 24 hours was the colony's age. The colony was standardized to McFarland's standard solution (1.5×108cell/ml) by inserting the plane tube into a dens check machine. A sample identification number was input into the computer software via a barcode after the standardized inoculums were loaded into the cassette. The sample ID number was therefore linked to the VITEK 2 card. The cassette was then put in the filler module, and when the cards were filled, it was moved to the reader incubator module. The device manages all of the subsequent processes, including controlling the incubation temperature, optically reading the cards, and continuously monitoring and transferring test data to the computer for analysis.

3-2-5-2-5- 1-Standardization

Following primary isolation, a straightforward inoculum preparation, standardization, and dilution stage reduced handling. After inserting the standardized inoculum into the cassette, a barcode was used to input the sample identification number into the computer program.

3-2-5-2-5- 2-Traceability

Following primary isolation, a straightforward inoculum preparation, standardization, and dilution stage reduced handling. After inserting the standardized inoculum into the cassette, a barcode was used to input the sample identification number into the computer program.

3-2-5-2-5 -3-Load and Go

The record Placed in the filler module. When the cards were filled, the cassette transferred to the reader/incubator module,. All subsequent steps were handled by the instrument.

3.2. 6: Molecular detection of genes encoded for antibiotic resistance:

3.2.6.1: DNA extraction

Following the manufacturer's instructions, DNA was extracted from pure colonies of identified coliform isolates using the Favor PrepTM Genomic DNA Mini Kit. Each isolate's inoculum was adjusted to a density of up to 109. Centrifugation was used for one minute at 14,000 rpm to extract the bacterial pellets. Following a thorough resuspension in 200 µl of FATG buffer, the collected cells were allowed to sit at room temperature for 10 minutes. The bacterial cells were treated with 200 µl of FABG buffer. The homogenous cell suspension that resulted was vortexed for 10 seconds every three minutes until the sample lysate became clear after 10 minutes of incubation at 70ºC. 96–100% ethanol was added to the homogenous suspension to extract the DNA, which was then moved to the FABG column that was put together inside a 2 ml collection tube and centrifuged for 5 minutes at 14000 rpm. The flow through was thrown away. A fresh two milliliter collection tube was filled with the FABG column. The FABG column was then assembled inside a fresh 2 ml collection tube, and 400 µl of W1 Buffer and 600 µl of Wash Buffer were added, respectively. The column membrane was then washed by centrifuging it for 30 seconds at 14,000 rpm. After that, the supernatant was decanted. To eliminate any remaining ethanol solution, the FABG column was subsequently centrifuged at 14,000 rpm for three minutes over a fresh 2 ml column collecting tube. Following the transfer of the dry FABG column to a fresh 1.5 ml microcentrifuge tube, 100µl of TE or warmed elution buffer was applied straight to the FABG column membrane for 3–5 minutes. The DNA was then eluted by centrifugation for 1 minute at 14000 rpm. Before being used, the eluted genomic DNA was kept at 20ºC after its purity was assessed using a nanodrob.

3.2.6.2-TBE Buffer (Tris-Borate-EDTA).

TBE was made and kept in stock as a 10× solution.

40 ml of 0.5 M EDTA, 55 g of boric acid, and 108 g of Tris base were dissolved in 1000 ml of D.W. to create the working solution. Next, 100 ml of 10× TBE buffer was added to 900 ml of sterile D.W. to create the final concentration of 1× TBE solution (Sambrook and Russell, 2001).

3-2-6-3-: Primers preparation

The primers used in this investigation were dissolved in TE Buffer (pH 8.0), which is made up of 10 mM Tris-HCl and 1 mM EDTA-Na2. The working solution was made from the primer stock tube after the primer stock tube had been constructed. To achieve a concentration of 100 picomole/microliter of primer stock solution, TE buffer was added in accordance with the instructions given by the primer manufacturer (Bioneer, Korea). After that, a working solution was made from stock by diluting it with TE buffer until it contained 10 picomole/microlite (Green and Sambrook, 2012).

3.2.6.4-Primer Pairs:

The present used primers are listed in Table 3-5

Table 3-5 Primer Sequencing (Macrogen/Korea).

Primers		**Sequences**	References
SHV	**F**	**AGGATTGACTGCCTTTTTG**	Gangoue-pieboji *et al.*,2005
	R	**ATTTGCTGATTTCGCTCG**	
TEM	**F**	**5-TTGGGTGCACGAGTGGGTTA-3**	Colom *et al.*,2003
	R	**R: 5- TAATTGTTGCCGGGAAGCTA-3**	
CTX-M	**F**	**F: 5- ACCGCCGATAATTCGCAGAT-3**	Kaftandzieva *et al.*,2011
	R	**R: 5-GATATCGTTGGTGGTGCCATAA-3**	
aac-Ib	**F**	**F: 5-TTGCGATGCTCTATGAGTGGCTA-3**	Robicsek *et al.*,2006
	R	**R: 5-CTCGAATGCCTGGCGTGTTT-3**	
qnrA	**F**	**F: 5-ATTTCTCACGCCAGGATTTG-3**	Robicsek *et al.*,2006
	R	**R: 5-GATCGGCAAAGGTTAGGTCA-3**	

3.2.6.5: Reaction mixture

Amplification of DNA was carried out in a final volume of 20 µl reaction mixture as mentioned in Table 3-6.

Table 3-6 Contents of the Reaction Mixture

No.	Contents of reaction mixture	Volume
1.	Green master mix	5 µl
2.	Upstream primer (10pmol/µl)	1.5µl
3.	Downstream primer (10pmol/µl)	1.5µl
4.	DNA template	3 µl
5.	Nuclease free water	9 µl
Total volume		20 µl

3.2.6.6.Polymerase chain reaction (PCR).

The thermal cycling conditions are mentioned in Table 3-7.

Table 3-7 Optimum thermal conditions for PCR.

Genes	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles number
BlaTEM	95 ˚C for 5 min	95 ˚C for 30 sec	55-60 ˚C 35 for sec	73 ˚C for 1 min	73 ◦C for 5 min	30
BlaSHV	95 ˚C for 5 min	95 ˚C for 30 sec	55-60 ˚C 35 for sec	73 ˚C for 1 min	73 ◦C for 5 min	30
CTX-M	95 ˚C for 5 min	95 ˚C for 30 sec	55-60 ˚C 35 for sec	73 ˚C for 1 min	73 ◦C for 5 min	30
qnr-A	95 ˚C for 5 min	95 ˚C for 30 sec	55-60 ˚C 35 for sec	73 ˚C for 1 min	73 ◦C for 5 min	30
Aac(6) Ib	95 ˚C for 5 min	95 ˚C for 30 sec	55-60 ˚C 35 for sec	73 ˚C for 1 min	73 ◦C for 5 min	30

3.2.6.7.Agarose Gel Electrophoresis

A 1.5%–2% gel was utilized to achieve good resolution for tiny PCR product fragments (0.2-1 kb), whereas a 1% gel was utilized to achieve good separation of genomic DNA (5–10 kb) following extraction. To 100ml of 1×TBE buffer, 1% weight of agarose was added, and the mixture was heated in the microwave until it turned transparent. After cooling the agarose to between 50 and 55°C, 100 ml of melting agarose gel was mixed with 5 µl of simply safe dye (10 mg/ml) to achieve a final concentration of 0.5 µg/ml (Sambrook and Russell, 2006). The comb was positioned correctly, the agarose was added to the gel tray with capped ends, and it was then allowed to dry. In one gel well, the samples were loaded

with loading dye, and in another, they were loaded with marker. The run was applied in accordance with the gel size and percentage once the electrodes were properly attached. Connect the electrodes and cover the gel box. DNA will travel towards the positive (red) electrode positioned away from the well. Turn on the power supply. Continue electrophoresis until the tracking dye moves at least 10 cm of the gel length. (The time of agarose gel electrophoresis is 1 hour for PCR product).

3-2-7- Biofilm formation

To investigate the ability of coliform bacteria for biofilm formation the, isolates were tested according to the method described by Bose *et al* (2009). With some modifications the, isolates were grown in 10 ml of nutrient broth medium by inoculating the medium with a loop full of each isolate individually, and tubes were incubated at a temperature of 37°C for 24 hours., and the tubes were washed with normal saline solution. Thereafter, tubes were left to dry then they were stained with 0.1% of Crystal Violet dye and left for 5-10 min. Finally tubes were turned over to dry until a layer appeared. Visible staining on the walls and base of the tube indicates a positive result.

3-2-8- Statistical analysis

The data were all displayed as mean± SD. Tukey's HSD multiple range post hoc testing was performed after two-way analysis of variance (ANOVA) to look for any significant variations in coliform counts between stations and seasons. The accepted statistical significance level was set at $P < 0.05$. IBM (Pennsylvania, USA) MiniTab statistical software version 17 was used to conduct the statistical study

Chapter: Four
Results and Discussion

4-Results and Discussion:

4-1-Total number of samples:

One hundred and fifty urine samples were collected from the children with urinary tract infection where their age between 1-10 years who visited the Children's Teaching Hospital at the city of Karbala. They were divided in to (100) samples females and (50) samples males, as shown in Figure 4-1.

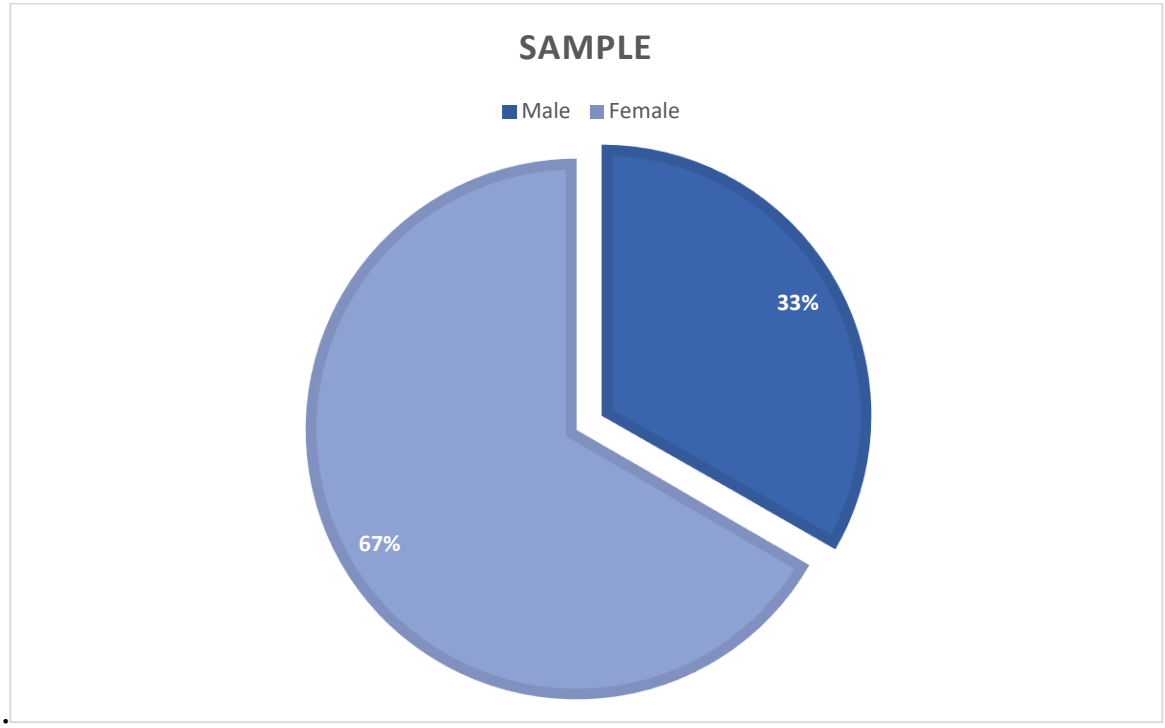

Figure 4-1 Distribution of the total number of samples according to sex.

The results of the current study regarding females being infected at a higher rate than males are consistent with the study (Abdul Ameer & Abdulla, 2022). The latter was conducted in the city of Baghdad, where the infection rate in females was (3-5%), which was higher than the infection rate in males (1%) and (7.8 %) of school-age girls and (1.6%) of school-aged boys. The current study is in agreement with the

study of Jamshidbeigi *et.al*, (2023) which was performed in Iran, the study revealed that females were 1.5 times more than infected males. Other study has found that the prevalence of UTIs in boys and girls was 0.47% and 0.37%, respectively, and that they could appear as asymptomatic (Gebretensaie *et al.*, 2023), these results are similar to what obtained from the present study.

4-2- The relationship of age with urinary tract infection percentage.

The results of the present study showed that the age group of (25-48) months among boys was higher for infections rate in comparison with other groups but no significant differences were shown. on the contrary, the lowest incidence of infection was found among boys in the age groups of (49-72) and (79-120) months Figure 4-2a.

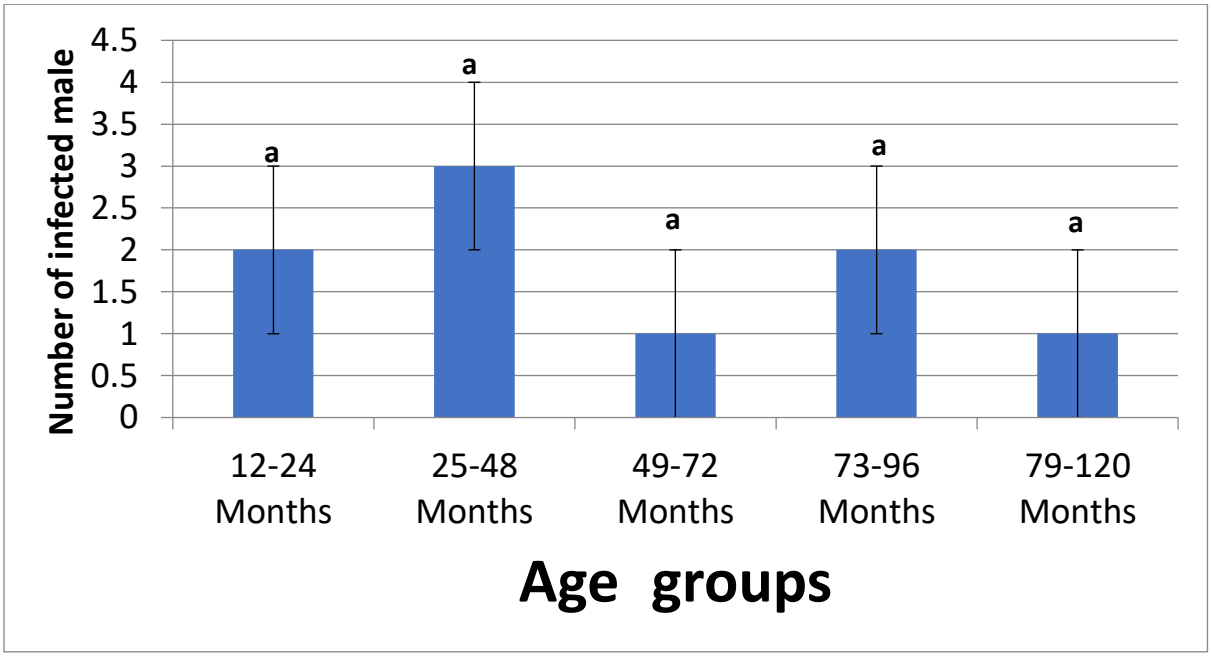

Figure (4-2a) Incidence of UTIs among male at the different age groups. No significant differences were found ($P > 0.05$).

The results of UTI infections among female are presented in Figure (4-2b).

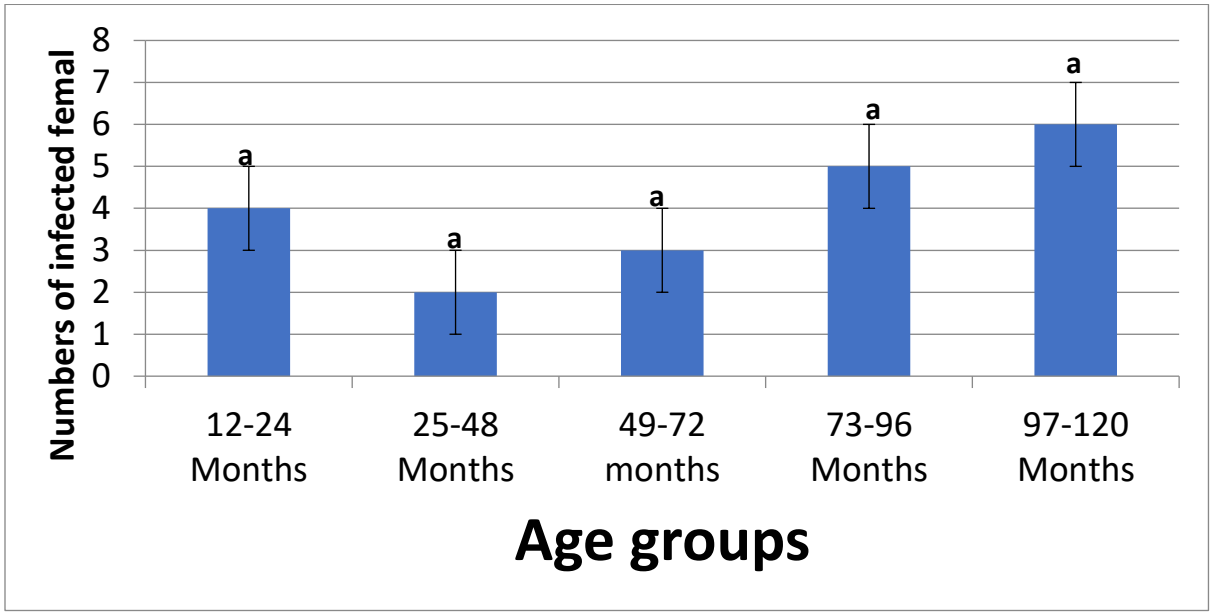

Figure (4-2b) Incidence of UTIs among femal at the different age groups. No significant differences were found ($P > 0.05$).

The period of age (97-120) months was th a ighest for incidence of UTI among female compared to other groups and no significant differences were revealed ($P \geq 0.05$). On the other hand, infections rate was lower in the age group of (25-48) months in comparison to the other groups.

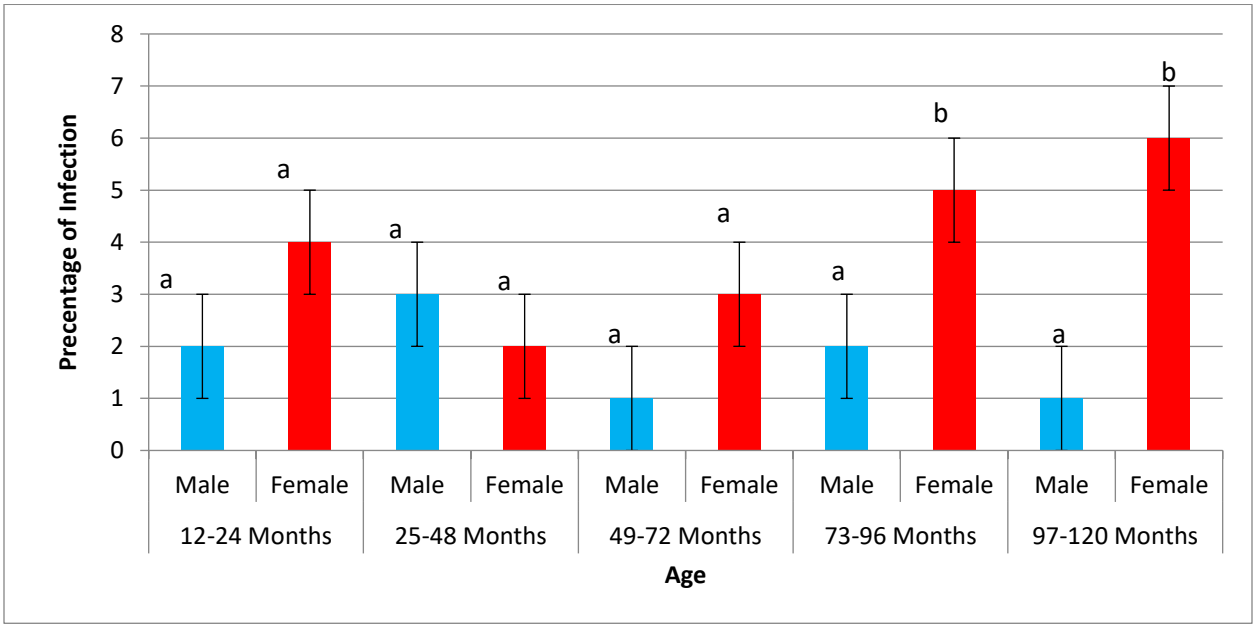

Figure (4-2c) Incidence of UTIs among children at the different age groups
Columns with same letters are not significantly different ($P \leq 0.05$).

The results of UTIs among children are illustrated in Figure 4-2c. The recurrence of infection among female was significantly higher compared to the boys with ages range of (73-96 and 97-120) months ($P \leq 0.05$). Although, female were highly subjected to UTIs in age of (12-24 and 49-72) months, no significant differences were found. The period of age (25-48) months was the only age groups shown higher infections in male than female with no significant differences.

The results of the current study regarding age groups are consistent with respect to age of group (12-24) months with local study of Noori &Hassan., (2021) in Baghdad, who found that the period was 12 months and less, males and females would be equal, and after this age, meaning more than the first year, the percentage would be inverse and the females would be higher. Barola *et al.*, (2024) stated that circumcision status, age, gender, and race all affect the prevalence of UTIs. Among

children under seven years, 1.7% of male and 8.4% of female get UTIs. When toilet training starts both male and female infants experience the highest frequency of UTIs throughout infancy Children's lower age, uncircumcised newborn male, and female sex are the main risk factors for UTIs which is similar to the current study. UTIs may be the initial sign of congenital kidney and urinary tract defects in male (3.7%) compared to female (2%) in the first year of life. The shorter length of the female urethra may help to explain why UTIs become more common in female after the first year of life This was proven by Daniell et al.,(2023) which is consistent with the present study.

UTI have two peaks: one during the 12 month of life and another between the ages of 24and 48month, which is when potty training begins In agreement, present data, obtanied from the study of Leung *et al* (2019) showed similarty for the 60 months group .Pervious studies demonstrated that male infected by UTI ,between the age of one and five years and female infected by UTI 10-20 times more frequently than male do during the preschool years which is different from the present study (Nasser *et al* .,2019;Nadi *et al.*,2024).

Respectively, in agreement with the data obtained from the current present study (Kumari *et. al.,* (2024) showed that the occurrences of UTIs are more in females than in males, and the reasons could be attributed to the behavioral variations, hormonal impacts, and structural differences.

Children in age of 84 -month old distributed as 1.7% of male and 7.8% of female predicted to have had a UTI this is what I have proven (Leung *et al.*, 2019) which is consistent with the present study Other study found that female in rate of 16% and male in rate of 10% infected of UTI Jamshidbeigi *et.al*,(2023) which is similar to our result.

4-3-Relationship between nature of housing, financial income and the incidence of UTI.

The obtained data from the present study illustrated that the number ofUTI infected children who's their fathers in free work, 93 (61.3%), was significantly higher in compared to the children whose fathers worked as state employees, 58 (38.6%), as presented in Figures(4-4) ($P \leq 0.05$).

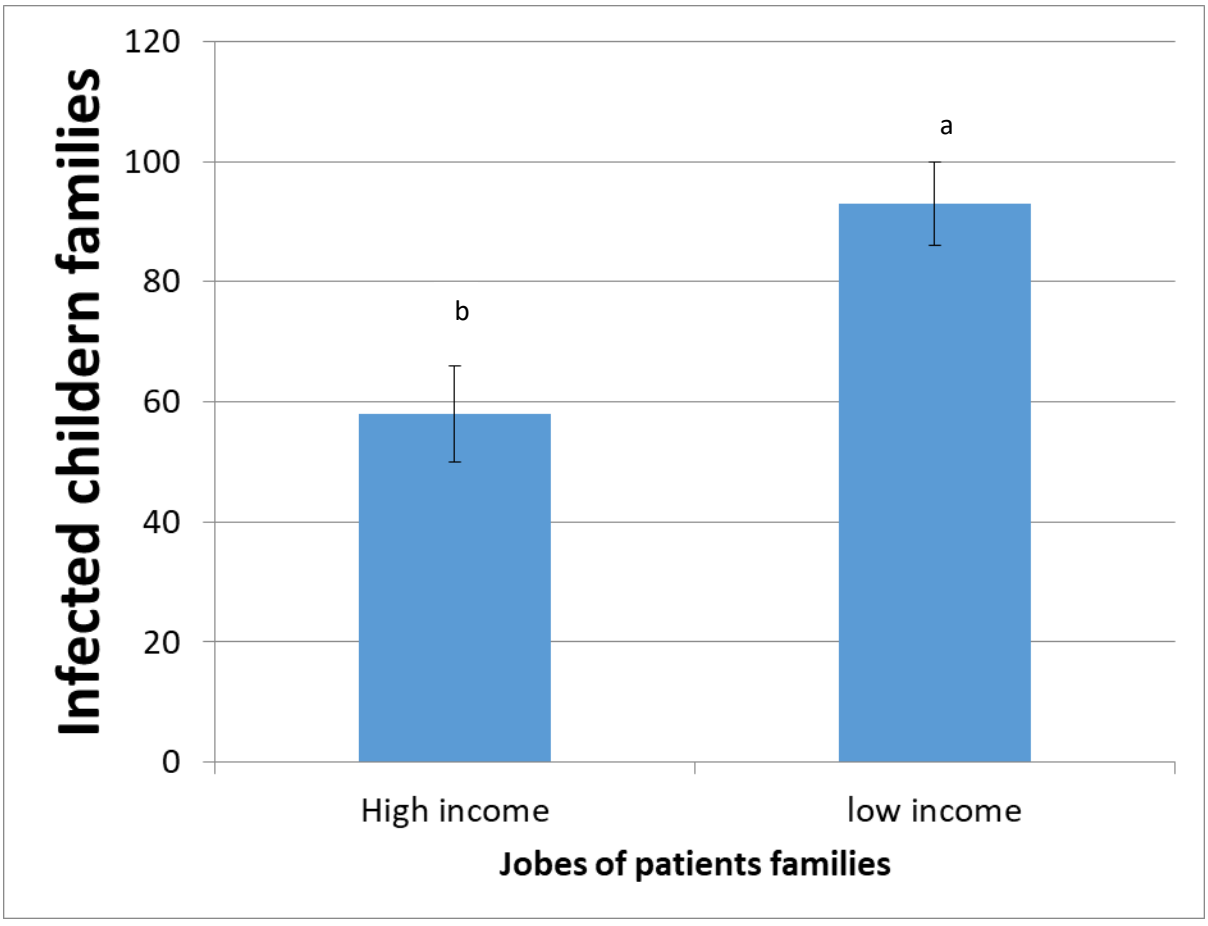

Figure 4-3 Nature of income for families of infected children. The results represent (Mean ± SD). Columns having different letters are significantly different (P =0.000).

The families of infected children by UTIs who lived in a city were significantly higher in comparison to the families lived in rural regions(Figure 4-4) ($P \leq 0.05$).

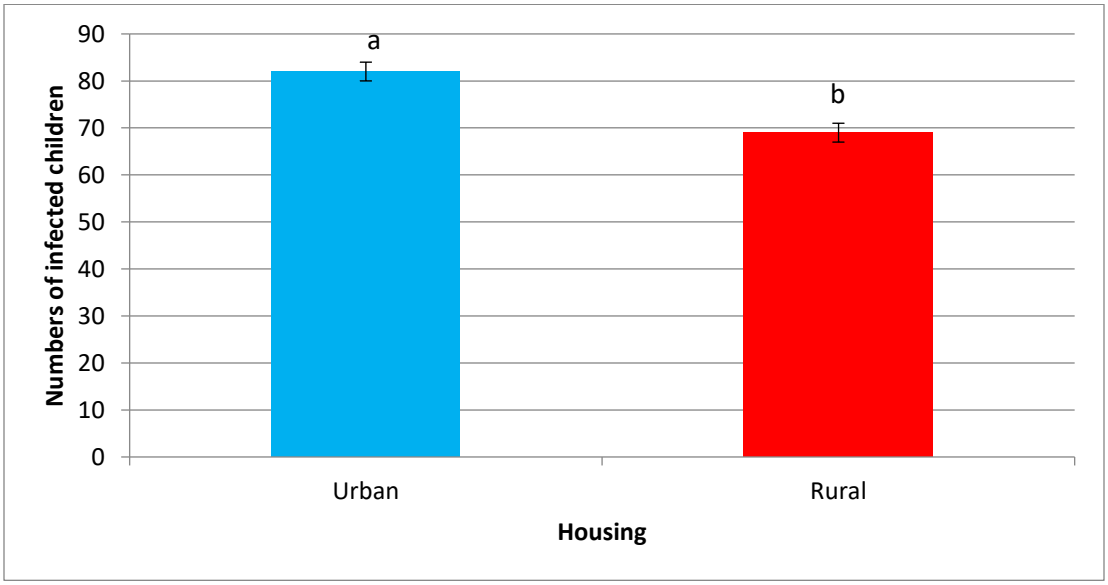

Figure 4-4 Nature housing for families of infected children. Columns having different letters are significantly different ($P = \leq 0.05$).

The present data, regarding housing and family's income, are not consistent with results obtained from a local study carried out by (Abdul Ameer & Abdulla., 2022) in Baghdad ,which showed that children who lived in rural areas had a higher risk of UTIs. A previous study also confirmed that a higher incidence of UTI was in males in rural areas compared to urban ones, this is because males who are prone to UTI have distinct urethral flora than males who are not (Hussein KA *et al.*, 2015).

Additionally, one of the biggest health issues that will have to deal with is antibiotic resistance, which is especially prevalent in low- to middle-income nations because of a lack of awareness of how serious the problem is and the overuse of antibiotics (Ara *et al.*, 2022).

In alocal study prevalence of UTIs among students in urban and rural areas in Erbil was significantly different in agreement with the present results and it also stated that hand washing was typical way to avoid UTIs in metropolitan settings, Moreover, school-age children from families with low incomes had a significantly greater chance of urinary tract infections Abdul Ameer & Abdulla., (2022).

4-4-Number of pathogenic coliform bacteria.

Thirty isolates of coliform bacteria were isolated from both gender who were infected with UTIs. The number of bacterial isolates was significantly higher (21) 70% isolates in females in comparison to (9) 30% isolates in males Figure 4-5 ($P \leq 0.05$).

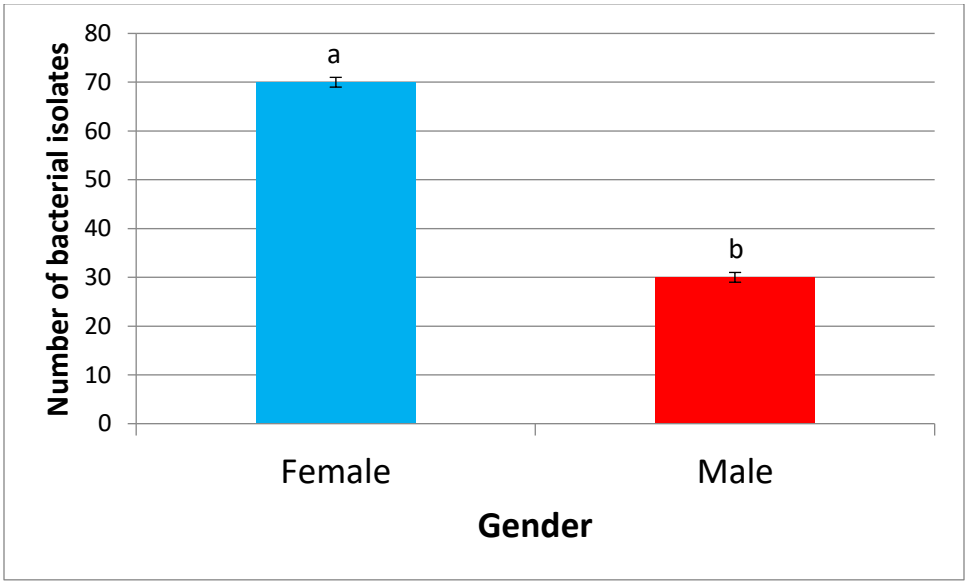

Figure (4-5a) Number of coliform bacteria isolated from urine samples among both gender. Columns having different letters are significantly different ($P = \leq 0.05$).

4-5-Morphological Identification.

The isolates of *E. coli* were initially identified based on their phenotypic characteristics, after their growing on both MacConkey agar (MA) and Eosin Methylene Blue agar (EMB) media.The figure (4-6a) shows colonies of *E. coli* on

MacConkey agar which are lactose fermenter and gives the pink color, which is help for initial identification of *E. coli* from other family member, especially the figure (4-6b) show gram negative bacteria on (EMB) medium with green metallic sheen color which is caused by the dyes' metachromatic quality (eosin and methylene blue) beside to the ability of these bacteria to ferment lactose, which raises the medium's pH to an acidic level. Thus, it is much easier to identify when the medium is made more selective for *E. coli* and diagnosis of these bacteria in the present study was according to (Basavaraju & Gunashree, 2022).

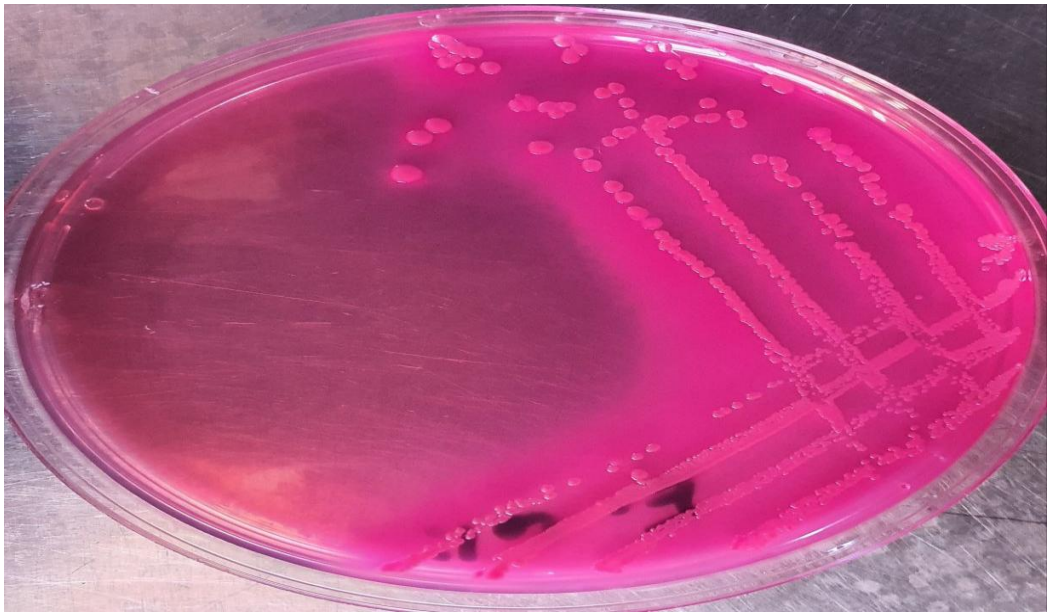

Figure (4-6a) The appearance of *E. coli* colonies on MacConkey agar medium as pink color and ferment lactose sugar.

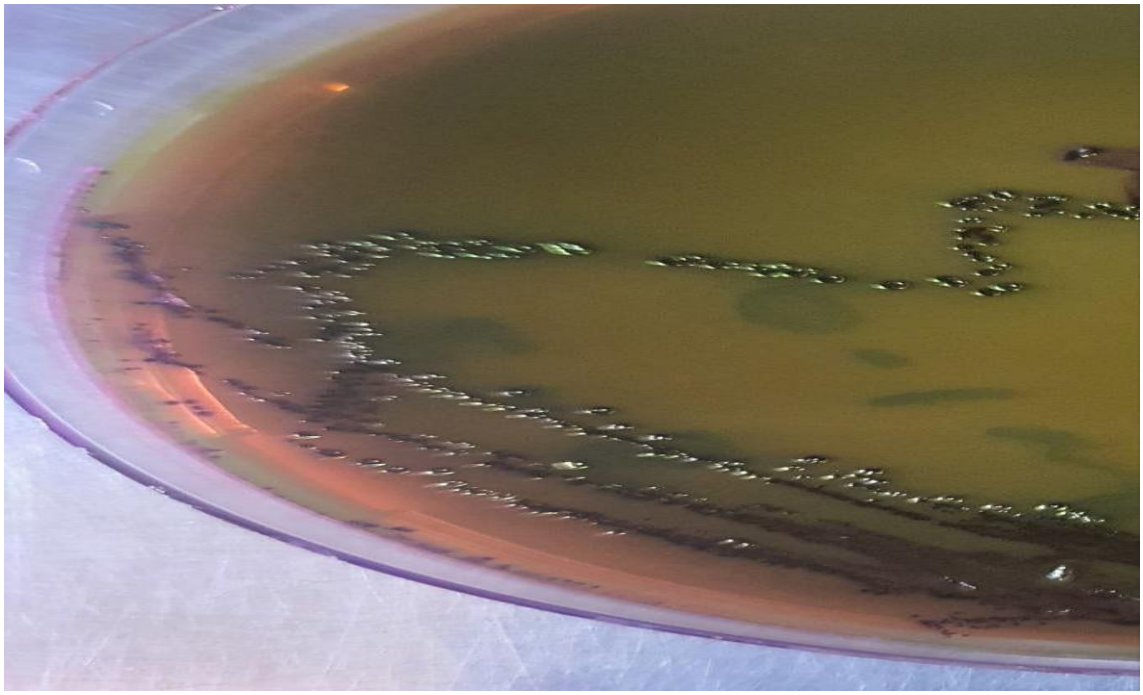

Figure (4-6) The appearance of *E. coli* colonies on Eosin methylene blue (EMB) as bright metallic green colonies.

Furthermore,the figure(4-6)showed that *klebsiella spp.* isolates appeared as mucoid colonies developed on MacConkey agar because of lactose fermentation, which was a distinguishing feature of *Klebsiella* spp. colonies that were very big and dome-shaped these results are consistent with what was mentioned by Khan *et. al*., (2022). on MacConkey agar; *Klebsiella* colonies have a huge, mucoid, red appearance with spreading red pigment that indicates glucose fermentation and acid production. Moreover, figure (4-6d) showed that *Enterobacter spp.* appeared as yellowish-centered pink colonies on MacConkey's agar of *Enterobacter* spp. Identification in the present study was based on diagnosis as mentioned in (Shahab *et. al*, 2017).

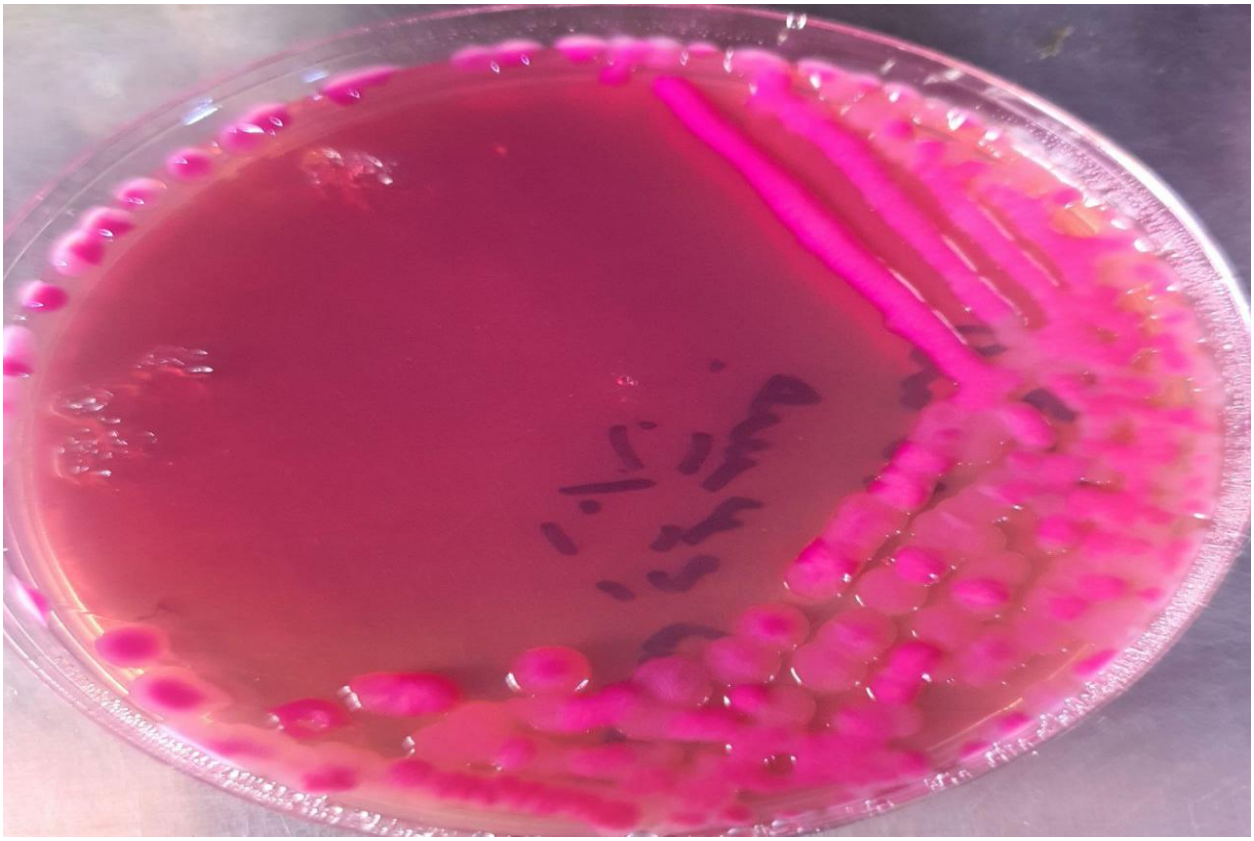

Figure (4-6c) Appearance of *Klebsiella pneumonia* on MacConkey Agar as pink color colonies big and dom shape, ferment lactose sugar and mucoid.

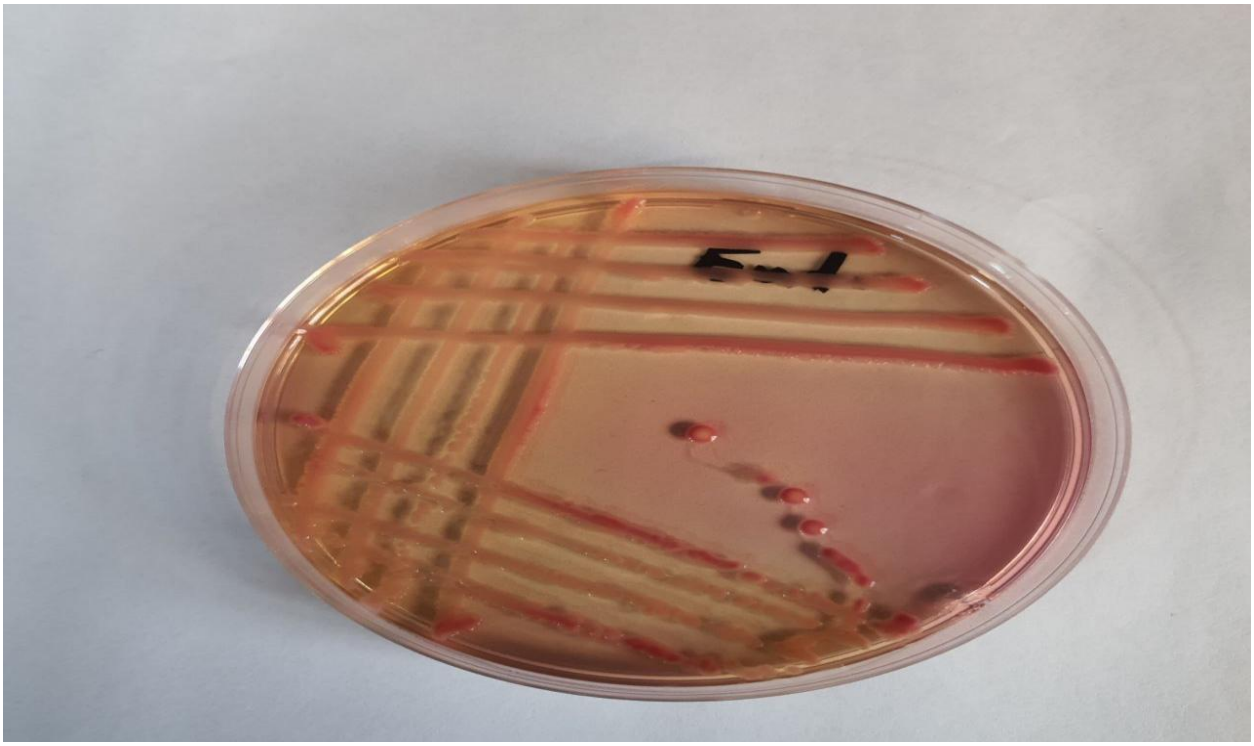

Figure (4-6) Appearance of *Enterobacter* spp on MacConkey Agar as Yellowish-centered pink colonies and ferment lactose sugar.

4-5-1-Microscopic Identification.

Selected pure colonies were subjected to Gram stain and examined using light microscopic, as all the *E. coli* colonies were Gram-negative, pink color, small rod shaped appearance, arranged in single or paired short and these features are similar as mentioned by (Islam *et al.*,2014). *Klebsiella pneumonia*, are Gram-negative straight rod, arranged singly, in pairs or in short chains these results are consistent with what was mentioned (Hmood &Swim, 2019).gAdditionally Enterobacter spp.isolates appeared under light microscope gram negative and rod shape and these feature are in agreement with study of Abbas &Radhi,(2016)

4-5-2- Biochemical Identification:

Results of biochemical tests IMVIC tests (Indole, methyl red, Voges-Proskauer and Citrate), showed that *E. coli* were positive for indole test, and methyl red test. Voges-Proskauer and Citrate tests were negative which according was to diagnosis as mentioned by Abdallah *et al.*, (2016). In additio, *Kleebsiella pneumonia*, were negative for Indole and Methyl Red tests, whereas Voges-Proskauer and Citrate tests were positive results. These result are consistent with what was mentioned by Modh *et al.*, (2021). The results of *Enterobacter* isolates regarding to the IMVIC tests were (-, -, +, -), respectively, these results are consistent with what was mentioned in Budiarso *etal*., (2023).

Table 4-1 The results of phenotypic and microscopic tests of coliform bacteria.

Type of bacteria	***E. coli***	***Klebsiella* spp.**	***Enterobacter* spp.**
MacConkey agar	Pink color and ferment lactose sugar	Pink color big and dom shape ferment lactose sugar and mucoid	Yellowish-centered pink colonies and ferment lactose sugar
EMB	Bright metallic green Colonies	—	—
Gram stain	Negative	negative	Negative
Shape of cells	Rod	rod	Rod
Indol test	Positive	Negative	Negative
Methyiene red test	Positive	negative	Negative
Vogas-proskaur test	Negative	positive	Positive
Citrate test	Negative	positive	Negative

4-6-Isolation bacteria from the water sample:

The results of coliform bacteria isolated during summer season by the first step of MPN, which is presumptive test, are presented in Figure 4-7a.

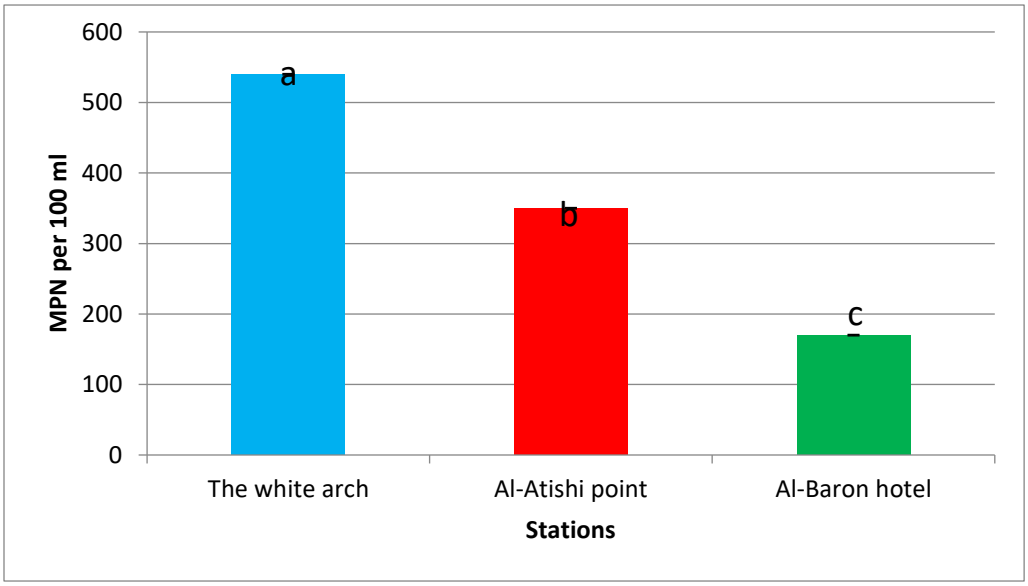

Figure (4-7a) Results of coliform bacteria isolated from the tested stations during the summer season. Columns having different letters are significantly different ($P \leq 0.05$).

It is clear to see significant differences in the number of bacterial coliform isolates among studied stations ($P \leq 0.05$). Coliform bacteria intensity was significantly higher in the white arch 540 MPN per 100ml in comparison to 350 and 170 MPN per 100 ml in Al-Atishi and Al-baron hotel stations, respectively. In contrast Al-Baron hotel station was significantly lower in the number of coliform 170 MPN per 100 ml compared with the other two stations Figure 4-7a ($P \leq 0.05$).

The results of coliform bacteria isolated during winter season by the first step of MPN, which is presumptive test, are explained in Figure 4-7b.

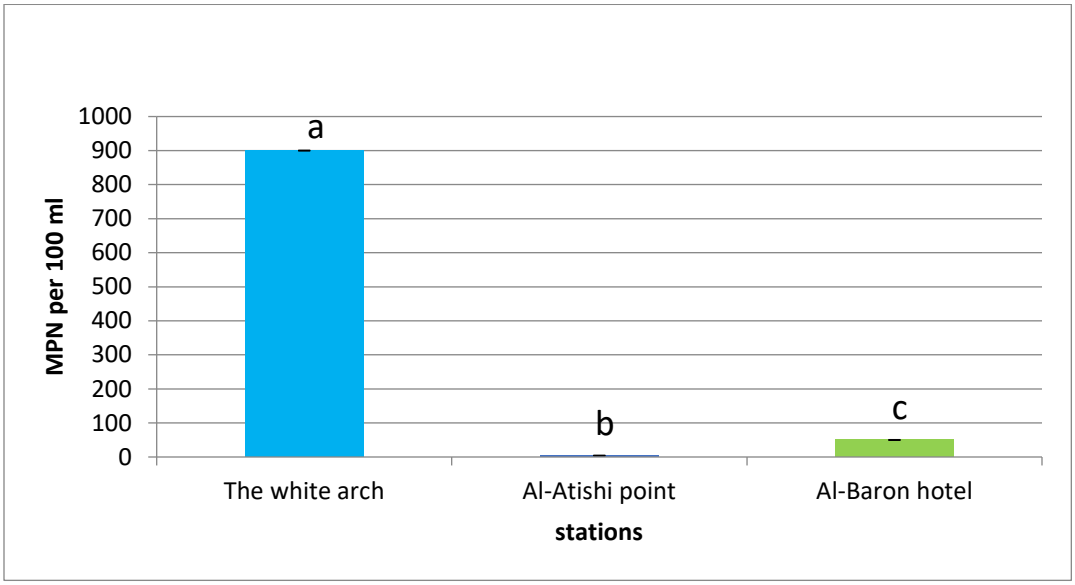

Figure (4-7b) Results of coliform bacteria isolated from the tested stations during the winter season. Columns having different letters are significantly different ($P \leq 0.05$).

It was similar to what was obtained from the summer season about the white arch which was significantly higher in the number of coliform bacteria as 900 MPN per 100 ml compared to 4 and 50 MPN per 100ml in Al-Atishi and Al-Baron hotel stations, respectively Figure 4-7b ($P \leq 0.05$). On the contrary, Al-Atishi point was singficantly lower in the number of coliform 4 MPN per 100ml in comparison to the other stations.

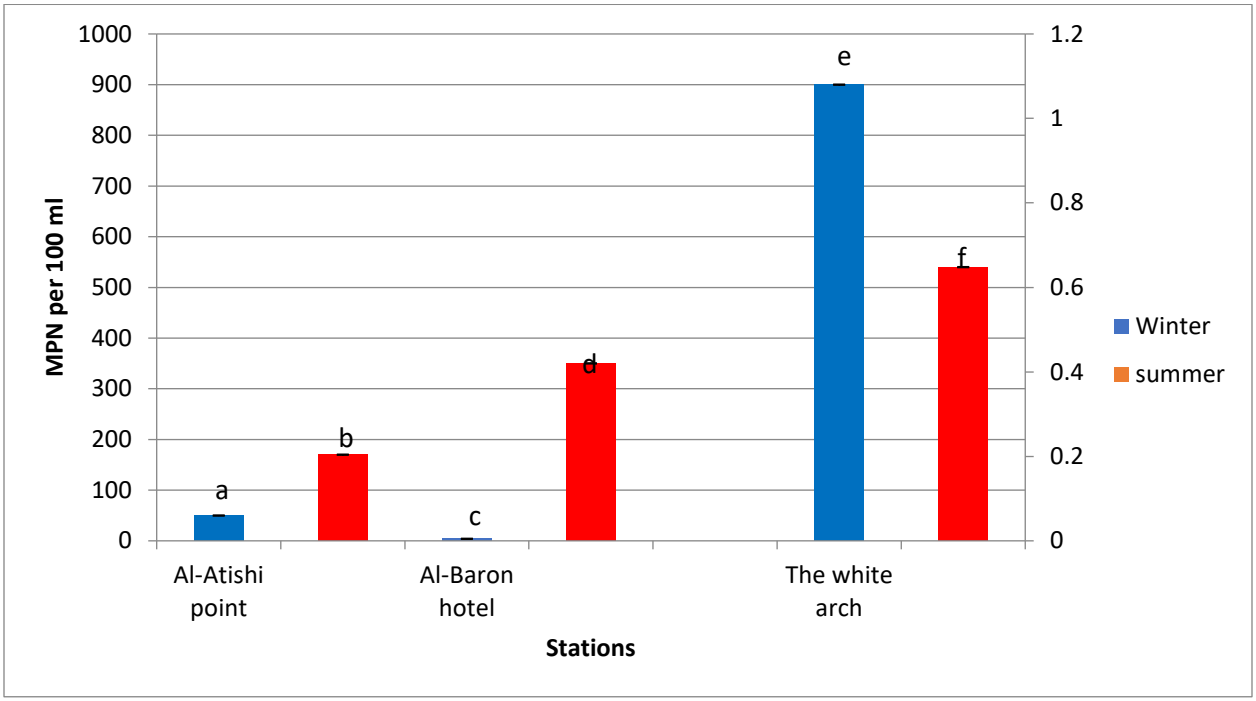

Figure (4-7c) Results of coliform bacteria isolated from the tested stations during the summer and winter seasons. Columns having different letters are significantly different ($P \leq 0.05$).

Statical analysis show two-way ANOVA for the data of coliform bacteria isolated from the three stations during the summer and winter seasons showed that there was a significant difference between two seasons in each station and between stations. The numbers of coliform bacteria were significantly higher in Al-Atishi and Al-Baron hotel stations during the summer season in comparison to their numbers during the winter seasons in the same stations Figure 4-7c ($P \leq 0.05$). On the other hand, the white arch was higher in coliform during both seasons compared to the other two stations, but coliform bacteria were significantly higher in the winter season compared to the summer season, which is not similar to the other stations Figure (4-7c) ($P \leq 0.05$).

Figure (4-8a+b) the positive result change to yellow color and production gas of presumptive test.

Seasonal variations were evident for coliform, with major peaks occurring in late winter/early spring and occasionally modest peaks in the fall season. Faecal Coliform levels were generally lower (25 MPN) in late spring, summer, and autumn than in late winter and early spring (chigbu *et al.*, 2005). This increase number of coliform bacteria in May could perhaps be linked to high temperatures and runoffs. High temperatures during the summer season in particular from June to August, could be contributed to higher than average levels of coliform bacteria (Bakaj&Dalipaj, 2020). This is similar to the result of the stations of Al-Atishi point and Al-Baron hotel.

In completed test, counting the number of positive tubes is how the results are read and the presence of metallic green colonies indicates that Brilliant Green Lactose Broth media is utilized because it is a unique medium that can be used to selectively grow bacteria other than coliform and identify gram-negative bacteria. Gram-negative bacteria can be inhibited and the coliform bacteria can grow more rapidly in this medium. The turbidity and gas production caused by the coliform group of bacteria fermenting lactose indicate to the presence of gram-negative coliform

bacteria and culturing on MacConkeyy agar that allow for growing *E.coli* as presented in Figure 4-8c .

Figure (4-8c) the confirmatory test

Water is a vital natural resource for life; but due to bacterial and heavy metal contamination brought on by subpar sewage infrastructure and procedures, water quality declines. Hospital wastewater is one of the possible causes of contaminating water and this is because hospital wastewater has significant concentrations of harmful microbes, various chemical compounds, and organic and inorganic

substances. contamination may have reached the water from water tanks, and this is what the researcher confirmed (Shaky *et al.*, 2021). If not adequately cleaned and maintained, domestic storage pots, tanks, and irrigation surrounding the residence may be sources of contamination even when water sources are free from contamination. The final stage of MPN method is the confirmatory, which showed colonies of *E. coli* on EMB media as metallic green shine colonies as shown in Figure (4-8d).

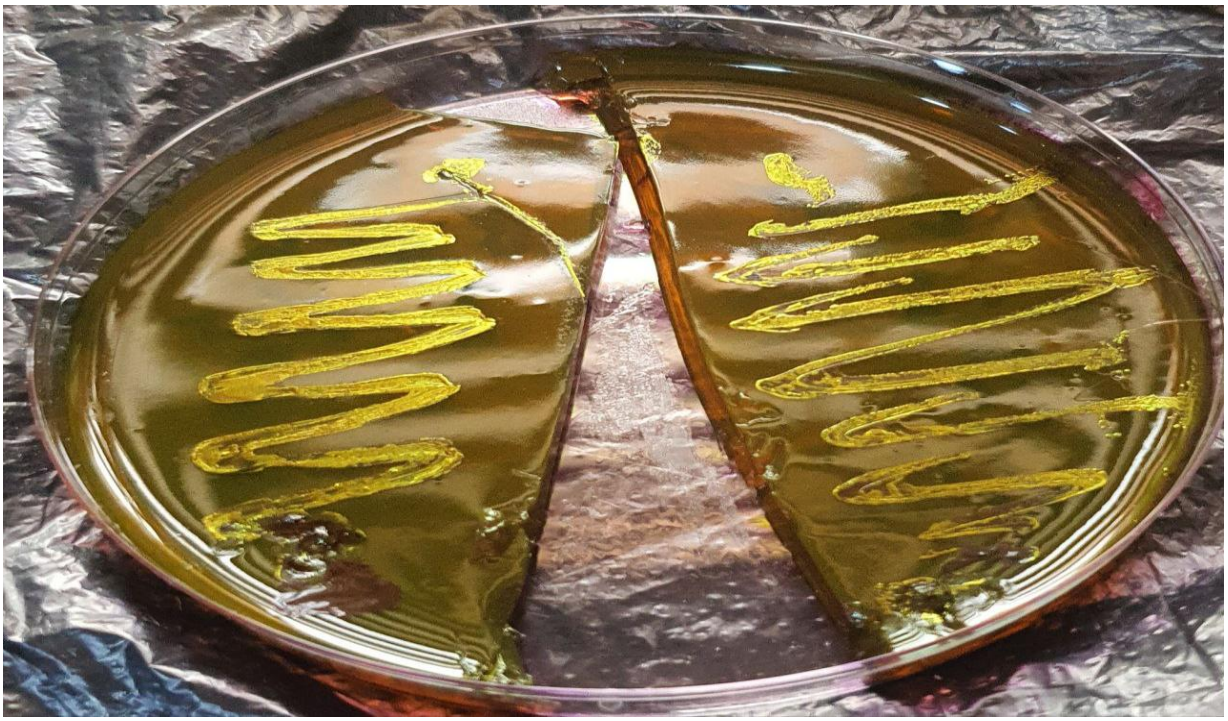

Figure (4-8d) Confirmatory steps in MPN test showed *E. coli* as shine metallic colonies on EMB media.

4-7- Bacterial resistance to antibiotics:

4-7-1 Bacterial resistance to antibiotics in the urine samples:

Twenty isolates, from the urine, were tested for their activity against 23 common types of antibiotics. These isolates were distributed as 14 isolates of *E.coli*, 5 isolates

of *Klebsiella pneumonia* and one isolates of *Enterobacter* spp. The bacterial isolates varied on their resistance to these antibiotics as shown in Figure 4-9a:

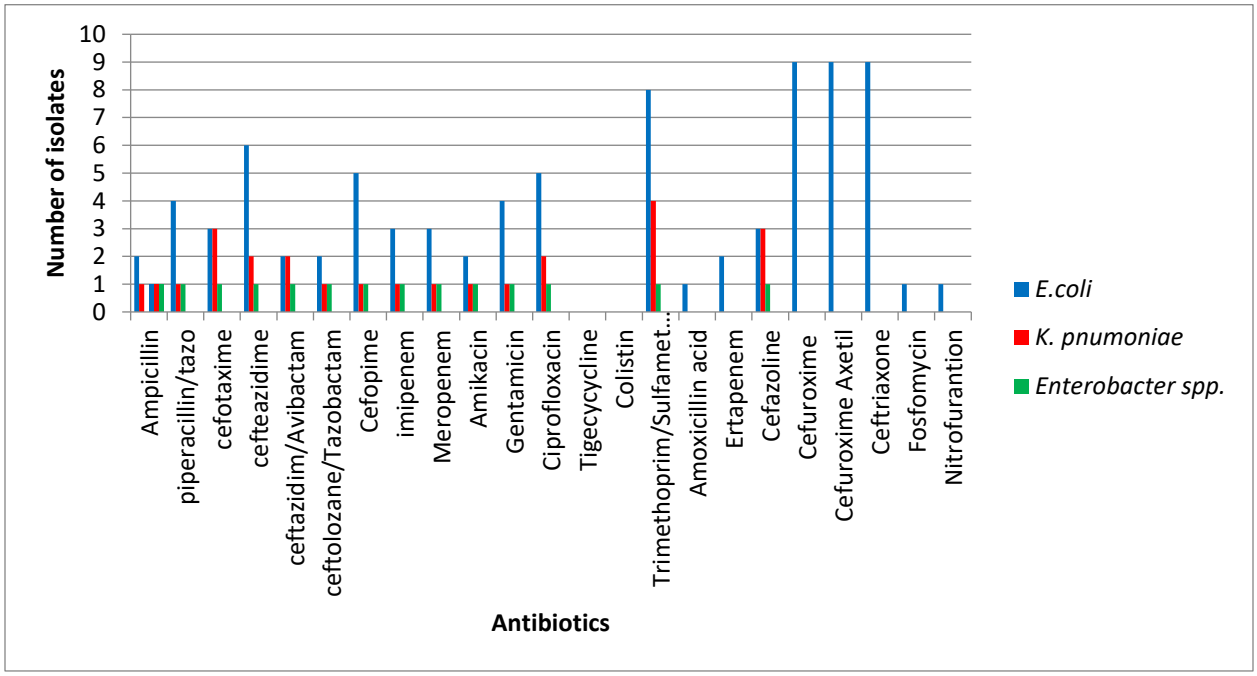

Figure 4-9 Antibiotic resistance activity of coliform bacteria isolated from the urine samples against different types of antibiotics.

Data showed that *E. coli* were more resistant of the used antibiotics, as they were the only microbe resistant to the antibiotics, including ceftriaxone, cefuroxime, cefuroxime-Axetil, Ertapenem, Amoxicillin acid, nitrofurantoin, and fosfomycin. In contrast other bacteria were not be able to show resistance to these antibiotics. On the other hand, all tested bacteria revealed resistance to the following antibiotics including cefepime, ciprofloxacin, Imipenem, piperacillin, meropenem, Ampicillin, cefazolin, cefeazidime, ceftazidime/Avibactam, Imipenem,Amikacin, Gentamycin

and cefazolin, trimethoprim and sulfamethoxazole in different number of their isolates.

The results of the current study regarding to trimethoprim are consistent with the results of Somorin *et al.*, (2022) who found that the most often isolated uropathogens were revealed strong trimethoprim resistance. In agreement with the present data, Agegnehu *et al.*, (2018) found that (90.6%) of total isolates of *E. coli* revealed a strong resistance activity against trimethoprim. Before prescribing an antibiotic, promptly identifying trimethoprim resistance may help reduce the chance of treatment failure, prolonged hospital stays, and recurrent UTIs (Somorin *et al.*, 2022).

Nowadays trimethoprim is only advised as a first-line treatment for UTIs in cases when the preferred antibiotic, nitrofurantoin, has a low risk of resistance. Nonetheless, gastrointestinal side effects are a common complaint among patients who receive nitrofurantoin prescriptions. Furthermore, it is not advised for people with impaired renal function to use nitrofurantoin (Somorin *et al.*, 2022).

Between *E. coli* and *Klebsiella*, high resistance rates have been seen to ampicillin, amoxicillin, trimethoprim/sulfamethoxazole (TMP/SMX), cefuroxime, and ciprofloxacin. The present study demonstrated that children with feverish or severe UTIs may benefit effectively from the use of nitrofurantoin, ceftriaxone, amikacin, and carbapenem which is similar to what found by Duicu *et al.*, (2021).

The resistance activity of *E. coli* against cefuroxime is in agreement with study of Vigi *et al.*, (2016) conducted in India, who concluded that 60% of the total isolates exhibited resistance to cefuroxime. In contrast, study accomplished by Abu Handan *et al.*, (2013) performed in Turkey showed that the rate of bacterial resistance to cefuroxime was (38.1%).

The data obtained from the present study regarding to ceftriaxone are similar to the study carried out in Iran which showed that the rate of bacterial resistance to ceftriaxone reached (61. 3%) (Ayatollahi *et al.*, 2022). A study conducted in Romania by Duicu *et al.*, (2021) found that the rate of bacterial resistance reached (8%) which is not in agreement with the present study. Bacterial resistance to antibiotics is a serious concern in both UTIs and other diseases due to antibiotic abuse, which can pose serious therapeutic challenges (Joya *et al.*, 2022).

Biofilms play an important role in bacteria gaining resistance to multiple antibiotics and are found in positive and negative bacteria (Uruen *et al.*, 2020). The overuse of antibiotics has led to the development of antibiotic resistance, which not only lowers the medication's therapeutic efficacy in treating infectious diseases that pose a serious threat to life but also raises treatment costs overall (Hamza &Omran, 2022).

β-lactamases are a class of widely distributed enzymes that are typically resistant to cephalosporin and penicillin. Two different pathways might result in acquired resistance: a mutation that happens in the cell's DNA during DNA transfer or replication bacteria can also develop resistance by transformation, transposition, and conjugation—all of which are referred to as horizontal gene transfer (Mancuso *et al.*, 2021). The difference in rates of bacterial resistance to the same antibiotic may be due to the use of medicinal herbs and plants commonly used in Kenya as an alternative to the use of antibiotics, thus reducing the emergence of resistance or its transmission between bacteria (Matai *et al.*, 2021).

Finally, in agreement with the present results of Amoxicillin bacterial resistance Hammond *et al.*, (2020) stated that the percentage of bacterial isolates resistant to this antibiotic was (14%), whereas the current study is not in agreement with the

study of Abu Handan *et al.*, (2013) in Turkey who found that the rate of bacterial resistance to this antibiotic was (51.7%).

The difference in resistance ratio is a result of genes encoding to the β-lactamase enzymes which are perhaps the most widely distributed; accidental mutations in these genes have resulted in modified catalysts with increasingly extended spectrum of resistance and outcomes of many years of constant selection pressure from human applications of antibiotics, via underuse, overuse, and misuse (Davies & Davies, 2010).

Four isolates of *K. pneumoniae* out of 5 were found to reveal resistance activity against to Trimethoprim which is not in agreement with study of Dulcu *et al.*, (2021) showed the percentage of bacterial resistance to this antibiotic was (3.92%).

Microorganisms assemble into biofilms when they develop their own extracellular matrix. By using several processes that rely on variables like the composition of the biofilm, they establish specific conditions that provide bacteria tolerance and resistance to antibiotics (Uruen *et al.*, 2020).

Three isolates were exhibited resistance activity to Ciprofloxacin and Cefteazidime.

Additionally, two isolates of *K. pneumoniae* out of 5 isolates were reported to exhibit resistance to cefotaxime and ciprofloxacin. Finally, one isolates only of K. *pneumoniae* revealed resistance of the rest of antibiotics including cefepime, Imipenem, piperacillin, Ampicillin, ceftazidime/Avibactam, Imipenem, Amikacin, and Gentamycin Figure 4-9.

Gram-negative bacteria are, in general, more resistant to antibiotics than Gram-positive ones due to the presence of the outer membrane, which reduces the permeability to many antibiotics (Uruen *et al.*, 2020).

Extended exposure to non-lethal antibiotic doses can cause microorganisms to acquire resistance. Bacteria can acquire antibiotic resistance (AMR)(antimicrobial resistance) by means of

mutations, horizontal gene transfer, and altered gene expression. (Uruen *et al.*, 2020).

The present study showed that the only isolate of *Enterobacter* spp. which was the only one that could be got was revealed resistance to Ciprofloxacin, Imipenem, piperacillin, Meropenem, Cefazolin, ceftazidime/Avibactam, Amikacin, Gentamycin, Trimethoprim and Sulfamethoxazole as presented in Figure 4-9.

The antibiotics resistance may be due to the differences in resistance ratio induced chromosomal enzymes, drug efflux, and membrane impermeability, to the current theory of mobile gene pools that predominantly control the spread of contemporary antibiotic resistance (Iredell *et al.*, 2020).

Poor health and hygiene education programmers, over prescription in clinical settings (primarily in developing nations where antibiotics are more easily accessible), and a lack of reliable diagnostic tools are the main causes of the current global antibiotic crisis (Abadi *et al.*, 2019).

The reason for the difference in the rates of bacterial resistance to the same antibiotic may be to reduce drug uptake, altered drug targets, drug inactivation, and activation of drug efflux pumps are the primary mechanisms of resistance (Mancuso *et al.*, 2021).

4-7-2- Antibiotics resistance activity of coliform bacteria isolated from the AL-Hussainiya river:

Thirty samples were collected from the AL-Hussainya river, these samples distributed as 15 during each season. Samples were cultured on proper media for their identification. Selection pure colonies according to their phenotype and some applying biochemical tests. Fifteen isolates were identified as 5 isolates for each *E. coli*, *K. pneumonia*, and *Enterobacter* spp. for each season. Vitek 2 system further was used for testing the antibiotics susceptibility activity for these isolates.

The present data regarding antibiotics resistance showed that all coliform bacterial isolated from the water sample during the summer season were not exhibited resistance to any type of the applied antibiotics.

The results of antibiotics resistance activity of coliform bacteria isolated from the AL-Hussainya river during the winter season are presented in Figure (4-10).

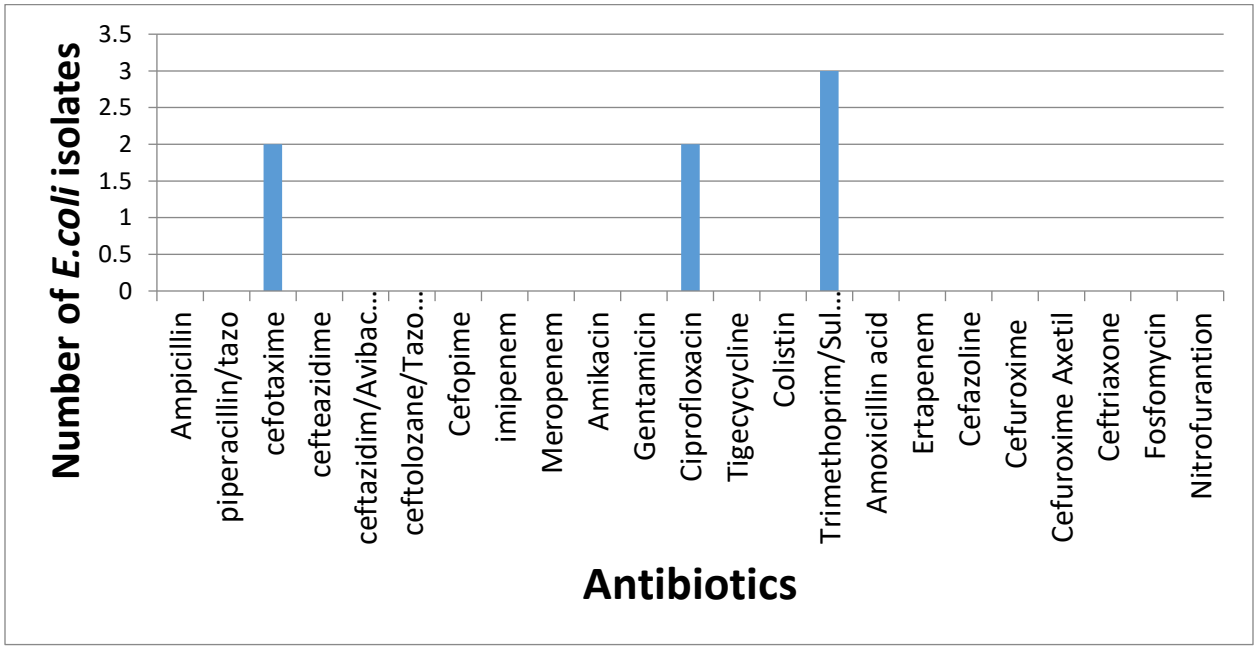

Figure 4-10 Antibiotic resistance activity of coliform bacteria isolated from the AL-Hussainya river samples during the winter season against different antibiotics.

Three isolates of *E. coli* out of the 5 isolates showed a resistance to Ciprofloxacin and 2 isolates were resist to Cefotaxime and Trimethoprim/Sulfamethoxazole (Figure (4-10). In contrast, all isolates of *K. pneumonia*, and *Enterobacter* spp. were not revealed antinotics resistance.

The present study is consistent with the previous study achieved by Adegoke *et al.*, (2020). The results of the current study was not in agreement with several previous studies which demonstrated that coliform bacteria were revealed resistance activity to a wide range of antibiotics including ceftazidime, cefuroxime and Gentamicin (Al-Badaii & Shuhaimi-Othman 2015; Mir *et al.*, 2021& Ahmed *et al.*, 2022).

Ahmed *et al.*, (2022) stated that that (Anti resistance bacteria) ARBis presented in sources of drinking water and these results explain how crucial the role of drinking water in the spread of (antimicrobial resistance)AMR is (Ahmed *et al.*, 2022). Moreover, Nishimura *et al.*, (2021) showed that during summer season, *E. coli* exhibited a high antibiotics resistance activity (46%) and the resistance rate dropped dramatically in winter season (4%), this is somewhat similar to obtained from the present study.

In contrast, many studies revealed that such tested antibiotics including ceftriaxone and Gentamicin were not resist by coliform bacteria, which is not in agreement with the current study. (Daly *et al.*, 2020 & Daly *et al.*, 2023).

It is worth to mention that many sources of pollution, in particular animal farms, untreated sewage, and agricultural practices, can result in the development of antibiotic-resistant and resistance of bacterial isolates to antibiotics increased

whenever the water quality indicators decreased (Al-Badaii & Shuhaimi-Othman., 2015).

4-8-Molecular detection of genes encoded for the antibiotic resistance in coliform bacteria isolated from the urine samples.

Polymerase chain reaction or PCR is made easier to identify the specific pathogen causing the UTIs, choose the best medications for treating the infection, and assess the bacteria's resistance to various antibiotics. Although, quantitative PCR is used for the measures the quantity of pathogen present, qualitative PCR determines if the pathogen is present or not (Zhao *et al.*, 2024).

PCR method was used in the current study to detect the presence of tested genes in the isolates of coliform bacteria from the urine samples.

Figure (4-11) shows the electrophoresis of PCR products, which showed that primers for genes were, amplified these genes through the appearance of a band in size of (588bp, 392bp and 467bp For *CTX-M*, *SHVbla* and *TEMbla*, respectively).

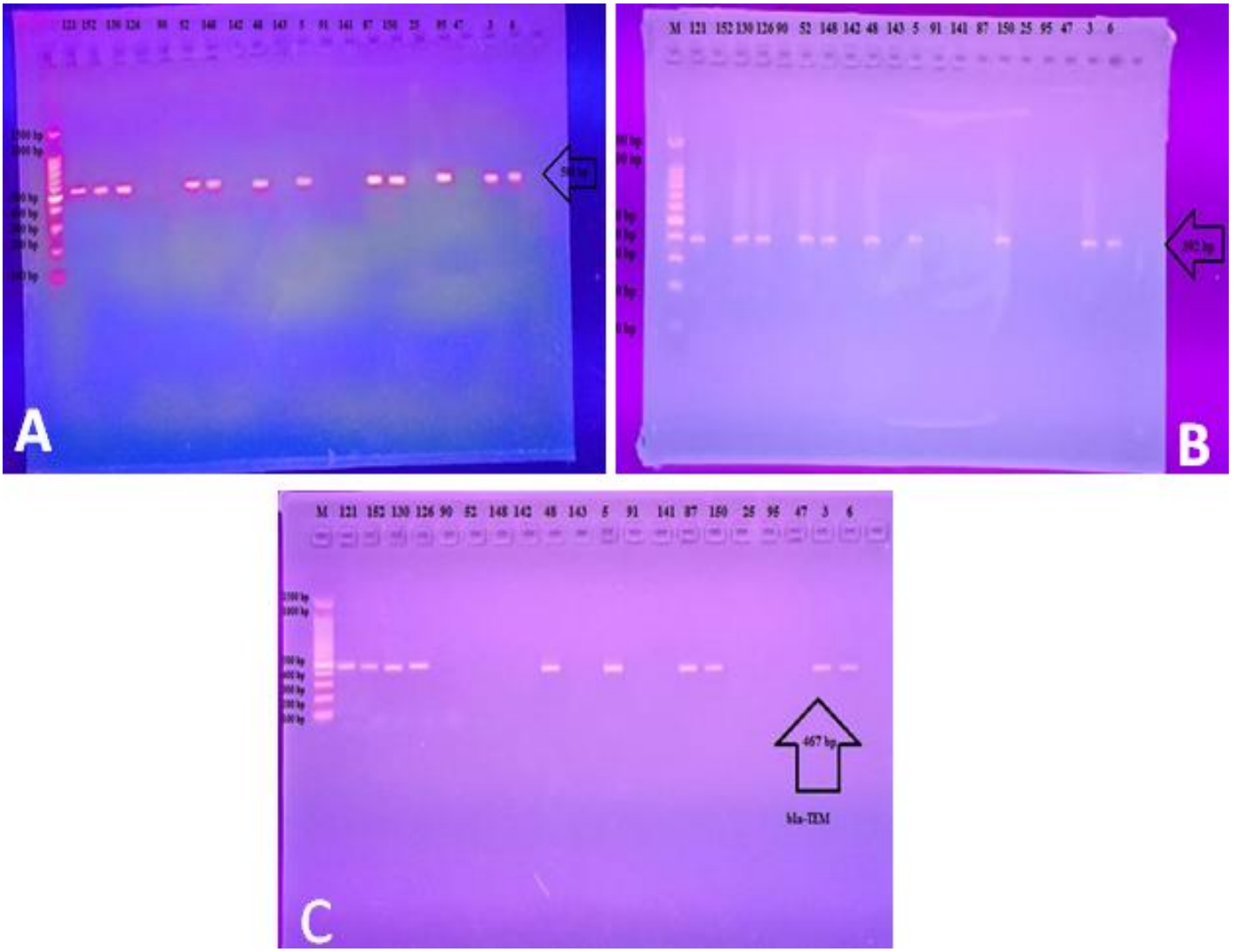

Figure 4-11 Electrophoresis of PCR products using primers specific for the genes at gel concentration of (1.5) and voltage (100v) for (30) min. Sample codes, A- *CTX-M*, B- *SHVbla*, and C-*TEMbla.*

The results of *CTX-M* gene, which amplified from the bacterial isolates of urine samples, are presented in Figure 4-11. The distribution of this gene on bacterial coliform was 5 isolates of *E .coli*, 3 isolates of *K. pneumonia* and one isolate of *Enterobacter* spp. Indeed, a significant differences were found between the number of *E .coli* and *Enterobacter* spp isolates Figure 4-12 ($P \leq 0.05$).

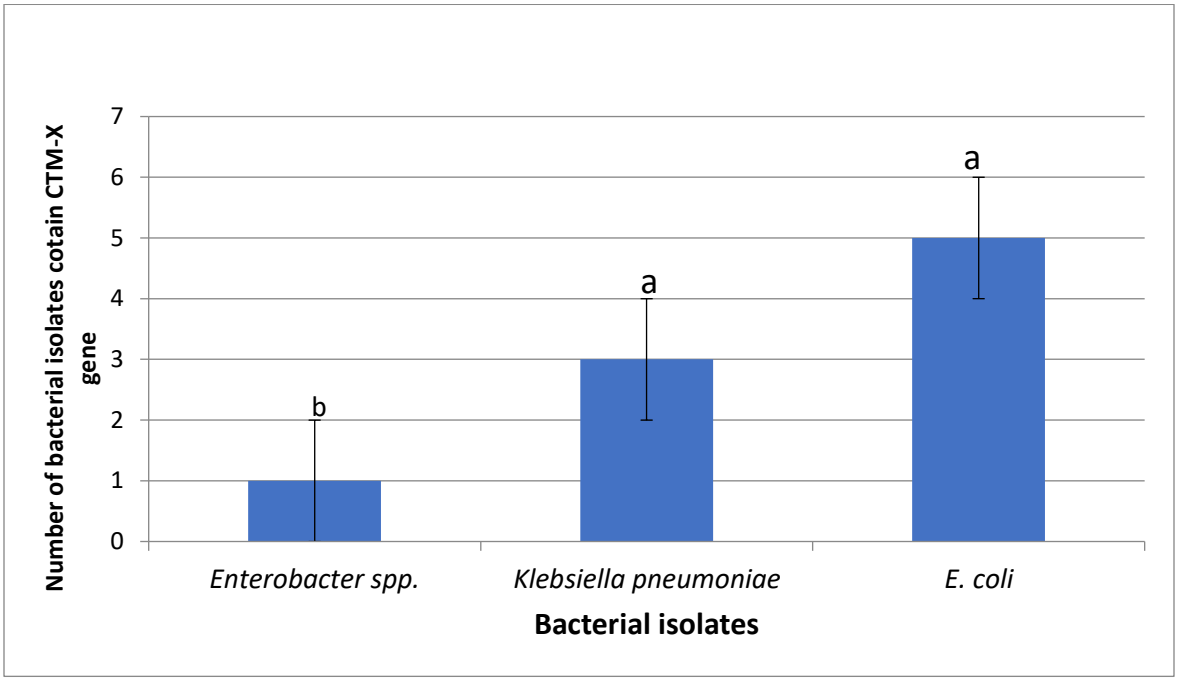

Figure 4-12 Number of bacterial coliform isolated identified from the urine samples carried *CTX-M* gene. Columns having different letters reflect a significant differences.

The present data are consistent with the observations of study conducted by Aziz *et al.*, (2015). The Enterobacter spp. isolate in the present study carried the *CTX-M* gene only and the rest of tested genes were not shown in this microbes. The present data is not in agreement with the study witch performed by David-Regli *et al.*,(2020),which demonstrated that Enterobacter spp. carried genes such as *CTX-M*,bla *SHV*, and bla *TEM* as shown infigure (4-12)

The number of coliform bacteria carried *SHV* bla gene are shown in Figure 4-13 and Table 4-2. The presence of this gene was significantly higher in *E. coli* (5 isolates out of 5) in comparison to the 2 isolates of *K. pneumoniae*out of 5 Figure 4-13 ($P \leq 0.05$). No isolate of *Enterobacter* was found to have *SHV*bla gene.

Table 4-2 Number of bacterial coliform isolated from the urine samples carried different genes encoded for antibiotic resistance.

Bacterial spp. / **Gene type**	***E. Coli***	***K. pneumonia***	***Enterbacter* spp.**
***SHV* bla**	5	2	_
***TEM* bla**	5	2	_
CTX-M	5	3	1
qur-A	_	_	_
***Aac*(6) Ib**	_	_	_

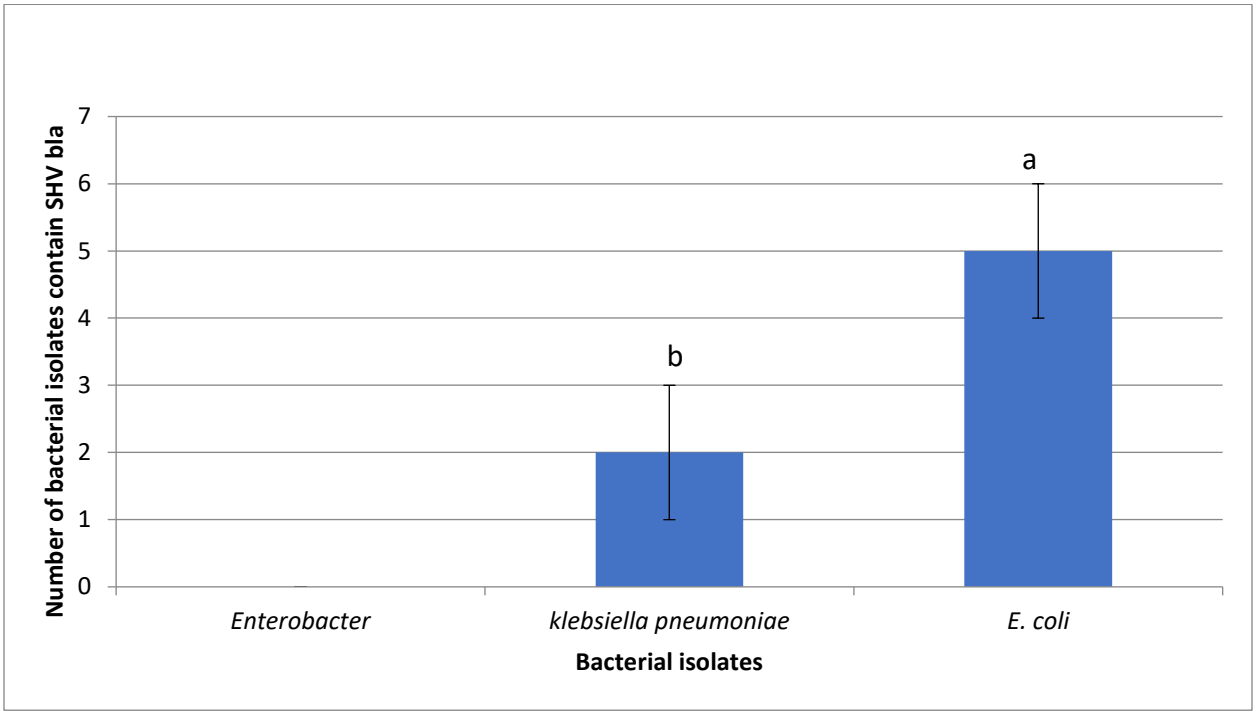

Figure (4-13) Number of bacterial coliform isolates of the urine samples carried *SHV*bla gene. Columns having different letters are significantly different ($P \leq 0.05$).

Literature has attributed the highest resistance for the presence of the *CTX-M* gene, as well as the *TEM*bla and *SHV*bla genes, which contributed to ESBL resistance in the region and around the world. Gene that encoded for the spreading of CTX-M enzymes and may result in more horizontal transmission in healthcare settings (Eltai *et al.*, 2018).

The number of coliform bacteria carried *TEM*bla ;gene are shown in Figure 4-14 and Table 4-2.

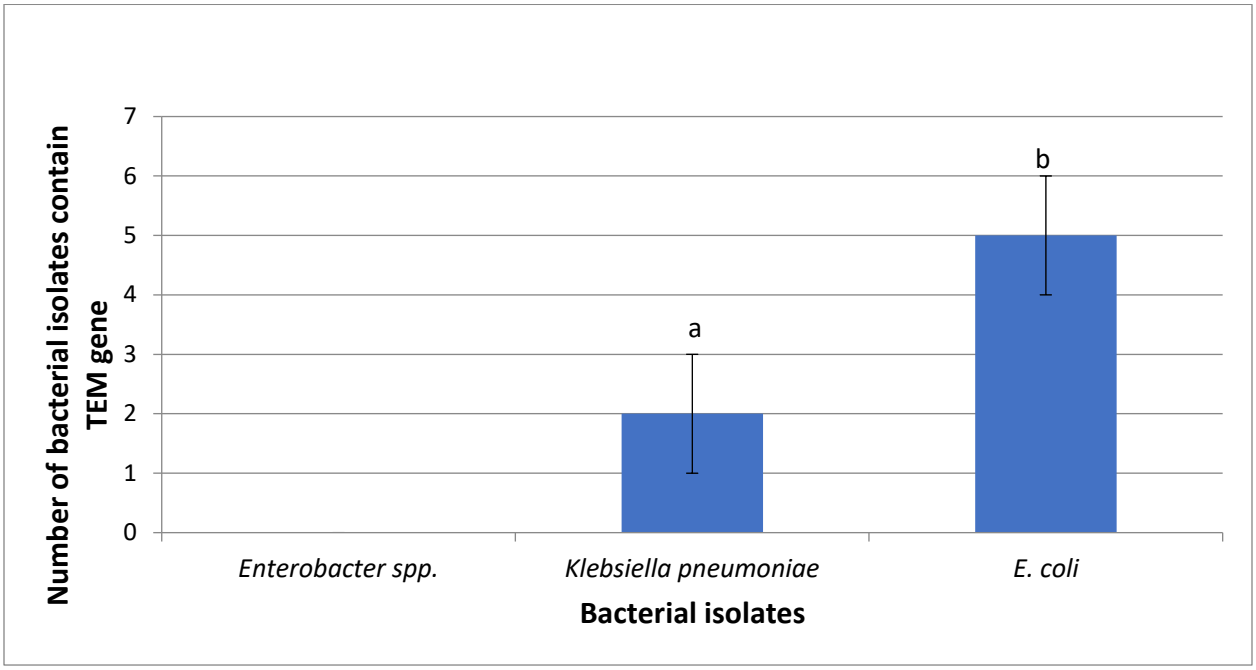

Figure (4-14) Number of bacterial coliform isolates of the urine samples carried *TEM*bla gene. Columns having different letters are significantly different.

The same tendency of *SHV*bla results was repeated with results of *TEM*bla. Five isolates of *E. coli* were found to have *TEM*bla gene compared to the 2 isolates of *K. pneumonia* with significant differences ($P \leq 0.05$) Figure 14-4. No isolate of *Enterobacter* spp. was found to have SHV gene.

The reason for this result could be children's high frequency of bla *CTX-MG1* gene which suggests that the plasmid-carried gene helps distribute *CTX-M* enzymes across the community and may result in further horizontal transmission in healthcare facilities (Eltai *et al.*, 2018).

4-9-Molecular detection of genes encoded for the antibiotic resistance in coliform bacteria isolated from Al-Hussainiya river water.

Figure 4-15 shows the electrophoresis of PCR products, which showed that primers for genes were, amplified these genes through the appearance of a band in size of (588bp, 392bp and 467bp For *CTX-M*, *SHV*bla and *TEM*bla, respectively).

The results of electrophoresis of PCR products, which showed the ability of primers used for amplification of genes by the appearance of a band in size of (516bp and 4826bp for qnr and aac, respectively) are presented in Figure 4-15.

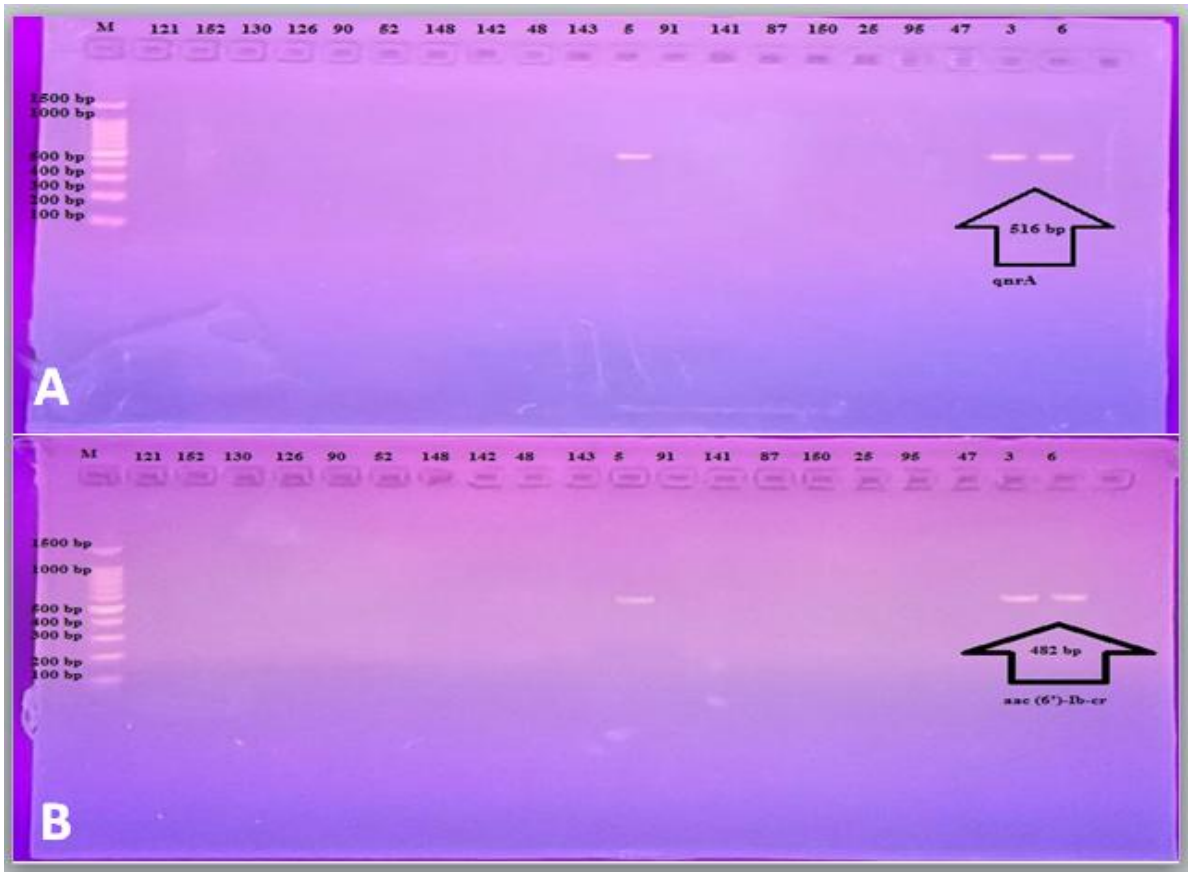

Figure 4-15 Electrophoresis of PCR products using primers specific for the genes at gel concentration of (1.5) and(100v) voltage for (30) min. Sample codes, A-*qnr-A*, B- *Aac(6)Ib*.

It is demonstrated that from the present results only 3 isolates of *E .coli* out of 5 carried all the tested genes Figure 4-16. These results lack statistical significance ($P \geq 0.05$). On the other hand, no isolates of other genera including *K. pneumoniae* and *Enterobacter* spp. were found to have any tested genes Table 4-3.

Table 4-3 Number of bacterial coliform isolated from river water samples during winter season carry different genes encoded for antibiotic resistance.

coliform bacteria / **Gene type**	***E. coli***	***K. pneumoniae***	***Enterobacter* spp.**
***SHV* bla**	3	_	_
***TEM* bla**	3	_	_
CTX-M	3	_	_
qur-A	3	_	_
Aac(6) Ib	3	_	_

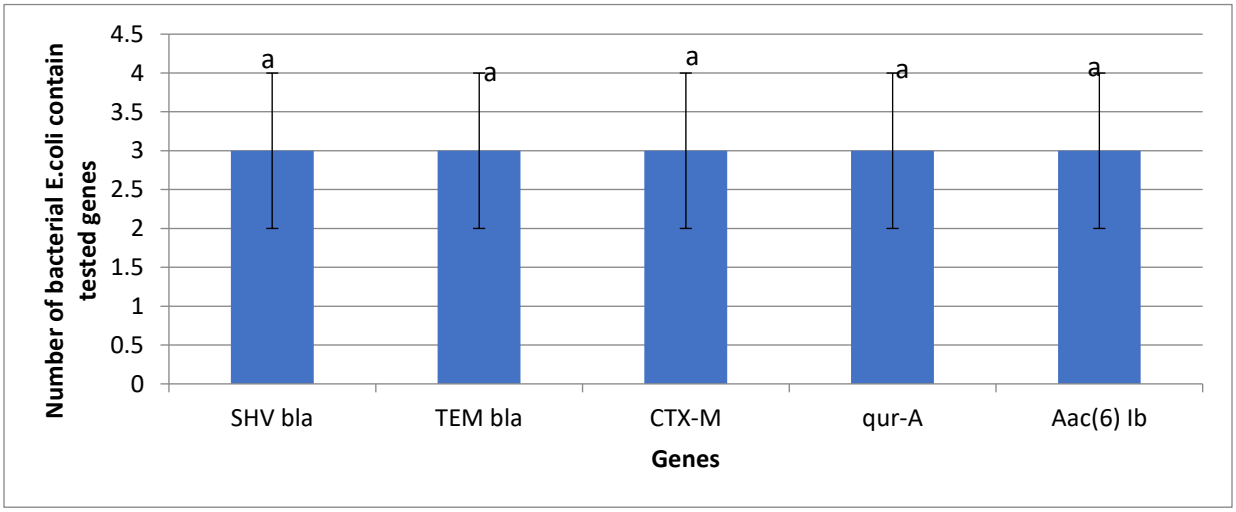

Figure 4-16 Number of bacterial coliform isolated from Al-Hussainiya river water during winter reason carrired *SHV* bla, *TEM* bla, *CTX-M*, *qnr-A* and *acc (6) Ib* genes. Columns having same letters exhibited no significant differences.

The *qnr-A* gene was not appear in *K. pneumoniae*, and Enterobacter spp. isolates, and these results are not in agreement with study of Betitra *et al.*, (2014). In other study conducted by YANDAI *et al.*, (2019) demonstrated that two strains of tested bacteria appeared to have the *qnrS* and *qnrB* genes which is similar to the present study.

Supporting the present data, *Talukdar et al., (*2013*)* stated that all isolates of *E .coli* had increased rates of resistance to the antibiotics b-lactam, Quinone, and fluoroquinolone. It is widely accepted that quinolone resistance gene (*qnr*) mediated by plasmids which has been found in several member of Enterobacteriaceae family including , *E. coli*.

This may be because of the residual effects of antibiotics , which have been widely employed in the human population and the food chain, producing a selective antibiotic pressure in the environment.

Furthermore, confirming to these findings Singh *et al.*, (2022) pointed out that *E. coli* isolated from global water bodies exhibited a high frequency of type *qnrS* gene.

The multi-drug resistance (MDR) *E. coli* could be a significant source of genetic antibiotic resistance determinants that are easily transmitted by horizontal gene transfer (HGT) to other environmental bacteria, including possible human diseases proven by the researcher(Singh *et al.*, 2022 a)

It was proven by Singh *et al.*, (2022 a) that the wastewater permits Antimicrobial-resistant bacteria (ARBs) with Antibiotic resistance genes (ARGs) to survive and spread the antiresistance genes(ARGs)U through many pathways; it continues to be a significant source of (antimicrobial resistance) AMR in the environment.

E. coli are unique among Enterobacteriaceae members in that they may transfer and receive antimicrobial resistance (AMR) genes (Singh *et al.*, 2022b). Consequently, it is recognized as a significant source of antimicrobial resistance(AMR) genes that can spread horizontally by gene transfer to other bacteria. Indeed, antimicrobial resistance (AMR) in *E. coli* are deemed as one of the world's worst problems for both humans and animals.

In contrast to the present results, *CTX-M*, *TEM*bla, and *SHV*bla genes were reported to carry on *Klebsiella* spp. (Sivaraman *et al.*, 2021). Additionally, *CTX-M* gene was existed in *Enterobacter* spp. isolates which is not in agreement to the present study.

The PCR method enhanced specificity, sensitivity, simplicity, and reduced time. The results are available in 24–48 h detecting specific pathogenic bacteria in the drinking water from sources such as groundwater, commercially bottled water, tap water, residential water tanks (Abada *et al.*, 2019).

Antibiotic or antimicrobial resistance in bacteria is a naturally occurring process and it is accelerating due to antibiotic abuse in both people and animals. Like all living things, bacteria multiply normally under certain conditions and can experience mutations that lead to resistance to specific antibiotics.

Antibiotic misuse that aims to eradicate bacteria puts additional selective pressure on bacteria, killing those susceptible to the drug while providing resistant bacteria with little opposition to live and grow. Natural selection encourages resistant bacteria and permits their growth and dissemination (Garcia., et al 2024).

4-10-Determine the ability of the tested coliform bacteria to produce biofilm.

biofilm is a complex structure made up of bacterial colonies wrapped in a polymer matrix comprising proteins, extracellular DNA, and polysaccharides. According to Olivares *et al.* (2013), bacteria in biofilms have up to 1000 times the antibiotic resistance of planktonic (free-swimming) bacteria. According to Arzanlou *et al.* (2017), the biofilm mode is the primary cause of chronic and device-related infections, while the planktonic form is indicative of acute infections.

The results of biofilm formation for coliform bacteria isolated from the urine samples revealed that all isolates of *E. coli* were able to produce biofilm which distributed as (42%) 5 out of 12 isolate were strong biofilm former and resistant to antibiotics and 7 isolates (58%) were moderate biofilm former (Table 4-4), this result is in agreement with the study performed by ZamaniI and Salehzadeh, (2018). Additionally, 3 (100%) isolates of *E.coli* which were sensitive to antibiotics and showed moderate biofilm formation and this result was supported by the study of Sudheendra & Basavaraj (2018).

Furthermore, all isolates of *Klebsiella* spp. (100 %) were strong biofilm formation and releated resistance for antibiotics, this result is consistence with Diriba *et al.*, (2020); AL-Fridawy *et. al*., (2020).

Table 4-4 Ability of tested coliform bacteria isolated from the urine samples to produce biofilm.

N	Isolates	Resistance	Sensitivity	Biofilm
1	***E. coli***	**R**	**_**	**moderate**
2	***E. coli***	**R**	**_**	**strong**
3	***E. coli***	**R**	**_**	**moderate**
4	***E. coli***	**R**	**_**	**moderate**
5	***E. coli***	**R**	**_**	**strong**
6	***E. coli***	**R**	**_**	**moderate**
7	***E. coli***	**R**	**_**	**moderate**
8	***E. coli***	**R**	**_**	**moderate**
9	***E. coli***	**R**	**_**	**moderate**
10	***E. coli***	**R**	**_**	**strong**
11	***E. coli***	**R**	**_**	**strong**
12	***E. coli***	**R**	**_**	**strong**
13	***E. coli***	**R**	**_**	**moderate**
14	***E. coli***	**R**	**_**	**strong**
15	***E. coli***	**R**	**_**	**strong**
16	***K. pneumoniae***	**R**	**_**	**strong**
17	***K. pneumoniae***	**R**	**_**	**strong**
18	***K. pneumoniae***	**R**	**_**	**strong**
19	***K. pneumoniae***	**R**	**_**	**strong**
20	***Enterobacter spp.***	**R**	**_**	**strong**

The results of coliform bacteria for biofilm production isolated from Al-Hussainiya river during summer season are illustrated in Table 4-5. The results showed that 2 (40%) isolates of *E. coli* were strong biofilm formation and sensitive for antibiotics

and 3 (60%) isolates with moderate biofilm formation. In agreement with the present results Castillo *et. al*., (2023) who pointed out multiple antibiotic treatments have not been shown to eradicate uropathogenicEscherichia coli(UPEC) biofilms. The virulence factors facilitate bacterial colonization and aid the organism in defeating the host defense mechanism. In addition, they allow organism to enter the urinary tract quite quickly. The Gram-negative bacteria *Escherichia coli, Klebsiella* spp., *Enterobacter*, *proteus*, and *pseudomonas* are frequently responsible for UTIs; as a result, *E. coli* are a significant organism in both community and hospital settings (Kottila & Hena., 2024).

Table 4-5 Ability of the tested coliform bacteria isolated from Al-Hussainiya river during the summer season to produce biofilm.

n	Isolates	Resistance	Sensitivity	Biofilm
1	*E. coli*	_	S	strong
2	*E. coli*	_	S	strong
3	*E. coli*	_	S	moderate
4	*E. coli*	_	S	moderate
5	*E. coli*	_	S	moderate
6	*K.pneumoniae*	_	S	moderate
7	*K.pneumoniae*	_	S	moderate
8	*K.pneumoniae*	_	S	strong
9	*Enterobacterspp*	_	S	strong
10.	*Enterobacterspp.*	_	S	moderate

The results of the present study demonstrated that 1 (33%) isolate of *K. pneumonia* were strong biofilm forming and 2 (67%) isolate were moderately biofilm forming and sensitive to antibiotic Table (4-5) which are not consistent with the results of the study Araujo *et al.*, (2024)& Nirwati *et al.*, (2019). . These results are not in agreement with study of Confirm Biofilm formation and antibiotic susceptibility do not statistically significantly correlate; while biofilm production makes bacteria more susceptible to antibiotics, drug resistance depends on a variety of other factors, including the presence of effusion pumps, degrading enzymes, and modifications in the site of action (Mahmoud *et al.*, 2024). In addition, 1 isolate (50%) of *Enterobacter* spp. was a strong biofilm former and the other isolate was moderately biofilm former and all isolates were sensitive for antibiotic, these data are not in agreement with the study conducted by Capita *et al* ., (2020).

The results of coliform bacteria for biofilm production isolated from Al-Hussainiya River during winter season are presented in Table 4-6.

Table 4-6 Ability of the tested coliform bacteria isolated from Al-Hussainiya river during the winter season to produce biofilm**.**

n	Isolates	Resistance	Sensitivity	Biofilm
1	*E.coli*	_	S	Strong
2	*E.coli*	_	S	Strong
3	*E.coli*	R		Strong
4	*E.coli*	R		Strong
5	*E.coli*	R		Strong
6	*K. pneumoniae*	_	S	Strong
7	*K. pneumoniae*	_	S	Strong
8	*Enterobacter* spp.	_	S	Strong
9	*Enterobacter* spp.		S	Strong
10	*Enterobacter* spp.		S	Moderate

As presented in Table 4-6 isolates of *E. coli* were strong biofilm producers and three of which were resistance to antibiotics and the reset were sensitive for antibiotics. The present results are not similar to what was found by Taj bakhsh *et al.*, (2016) who pointed out that multiple antibiotic treatments have not been shown to eradicate uropathogenic Escherichia coli(UPEC) biofilms. In addition, the cells in a biofilm can transfer genes encoding for antibiotic resistance horizontally more easily when they are close to one another, which increases the spread of antibiotic resistance and virulence traits. Biofilm-forming bacteria are still proliferating in waterways, raising the possibility of an epidemic (Koskeroglu *et. al.*, 2023). In addition, two isolates of ***K. pneumoniae*** were only tested for their ability to produce biofilm and were appeared to be strong biofilm producer and were sensitive to antibiotics which is in agreement to the study of Araujo *et al.*, (2024). Additionally, 2 isolates of *Enterobacter* spp. during winter season were strong formation biofilm and sensitive to the antibiotics and the other one was moderate biofilm formation and sensitive to the antibiotics which is not similar to the study of Koskeroglu *et al.*, (2023).

Bacterial aggregation known as biofilms are affixed to extracellular matrix consisting of proteins, nucleic acids, polymers, polysaccharides, and enzymes. Antibiotic resistance in the biofilm-forming strain can result in the development of widespread multidrug resistance, completely drug-resistant bacteria. Biofilms may play a role in increasing bacterial resistance to antibiotics as stated Al-Janabi *et. al*., (2018) who statated that the ability of the organism to overcome the host's innate and acquired immune defense mechanisms as well as to resist antimicrobial therapy is facilitated by the creation of biofilms and research revealed that the primary factors influencing chronic infection were biofilm formation.

Conclusions & Recommendation

Conclusions :

1-In the present study, *E. coli* are deemed as the most common causative agents of the UTIs among coliform bacteria.

2-Coliform bacteria isolated from UTIs infected children with revealed antibiotics resistance activity is connected with ability of these bacteria to produce biofilm.

3-Isolation of coliform isolates from the river water hint to the possibility of transferring these isolates to the people in different ways if the water treatment is not enough.

4-Variation of encoding genes to the antibiotics resistance in coliform bacteria isolated from urine and water samples indicated for ability of these bacteria to resist different types of antibiotics.

Recommendation

1-It is preferable to use molecular methods such as (cloning and denaturation gradient gel electrophoresis DGGE) to target urine samples directly.

2-For detection the source of contamination in water (Human or/and animals), other bacterial indicators should be used example *Streptococcus* spp.

3-More water bodies should be covered in ordered to obtain wide understanding about existence of coliform bacteria

References:

References :

Abada E, Al-Fifi Z, Al-Rajab AJ, Mahdhi M, Sharma M. (2019). Molecular identification of biological contaminants in different drinking water resources of the Jazan region, Saudi Arabia. *J Water Health* 17(4):622-632.

Abbas Y A and Radhi G F (2016) Rapid identification of Enterobacter spp. Isolated from hospitals in Basrah Province By Automated Szstem (VITEK 2) international *journal of microbiology Genetics and monocular Biology Research*. vol 29no.opp ,9-20.

Abd Elhameed A. O., Khalie S. A. l, Torky H. A..(2023) . Characterization of Klebsiella pneumoniae isolated from Contaminated Sheep and Goat Meat at Matrouh Governorate and Its Antibiotic Resistance. *AJVS*., 79 (1): 37-43

Abdallah M S, Mustapha T ,Gambo,A and Ishaq ,S.,(2016) Biochemical Identification and cultural characterization of some gram –negative bacteria obtained from fecal DiarrHOEal samples cibtech *journal of microbiology* (ssn 2314-3867)vol (5)(1) pp ,31-34

Abdi S, Ranjbar R, Hakemi Vala M, Jonaidi N, Baghery Bejestany O. (2014): Frequency of bla TEM, bla SHV, bla CTX-M, and qnrA Among Escherichia coli Isolated From Urinary Tract Infection. *Arch Clin Infect* Dis.;9(1):18690. https://doi.org/10.5812/archcid.18690**.**

Abdulameer AJ and Abdulla DE(2022) Prevalence of Urinary Tract Infection and its Related Factors in a Sample of School Age Children from Baghdad/ Iraq, 7(2): 000167.

Abuhandan M, Güzel B, Oymak Y, Çiftçi H.(2013) Antibiotic sensitivity and resistance in children with urinary tract infection in Sanliurfa. *Turk J Urol.* Jun;39(2):106-10.

Adegoke, A.A., Madu, C.E., Aiyegoro, O.A. *et al.* (2020) Antibiogram and beta-lactamase genes among cefotaxime resistant E. coli from wastewater treatment plant. *Antimicrob Resist Infect Control* 9, 46.

Adeoye, T., Folorunso, V. T., Banjo, A. O., Akinlolu, M. A., Akintomide, R. A., Adeleke, B. S., &Teniola, O. D. (2020). isolation and screening of antibiotic

producing bacillus species from soil samples in okitipupa, nigeria. *journal of the faculty of science*, 2(2).

Agegnehu A, Worku M, Nigussie D, Lulu B, Tadesse BT(2020). Pediatric Febrile Urinary Tract Infection Caused by ESBL Producing *Enterobacteriaceae* Species. *Biomed Res Int.* Dec 2;2020:6679029.

Ahmed ,N.; Ali ,Z.; Riaz ,M.; Zeshan ,B.; Wattoo ,J.I.; Aslam ,M.N.(2020). Evaluation of Antibiotic Resistance and Virulence Genes among Clinical Isolates of Pseudomonas aeruginosa from Cancer Patients. *Asian Pac J Cancer Prev*. 1;21(5):1333-1338.

Ahmed H, Zolfo M, Williams A, Ashubwe-Jalemba J, Tweya H, Adeapena W, Labi AK, Adomako LAB, Addico GND, Banu RA, Akrong MO, Quarcoo G, Borbor S, Osei-Atweneboana MY.(2022) Antibiotic-Resistant Bacteria in Drinking Water from the Greater Accra Region, Ghana: A Cross-Sectional Study, December 2021-March 2022. *Int J Environ Res Public Health*. 28;19(19):12300.

Akter T, Shahriar A, Alo M, Rahman T, Emran TB (2020). Survival assessment of pathogenic bacteria with antibiotic resistance traits from fresh summer royal grape: In vitro microbial challenge test. *J Microbiol Biotechnol Food Sci*;10:344–9, http://dx.doi.org/10.15414/jmbfs.2020.10.3.344-349.

Al-Abdan, M. A., Bin-Jumah, M. N., Ali, D., & Alarifi, S. (2021). Investigation of biological accumulation and eco-genotoxicity of bismuth oxide nanoparticle in fresh water snail *Lymnaea luteola*. *Journal of King Saud University - Science*, *33*(2), 101355.

Al-Badaii F· Shuhaimi-Othman M .(2015)Water Pollution and its Impact on the Prevalence of Antibiotic-Resistant E. coli and Total Coliform Bacteria: A Study of the Semenyih River, Peninsular Malaysia *Water Qual Expo Health* 7:319–330.

AL-Fridawy,R .A. K ., Al-Daraghi,W A H , Alkhafaji M H(2020)Isolation and Identification of Multidrug Resistance Among Clinical and Environmental Pseudomonas aeruginosa Isolates. *Iraqi Journal of Biotechnology*Vol 19 No 2

AL-Janabi, R.S,Ali,A.J.Hussein H .H (2018) ;Immunological and molecular study of staphylococcus epidermies isolated from Devices associated in karbala proviance Phd thes is 70 pp.

Allen, H. K., Donato, J., Wang, H. H., Cloud-Hansen, K. A., Davies, J., &Handelsman, J. (2010). Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology*, 8(4), 251-259.

Ara R, Mohammad Nasrullah S, Tasnim Z, Afrin S, Saif-Ur-Rahman KM, Hawlader MDH.(2022) Effective antimicrobial therapies of urinary tract infection among children in low-income and middle-income countries: protocol for a systematic review and meta-analysis. *BMJ Open*. Apr 12;12(4): e060568.

Araújo, S.; Silva, V.; de Lurdes Enes Dapkevicius, M.; Pereira, J.E.; Martins, Â.; Igrejas, G.; Poeta, P. (2024)Comprehensive Profiling of Klebsiella in Surface Waters from Northern Portugal: Understanding Patterns in Prevalence, Antibiotic Resistance, and Biofilm Formation. *Water* 16, 1297.

Arzanlou, M.; Chai, W.C(2017). Venter, H. Intrinsic, adaptive and acquired antimicrobial resistance in Gram-negative bacteria. *Essays Biochem*., 61, 49–59

Auguet, O., Pijuan, M., Borrego, C. M., Rodriguez-mozaz, S., Triadó-margarit, X., Varela, S., *et al.* (2017). Sewers as potential reservoirs of antibiotic resistance. *Sci. Total Environ.* 605–606, 1047–1054. doi: 10.1016/j.scitotenv.2017.06.153

Ayatollahi J, Fotouhi F , Akhondimeybodi Z, Hamidfar M , Hossein S.(2022) Determination of antibiotic resistance of E. coli isolated from urine culture samples,*The Journal of Medical Research*; 8(5):162-164.

Ayatollahia J , Sharifyazdib M , Fadakarfardb R, Hossein Shahcheraghi S(2020) Antibiotic resistance pattern of Klebsiella pneumonia in obtained samples from Ziaee Hospital of Ardakan, Yazd, Iran during 2016 to 2017 . *Iberoamerican journal of medicine* 32-36.

Aziz Z S. J A S and Addoos S A ,(2015)Identification of ESBL CTX-M-15 genes from isolates of urinary tract infections. *Magazin of Al-Kufa University for Biology* / VOL.7/ NO. ISSN: 2311-6544.

Bakaj A, DalipajX(2020) Assessment of Microbiological Indicators and Physic-chemical Parameters of Orikumi Lagoon, Vlora, Albania Conference Paper · February t: https://www.researchgate.net/publication/368247425.

Bako, E., Kagambèga, A., Traore, K. A., Bagre, T. S., Ibrahim, H. B., Bouda, S. C., Bonkoungo, I. J. O., Kabore, S., Zongo, C., Traore, A. S., & Barro, N. (2017). Characterization of diarrheagenic Escherichia coli isolated in organic waste products (cattle fecal matter, manure and slurry) from cattle's markets in Ouagadougou, Burkina Faso. *International Journal of Environmental Research and Public Health*, *14*(10), 1100.

Barola S, Grossman OK, Abdelhalim A. Urinary Tract Infections In Children. (2024). Jan 11. In: StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; *PMID*: 38261676.

Basaqvaraju M and Gunashree B S (2022). Escherichia coli :An Overview of Main characteristics Dol :10.5772/intechopen : 105508.

Bashir I, Lone FA, Bhat RA, Mir SA, Dar ZA, Dar SA(2020). Concerns and threats of contamination on aquatic ecosystems. *Bioremed Biotechnol*; 27:1-26. DOI: https://doi.org/10.1007/978-3-030-35691-0_1.

Belete, M.A and Saravanan, M. A.(2020). Systematic Review on Drug Resistant Urinary Tract Infection Among Pregnant Women in Developing Countries in Africa and Asia; 2005-2016" . *Infect Drug Resist*, vol. 18(13),pp.1465-1477.

Betitra Y, Teresa V, Miguel V, Abdelaziz T.(2014) Determinants of quinolone resistance in Escherichia coli causing community-acquired urinary tract infection in Bejaia, Algeria. *Asian Pac J Trop Med*.;7(6):462-7.

Bevan, E.R.; Jones, A.M.; Hawkey, P.M(2017). Global epidemiology of CTX-M β-lactamases: Temporal and geographical shifts in genotype. *J. Antimicrob. Chemother. 72*, 2145–2155. (**Google Scholar**) (**CrossRef**)(**Green Version**).

Bhattacharjee MK(2016). Antibiotics that inhibit nucleic acid synthesis. In: Chemistry of antibiotics and related drugs. *Springer International Publishing*;. p. 109–28, http://dx.doi.org/10.1007/978-3-319-40746-3 5.

Bird ,P., Bastin ,B., Klass ,N., Crowley ,E., Agin ,J., Goins ,D., Bakken, H., Lingle ,C. and Schumacher ,A .(2021) Evaluation of the 3MTM PetrifilmTM Rapid E. coli/ Coliform Count Plate for the Enumeration of E. coli and Coliforms: Collaborative Study, First Action:13 J. *AOAC Int.* 103 513–22.

Bodendoerfer, E.; Marchesi, M.; Imkamp, F.; Courvalin, P.; Böttger, E.C.; Mancini, S.(2020). Co-occurrence of aminoglycoside and β-lactam resistance mechanisms in aminoglycoside-non-susceptible Escherichia coli isolated in the Zurich area, Switzerland. *Int. J. Antimicrob*. Agents, 56, 106019. (CrossRef).

Bonkat, G.; Bartoletti, R.; Bruyère, F.; Cai, T.; Geerlings, S.E.; Köves, B.; Schubert, S.; Pilatz, A.; Veeratterapillay, R.; Wagenlehner, F.(**2023)**. EAU Guidelines on Urological Infections. *Eur. Assoc. Urol. 182*, 237–257. (**Google Scholar**).

Bonnet R. (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother* 48:1–14.

Branger, C.; Ledda, A.; Billard-Pomares, T.; Doublet, B.; Fouteau, S.; Barbe, V.; Roche, D.; Cruveiller, S.; Médigue, C.; Castellanos, M.; *et al.* (**2018)**. Extended-spectrum β-lactamase-encoding genes are spreading on a wide range of Escherichia coli plasmids existing prior to the use of third-generation cephalosporins. *Microb. Genom. , 4,* e000203. (**Google Scholar**) (**CrossRef**).

Brauner, A.; Fridman, O.; Gefen, O.; Balaban, N.Q(2016). Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nat. Rev. Microbiol*., 14, 320–330. (CrossRef) (PubMed).

Brian Veauthier, MD, and Michael V. Miller, DO (2020) Urinary Tract Infections in Young Children and Infants: Common Questions and Answers. American Family Physician.Volume 102, Number 5

Budiarso T.Y, Amarantini C , Restiani,R.(2023). Isolation and Biochemical chaeacterization of Enterobacter cloacae isolates from ready –to eat food *AIP Conference Proceedings* 2556(1).

Bush, K., and Bradford, P. A. (2020). Epidemiology of β-lactamase-producing pathogens. *Clin. Microbiol. Rev*. 33:e00047-19.

Cabral JPS, (2010). Water microbiology. Bacterial pathogens and water. *Int J Environ Res Public Health* 7: 3657–3703. (PMC free article) (PubMed) (Google Scholar).

Canton R, Gonzalez-Alba JM, Galan JC. (2012). CTX-M enzymes: origin and diffusion. *Front Microbiol* 3:110.
Carattoli, A.(2013). Plasmids and the spread of resistance. Int. J. Med. Microbiol., 303, 298–304.

Castillo F Y R, Guerrero Barrera AL, Harel J, Avelar González FJ, Vogeleer P, Arreola Guerra JM, González Gámez M.(2023). Biofilm Formation by *Escherichia coli* Isolated from Urinary Tract Infections from Aguascalientes, Mexico. *Microorganisms.* Nov 25;11(12):2858.

Chakrabarty, S. , Mishra, M. P and Bhattacharyay, D.(2022). "Targeting Microbial Bio-film: an Update on MDR Gram-Negative Bio-film Producers Causing CatheterAssociated Urinary Tract Infections". *Applied Biochemistry and Biotechnology*,.194, (6), 2796- 2830.

Chalachew Y, Melese K, Melkamu M(2020). Antimicrobial-resistance pathogen load and public health risk of drinking water in South Gondar. *Res Square*.;1:1–14.

Chao, Y.-S.; Farrah, K(2019). Fluoroquinolones for the Treatment of Urinary Tract Infection 2; *Canadian Agency for Drugs and Technologies in Health*: Ottawa, ON, Canada,.

Chigbu P, Gordon S, Tchounwou PB. (2005) The seasonality of fecal coliform bacteria pollution and its influence on closures of shellfish harvesting areas in Mississippi Sound. *Int J Environ Res Public Health*. 2(2):362-73.

Colom ,K., Pérez ,J., Alonso ,R., Fernández-Aranguiz ,A., Lariño ,E., Cisterna ,R.(2003). Simple and reliable multiplex PCR assay for detection of blaTEM, bla (SHV) and blaOXA-1 genes in *Enterobacteriaceae*. *FEMS Microbiol Lett.* 223:147–51. https://doi.org/10.1016/S0378-1097(03)00306-9.

Cox, G.; Wright, G.D.(2013). Intrinsic antibiotic resistance: Mechanisms, origins, challenges and solutions. *Int. J. Med. Microbiol*., 303, 287–292.

Czaplewski L, Bax R, Clokie M, Dawson M, Fairhead H, Fischetti VA, *et al*.(2016). Alternatives to antibiotics—a pipeline portfolio review. *Lancet Infect Dis*;16:239–51, http://dx.doi.org/10.1016/S1473-3099(15)00466-1.

D'Andrea, M. M., Arena, F., Pallecchi, L., and Rossolini, G. M. (2013). CTX-M-type β-lactamases: a successful story of antibiotic resistance. *Int. J. Med. Microbiol*. 303, 305–317. doi: 10.1016/j.ijmm.2013.02.008.

Da Cunha BR, Fonseca LP, Calado CRC(2019). Antibiotic discovery: where have we come from, where do we go? *Antibiotics*;8, http://dx.doi.org/10.3390/ antibiotics8020045.

Daly M, Powell J, O'Connell NH, Murphy L, Dunne CP.(2023) Antimicrobial Resistance Is Prevalent in *E. coli* and Other Enterobacterales Isolated from Public and Private Drinking Water Supplies in the Republic of Ireland. *Microorganisms*. 6;11(5):1224

Daniel M, Szymanik-Grzelak H, Sierdziński J, Podsiadły E, Kowalewska-Młot M, Pańczyk-Tomaszewska M.(2023) Epidemiology and Risk Factors of UTIs in Children-A Single-Center Observation. *J Pers Med*. 10;13(1):138.

David-Regli A, Lavigne JP, Pagès JM.(2019) *Enterobacter* spp.(2019) Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. *Clin Microbiol Rev*. 17;32(4)

Davies JE (2006). Aminoglycosides: ancient and modern. *J Antibiot (Tokyo)*;59:529–532. doi: 10.1038/ja.2006.73.

Davin-Regli A, Lavigne JP, Pagès JM. *Enterobacter* spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance. Clin Microbiol Rev. 2019 Jul 17;32(4):e00002-19. doi: 10.1128/CMR.00002-19. PMID: 31315895; PMCID: PMC6750132.

DiGiandomenico A, Sellman BR(2015). Antibacterial monoclonal antibodies: the next generation? *Curr Opin Microbiol*;27:78–85, http://dx.doi.org/10.1016/j.mib.2015.07.014.

Dinos GP, Athanassopoulos CM, Missiri DA, Giannopoulou PC, Vlachogiannis IA, Papadopoulos GE, *et al.*(2016). Chloramphenicol derivatives as antibacterial and anticancer agents: historic problems and current solutions. *Antibiotics*;5, http://dx.doi.org/10.3390/antibiotics5020020.

Diriba K, Kassa T, Alemu Y, Bekele S.(2020). In Vitro Biofilm Formation and Antibiotic Susceptibility Patterns of Bacteria from Suspected External Eye Infected Patients Attending Ophthalmology Clinic, Southwest Ethiopia. *Int J Microbiol*. 18;2020:8472395.

Dodds DR.(2017). Antibiotic resistance: a current epilogue. *Biochem Pharmacol*;134:139–46, http://dx.doi.org/10.1016/j.bcp.2016.12.005.

Dorado-García, A., Smid, J. H., van Pelt, W., Bonten, M. J. M., Fluit, A. C., van Den Bunt, G., Wagenaar, J. A., Hordijk, J., Dierikx, C. M., Veldman, K. T., de Koeijer, A., Dohmen, W., Schmitt, H., Liakopoulos, A., Pacholewicz, E., Lam, T. J. G. M., Velthuis, A. G., Heuvelink, A., Gonggrijp, M. A., van Duijkeren, E., van Hoek, A. H. A. M., & Husman, A. M. R. (2018). Molecular relatedness of

ESBL/AmpC-producing Escherichia coli from humans, animals, food and the environment: A pooled analysis. *The Journal of Antimicrobial Chemotherapy*, 73(2), 339-347. http://dx.doi.org/10.1093/jac/dkx397. PMid:29165596 .

Drawz SM, Papp-Wallace KM, Bonomo RA. (2014). New beta-lactamase inhibitors: a therapeutic renaissance in an MDR world. *Antimicrob Agents Chemother* 58:1835–1846.

Duicu C, Cozea I, Delean D, Aldea AA, Aldea C.(2021). Antibiotic resistance patterns of urinary tract pathogens in children from Central Romania. *Exp Ther Med*.;22(1):748.

ECDC (2020). Antimicrobial consumption in the EU/EEA – annual epidemiological report (2019). Stockholm: ECDC.

Elnagar W., Habib E., Shokralla S., Barwa R., Abdel Galil K. & Abdulmageed E. (2010). Phenotypic and genotypic characterization of Extended Spectrum Beta-lactamases produced by Enterobacteriaceae. *The Egyptian J. Med. Microbiol*.. 19: 59–66.

Eltai N O , Al ThaniA A, Al-Ansari K , Deshmukh A S , Wehedy E, Al-Hadidi S H and Yassine H M,(2018). Molecular characterization of extended spectrum β - lactamases enterobacteriaceae causing lower urinary tract infection among pediatric population ,Antimicrobial Resistance and Infection Control ,7:90

Esmaeel, N.E.; Gerges, M.A.; Hosny, T.A.; Ali, A.R.; Gebriel, M.G(**2020)**. Detection of Chromosomal and Plasmid-Mediated Quinolone Resistance Among *Escherichia coli* Isolated from Urinary Tract Infection Cases; Zagazig University Hospitals, Egypt. *Infect. Drug Resist.* , *13*, 413–421.

FAO Fishstat J(2016): Global Fishery and Aquaculture Production Statistics. Rome: FAO.

Falco Restrepoa A. D., Velásquez Nieves M. A. , Takiff H.(2017). Molecular characterization of KPC-producing Klebsiella pneumoniae isolated from patients in

a Public Hospital in Caracas, Venezuela. *Enferm Infecc Microbiol Clin*.;35(7):411–416.

Fatahi-Bafghi, Mehdi. (2017). "Phenotypic and molecular identification of nocardia in brain abscess." *Advanced biomedical research* no. 6.

Fernández-Villa D, Aguilar MR, Rojo L.(2019). Folic acid antagonists: antimicrobial and immunomodulating mechanisms and applications. *Int J Mol Sci*;20, http://dx.doi.org/10.3390/ijms20204996.

Flores-Mireles, A. L. , Walker, J. N ., Caparon, M and J.Hultgren, S. (2015). "Urinary tract infections: epidemiology, mechanisms of infection and treatment options". *Nature reviews microbiology*, vol.13,pp.(5), 269-284.

Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). Diagnostic microbiology (pp. 288-302). St Louis: Mosby.

Fortman JL, Mukhopadhyay A.(2016). The future of antibiotics: emerging technologies and stewardship. *Trends Microbiol*;24:515–7, http://dx.doi.org/10.1016/j.tim.2016.04.003.

Fuga, B., Sellera, F. P., Cerdeira, L., Esposito, F., Cardoso, B., Fontana, H., Moura, Q., Cardenas-Arias, A., Sano, E., Ribas, R. M., Carvalho, A. C., Tognim, M. C. B., de Morais, M. M. C., Quaresma, A. J. P. G., Santana, Â. P., Reis, J. N., Pilonetto, M., Vespero, E. C., Bonelli, R. R., Cerqueira, A. M. F., Sincero, T. C. M., & Lincopan, N. (2022). WHO critical priority Escherichia coli as one health challenge for a post-pandemic scenario: Genomic surveillance and analysis of current trends in Brazil. *Microbiology Spectrum*, 10(2), e01256-21. http://dx.doi.org/10.1128/spectrum.01256-21. PMid:35234515.

Gajdács M, Albericio F.(2019). Antibiotic resistance: from the bench to patients. *Antibiotics*;8, http://dx.doi.org/10.3390/antibiotics8030129.

Gangoué-Piéboji ,J., Bedenic ,B., Koulla-Shiro ,S., Randegger ,C., Adiogo ,D., Ngassam P., *et al*.(2005). Extended-spectrum-beta-lactamase-producing *Enterobacteriaceae* in Yaounde, Cameroon. *J Clin Microbiol.* 43:3273–7.

García P · López D · M A S M. Sanz ·. Hermoso J A . (2024). BacterialResistance toAntibioticsSciencefor PublicPolicy *NIPO*: 155-24-095-9e-NIPO: 155-24-094-3Legal deposit: M-11805-

Golkar Z, Bagasra O, Gene Pace D.(2014). Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *J Infect Dev Ctries*;8:129–36, http:// dx.doi.org/10.3855/jidc.3573.

Gomes, T.A.T.; Elias, W.P.; Scaletsky, I.C.A.; Guth, B.E.C.; Rodrigues, J.F.; Piazza, R.M.F.; Ferreira, L.C.S.; Martinez, M.B.(2016). Diarrheagenic Escherichia coli. *Braz. J. Microbiol*., 47, 3–30.

Grossman TH(2016). Tetracycline antibiotics and resistance. Cold Spring Harb Perspect Med;6, http://dx.doi.org/10.1101/cshperspect.a025387.

Gupta A., Ampofo K., Rubenstein D. & Saiman L. (2003). Extended spectrum β lactamase-producing Klebsiella pneumoniae infections' review of the literature. *Journal of erinatology*.. 23: 439.

Gyles, C.L.; Prescott, J.F.; Songer, J.G.; Thoen, C.O.(2010). Pathogenesis of Bacterial Infections in Animals, 4th ed.; Wiley-Blackwell: *Hoboken, NJ,* USA,; ISBN 9780813812373.

Haaber, J.; Penades, J.R.; Ingmer, H .(2017). Transfer of Antibiotic Resistance in Staphylococcus aureus. *Trends Microbiol*., 25, 893–905. (CrossRef)

Haasdijik, R. A., & Ingen, J. V. (2018). Escherichia hermannii as the sole pathogen in urosepsis: Case report. *New Microbes New Infections, 22*, 100–101.

Hammond A, Stuijfzand B, Avison MB, Hay AD.(2020) Antimicrobial resistance associations with national primary care antibiotic stewardship policy: Primary care-based, multilevel analytic study. *PLoS One.* May 14;15(5)

Hassan KS, Noori TA.(2021). Descriptive study onurinary tract infections among children. *Iraqi J Community Med* ;34:54-7.

Hocquet, D.; Muller, A.; Bertrand, X.(2016). What happens in hospitals does not stay in hospitals: Antibiotic-resistant bacteria in hospital wastewater systems. *J. Hosp. Infect.*, 93, 395–402.

Huddleston, J.R.(2014). Horizontal gene transfer in the human gastrointestinal tract: Potential spread of antibiotic resistance genes. *Infect. Drug Resist.*, 7, 167–176.

Hughes, H., Sambrook, J., & MacCallum, P. (2012). Molecular cloning: a laboratory manual. In Molecular cloning: a laboratory manual (pp. 1890-1890).

Hussein K A ,Palpitany SA, Ahmed S H.(2014). Prevalence of Urinary Tract Infection among Secondary School Students in Urban and Rural in Erbil: Comparative Study,. *Kufa Journal For Nursing Sciences* Vol.4 No. 3 201

Invik ,J., Barkema ,H. W., Massolo ,A., Neumann ,N. F, and Checkley ,S. (2017). Total coliform and Escherichia coli contamination in rural well water: Analysis for passive surveillance *J. Water Health* 15 729–40.

Iredell J, Brown J, Tagg K. (2016):Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. *BMJ*. Feb 8;352:h6420.

Isfahani, Bahram Nasr, Hossein Fazeli, Zeinab Babaie, Farkhondeh Poursina, Sharareh Moghim, and Meisam Rouzbahani. (2017). "Evaluation of polymerase chain reaction for detecting coliform bacteria in drinking water sources." Advanced biomedical research no. 6.

Islam M M,Islam M N ,Sharifuzzaman and Fakhruzzaman M.(2017):Isolation and Identification of Escherichia coli and Salmonella from poultry litter and feed ,*international journal of natural and social sciences* 1-7.

Ismael, A. L. , Aubaid, S. H and Nasir, H. M. (2022). "Estimating the Level of Interleukin-22 in Sera of Patients with Uropathogenic Escherichia coli Infection in Mosul City". *Rafidain journal of science*, vol.31,(2).

Jang J, Hur H-G, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S, (2017). Environmental *Escherichia coli*: ecology and public health implications-a review. *J Appl Microbiol* 123: 570–581. (PubMed) (Google Scholar).

Jin, W.; Wachino, J.; Kimura, K.; Yamada, K.; Arakawa, Y.(**2015)**. New plasmid-mediated aminoglycoside 6′-N-acetyltransferase, AAC(6′)-Ian, and ESBL, TLA-3, from a Serratia marcescens clinical isolate. *J. Antimicrob. Chemother.* , *70*, 1331–1337. (**Google Scholar**) (**CrossRef**)(**Green Version**).

Johnston, C.; Martin, B.; Fichant, G.; Polard, P.; Claverys, J.P.(2014). Bacterial transformation: Distribution, shared mechanisms and divergent control. *Nat. Rev. Microbiol.*, 12, 181–196. (CrossRef).

Joya M, Aalemi AK, Baryali AT.(2022). Prevalence and Antibiotic Susceptibility of the Common Bacterial Uropathogen Among UTI Patients in French Medical Institute for Children. *Infect Drug Resist*. Aug 6;15:4291 -4297.

Kaftandzieva ,A., Trajkovska-Dokic ,E., Panovski ,N.(2011). Prevalence and molecular characterization of ESBLs producind *Escherichia coli* and *Klebsiella pneumonia*. *Biol Med Sci*. 32:129–41. PMID: 22286618.

Kairunnisa ,D., Hendrawan ,D. I., Fachrul ,M .F and Rinanti ,A .(2021). Distribution pattern of E. coli and total coliform at Tanah Sareal District, Bogor City, Indonesia IOP Conf. Ser. *Earth Environ. Sci*. 802

Karkman, A., Do, T. T., Walsh, F., and Virta, M. P. J. (2018). Antibiotic-Resistance Genes in Waste Water. *Trends Microbiol*. 26, 220–228.

Khan R , Wali S and Pari B (2023) Comprehensive Analysis of Klebsiella Pneumonia culture identification and antibio granin :Implication for Antimicrobial Susceptipility Patterns from sputum sample in Mardan Khyber Pakhtunkhwa,Pakistan.

Khan, F. A., Söderquist, B., and Jass, J. (2019). Prevalence and diversity of antibiotic resistance genes in swedish aquatic environments impacted by household and hospital wastewater. *Front. Microbiol.* 10:688. doi: 10.3389/fmicb.2019.00688.

Khosravi, A. D., Hoveizavi, H., & Mehdinejad, M. (2013). Prevalence of Klebsiella pneumoniae encoding genes for CTX-M-1, TEM-1 and SHV-1 Extended-Spectrum Beta Lactamases (ESBL) enzymes in clinical specimens. *Jundishapur Journal of Microbiology*, 6(10), 8256. http://dx.doi.org/10.5812/jjm.8256

Kim Y, Lee S-M, Nong LK, Kim J, Kim SB and Kim D (2023) Characterization of Klebsiella pneumoniae bacteriophages, KP1 and KP12, with deep learning-based structure prediction. Front. Microbiol. 13:990910. doi: 10.3389/fmicb.2022.990910.

Koskeroglu K , Barel M, Hizlisoy H, Yildirim Y .(2023). Biofilm formation and antibiotic resistance profiles of water-borne pathogens , *Research in Microbiology* ,174 ,104056.

Kottila R, HenaV.(2024). Antibiotic susceptibility and biofilm formation among lactose fermenting Gram negative bacilli causing urinary tract infection. *J Microbiol Infect Dis*.; 14(1): 1-7.

Krause KM, Serio AW, Kane TR, Connolly LE.(2016). Aminoglycosides: an overview. *Cold Spring Harb Perspect Med*;6, http://dx.doi.org/10.1101/ cshperspect.a027029.

Kumari N, Kuma S, Jha G, KumarR, Kumar Singh B, Athar Ansari M (2023) Epidemiology of Urinary Tract Infection in paediatric population: A single institution study International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 1.

Kuo, P.Y.; Lo, Y.T.; Chiou, Y.J.; Chen, C.A.; Hidrosollo, J.H.; Thuy, T.T.D.; Zhang, Y.Z.; Wang, M.C.; Lin, T.P.; Lin, W.H.; et al (**2022)**. Plasmid-mediated quinolone resistance determinants in fluoroquinolone-nonsusceptible *Escherichia*

coli isolated from patients with urinary tract infections in a university hospital, 2009–2010 and 2020. *J. Glob. Antimicrob. Resist.*, *30*, 241–248.

Latipour M., Gholipour A. & Damavandi M. S.(2016). Prevalence of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates in nosocomial and community-acquired urinary tract infections. *Jundishapur journal of microbiology*.. 12: 9(3):e31179.

Leung AKC, Wong AHC, Leung AAM, Hon KL.(2019) Urinary Tract Infection in Children. *Recent Pat Inflamm Allergy Drug Discov*.;13(1):2-18.

Livermore, D.M.; Woodford, N. (2006). The Beta-Lactamase Threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. *Trends Microbiol*., 14, 413–420.

MacFaddin, J. F. (2000). Biochemical Test for Identification of Medical Bacteria.3rd ed., Williams and wilkins – baltimor., 321-400.

Machuca, J.; Ortiz, M.; Recacha, E.; Díaz-De-Alba, P.; Docobo-Perez, F.; Rodríguez-Martínez, J.-M.; Pascual, Á(**2016)**. Impact of AAC(6′)-Ib-cr in combination with chromosomal-mediated mechanisms on clinical quinolone resistance in *Escherichia coli*. *J. Antimicrob. Chemother.* , *71*, 3066–3071. (**Google Scholar**) (**CrossRef**) (**PubMed**).

Mahmouda AA, ,. Hajic L P, Mwakyomad A A , Debora C. Kajegukaa (2024)Detection of Biofilm Formation and Antibiotic Susceptibility in Escherichia coli Isolated From the Urine of Pregnant Women at Mnazi Mmoja Hospital, Zanzibar, *Tanzania East Africa Science* | Vol. 6 | No. 1

Mai-Prochnow A, Clauson M, Hong J, Murphy AB.(2016). Gram positive and gram negative bacteria differ in their sensitivity to cold plasma. *Sci Rep*;6, http://dx.doi.org/10.1038/srep38610.

Mancuso G, Midiri A, Gerace E, Biondo C.(2021): Bacterial Antibiotic Resistance: *The Most Critical Pathogens. Pathogens*. 12;10(10):1310.

Martinez, J. L., and Baquero, F. (2017). "When pathogens and environmental organisms meet," in *Antimicrobial resistance in wastewater treatment processes*, (Hoboken, NJ: John Wiley & Sons, Inc), 15–33.

Mawlood A. H., Omer S. A., Jalal S. T. & Haji S. H.(2018).Molecular detection of SHV-Type ESBL in E.coli and K. pneumoniae and their antimicrobial resistance profile. *Zanco Journal of Medical Sciences*. 22, 262–272.

Meng, L., Liu, H., Lan, T., Dong, L., Hu, H., Zhao, S., *et al.* (2020). Antibiotic Resistance Patterns of Pseudomonas spp. Isolated From Raw Milk Revealed by Whole Genome Sequencing. *Front. Microbiol*. 11:1005.

Mir N, Saleem S, Imran M, Ghazal A, Usman M,(2021) Isolation of Multi Drug Resistant Escherichia coli from drinking water of Lahore City, Pakistan *P J M H S* Vol. 15, NO. 5.

Modh K S , Parmar B.C;Chandhary B handeri B.B,.Thakur S,Pargiz .B and Patel N;M(2021). Molecular Detection of Klebsiella spp. From Poultry Meat.*ind.J.pure APP .Biosci* 9 (1) ,511-518.

Moghaddam ,M.N., Beidokhti ,M.H., Jamehdar SA, Ghah-raman M.(2014). Genetic properties of blaCTX-M and blaPER β-lactamase genes in clinical isolates of Enterobacteri-aceae by polymerase chain reaction. *Iran J Basic Med Sci*;17(5):378-383.

Mohammad R.Z., Mohammad A., Salomeh K., Reza A. G., Hossein S., Maryam S., Azam A., Mana S., Negin N., and Reza K. A.(2014). "Rapid detection of coliforms in drinking water of Arak city using multiplex PCR method in comparison with the standard method of culture (Most Probably Number)." *Asian Pacific journal of tropical biomedicine* no. 4 (5):404-409.

Mohammed I. and Abass E.(2019). Phenotypic detection of Extended Spectrum β-Lactamases (ESBL) among gram negative uropathogens reveals highly

susceptibility to imipenem. *Pak J Med Sci*.. 35(4): 1104–1109. https://doi.org/10.12669/pjms.35.4.207 PMID: 31372151 9.

Mohr Kathrin I.(2016). History of antibiotics research. *Curr Top Microbiol Immunol*;358:3–32.

Mohr KI.(2016). History of Antibiotics Research. *Curr Top Microbiol Immunol*.;398:237-272. doi: 10.1007/82_2016_499. PMID: 27738915.

Naboka, Y.L., Mavzyutov, A.R., Kogan, M.I., Gudima, I.A., Dzhalagoniya, K.T., Ivanov, S.N., Naber, K.G.(2021). The gene profile of Enterobacteriaceae virulence factors in relation to bacteriuria levels between the acute episodes of recurrent uncomplicated lower urinary tract infection. *Expert Rev. Anti-Infect. Ther.*, 19, 1061–1066.

Nadi H .,shalan Z .,Al-Qatan H and Alotaibi (2024) :Urinary Tract Infection in boys less than five years of age :Ageneral pediatric presepective :*kuwait Med*;38(3);220-225

Nainu F, Masyita A, Bahar MA, Raihan M, Prova SR, Mitra S, *et al*. Pharmaceutical prospects of bee products: Special focus on anticancer, antibacterial, antiviral, and antiparasitic properties. *Antibiotics* 2021;10:822, http://dx.doi. org/10.3390/antibiotics10070822.

Nasser R, Mohammed MTP, Veettil RK, (2019). Clinical Characteristics and Microbiological Analysis of Urinary Tract Infection in Children of 2 Months to 12 Years: A Prospective Study. *Pediatr Inf Dis*;1(3):79–81.

Nirwati H, Sinanjung K, Fahrunissa F, Wijaya F, Napitupulu S, Hati VP, Hakim MS, Meliala A, Aman AT, Nuryastuti T.(2019) Biofilm formation and antibiotic resistance of *Klebsiella pneumoniae* isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. 16;13(Suppl 11):20.

Nishimura E, Nishiyama M, Nukazawa K, Suzuki Y.(2021). Comparison of Antibiotic Resistance Profile of *Escherichia coli* between Pristine and Human-Impacted Sites in a River. *Antibiotics* (Basel). May 13;10(5):575

Niumsup ,P.R., Tansawai ,U., Na-Udom ,A., Jantapalaboon ,D., Assawatheptawee, K., Kiddee ,A, *et al.*(2018). Prevalence and risk factors for intestinal carriage of CTX-M-type ESBLs in Enterobacteriaceae from a Thai community. *Eur J Clin Microbiol Infect Dis*;37(1):69-75.

Nordmann ,P and Poire ,L.(2019). Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. *Clin Infect Dis* 2019;69(7):521–8.

Olivares, J.; Bernardini, A.; Garcia-Leon, G.; Corona, F.B.; Sanchez, M.; Martinez, J.L.(2013). The intrinsic resistome of bacterial pathogens. *Front. Microbiol*., 4, 103.

Palmeira, J. D., & Ferreira, H. M. N. (2020). Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in cattle production - a threat around the world. Heliyon, 6, 03206.

Palpitany, S. A., Ahmed, S. H., & Hussein, K. A. (2014). Prevalence of Urinary Tract Infection among Secondary School Students in Urban and Rural in Erbil: Comparative Study. *Kufa Journal for Nursing Sciences*, *4*(3), 129–135. https://doi.org/10.36321/kjns.vi20143.2766l;22(1):748.

Panneerselvam, A, and G Arumugam. (2012). "Isolation and identification of bacteria from lake water in and around ranipet area, vellore district." *International Journal of Pharmaceutical and Biological Archives* no. 3 (4):1008-1011.

Papich MG (2016). Linezolid. In: Saunders handbook of veterinary drugs. Elsevier;. p. 451–2, http://dx.doi.org/10.1016/B978-0-323-24485-5.00339-9.

Parnham MJ, Haber VE, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. (2014).Azithromycin: mechanisms of action and their relevance for clinical applications. *Pharmacol Ther* ;143:225–45, http://dx.doi.org/10.1016/j. pharmthera.2014.03.003.

Paterson, D. L., and Bonomo, R. A. (2005). Extended-spectrum β-lactamases: a clinical update. *Clin. Microbiol. Rev*. 18, 657–686. doi: 10.1128/cmr.18.4.657- 686.

Peirano G, Pitout J. (2019). Extended-spectrum beta-lactamase-producing *Enterobacteriaceae:* update on molecular epidemiology and treatment options. *Drugs* 79:1529–1541.

Peirano, G.; Schreckenberger, P.C.; Pitout, J.D.D.(2011). Characteristics of NDM-1-Producing Escherichia coli Isolates That Belong to the Successful and Virulent Clone ST131. *Antimicrob. Agents Chemother*., 55, 2986–2988.

Pérez-Etayo, L.; González, D.; Vitas, A.I.(2020). The Aquatic Ecosystem, a Good Environment for the Horizontal Transfer of Antimicrobial Resistance and Virulence-Associated Factors Among Extended Spectrum β-Lactamases Producing E. Coli. *Microorganisms*, 8, 568. (CrossRef).

Perry, M. R., Lepper, H. C., McNally, L., Wee, B. A., Munk, P., Warr, A., *et al.* (2021). Secrets of the hospital underbelly: Patterns of abundance of antimicrobial resistance genes in hospital wastewater vary by specific antimicrobial and bacterial family. *Front. Microbiol.* 12:703560. doi: 10.3389/fmicb.2021.703560

Peterson, E.; Kaur, P (2018). Antibiotic Resistance Mechanisms in Bacteria: Relationships between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. *Front. Microbiol*., 9, 2928.

Phan, M.D.; Peters, K.M.; Fraga, L.A.; Wallis, S.C.; Hancock, S.J.; Nhu, N.T.K.; Forde, B.M.; Bauer, M.J.; Paterson, D.L.; Beatson, S.A.; et al,.(**2022**). Plasmid-Mediated Ciprofloxacin Resistance Imparts a Selective Advantage on *Escherichia coli* ST131. *Antimicrob. Agents Chemother.* , *66*, e02146-21. (**Google Scholar**) (**CrossRef**).

Pilmis, B., Le Monnier, A., and Zahar, J. (2020). Gut microbiota, antibiotic therapy and antimicrobial resistance: A narrative review. *Microorganisms* 8:269. doi: 10.3390/microorganisms8020269.

Pilmis, B.; Cattoir, V.; Lecointe, D.; Limelette, A.; Grall, I.; Mizrahi, A.; Marcade, G.; Poilane, I.; Guillard, T.; Bourgeois Nicolaos, N.; et al(2018). Carriage of ESBL-producing Enterobacteriaceae in French hospitals: The PORTABLSE study. *J. Hosp. Infect.*, 98, 247–252.

Pishtiwan A. H. & Khadija K. M.(2019). Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBLProducing Klebsiella pneumoniae and Escherichia coli isolated from Thalassemia Patients in Erbil, Iraq. *Mediterranean journal of hematology and infectious diseases*.. 11(1):e2019041.

Pitout, J.D.D.; Laupland, K.B.(2008). Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae: An Emerging Public-Health Concern. *Lancet Infect. Dis.*, 8, 159–166.

Poirel L, Bonnin RA, Nordmann P.(2012). Genetic support and diversity of acquired extended-spectrum β-lactamases in Gram-negative rods. *Infect Genet Evol* 12:883–893.

Poli, G.; Cocilovo, A.; Dell'Ara, P.E.; Martino, P.A.; Ponti, W.(2005). Microbiologia e immunologia veterinaria. *In Microbiologia e Immunologia Veterinaria*; UTET Scienze Mediche: Milan, Italy,; ISBN 8802070342.

Rabello, R. F., Bonelli, R. R., Penna, B. A., Albuquerque, J. P., Souza, R. M., & Cerqueira, A. M. F. (2020). Antimicrobial resistance in farm animals in Brazil: An update overview. *Animals* (Basel), 10(4), 552. http://dx.doi.org/10.3390/ani10040552. PMid:32224900.

Rahman, S., Ali, T., Ali, I., Khan, N. A., Han, B., & Gao, J. (2018). The growing genetic and functional diversity of extended spectrum beta-lactamases. Biomedical Research International, 2018, 9519718. *PMid:*29780833.

Ramadan, A.A.; Abdelaziz, N.A.; Amin, M.A.; Aziz, R.K.(**2019)**. Novel blaCTX-M variants and genotype-phenotype correlations among clinical isolates of extended

spectrum beta lactamase-producing Escherichia coli. *Sci. Rep.* , *9,* 4224. (**Google Scholar**) (**CrossRef**).

Ramirez D, Giron M. Enterobacter Infections. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559296/

Ramirez MS, Tolmasky ME.(2010). Aminoglycoside modifying enzymes. *Drug Resist Updat*;13:151-71

Ranjan, A., Sridhar, S. T., Matta, N. , Chokkakula, S. and. Ansari, R. K. (2017). "Prevalence of UTI among pregnant women and its complications in newborns". *Indian Journal of Pharmacy Practice*,vol.10,pp .(1).

Reygaert, W.C.(2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiol*., 4, 482–501. (CrossRef) (PubMed).

Robicsek ,A., Strahilevitz ,J., Sahm ,D.F., Jacoby ,G.A., Hooper ,D.C.(2006). qnr prevalence in ceftazidime-resistant Enterobacteriaceae isolates from the United States. Antimicrob Agents Chemother . ;50:2872-4. doi: 10.1128/AAC.01647-05. PubMed PMID: 16870791; *PubMed Central PMCID*: PMC1538681.

Rodriguez-mozaz, S., Chamorro, S., Marti, E., and Luis, J. (2014). Occurrence of antibiotics and antibiotic resistance genes in hospital and urban wastewaters and their impact on the receiving river. *Water Res.* 69, 234–242. doi: 10.1016/j.watres.2014.11.021.

Rossolini, G., Arena, F., & Giani, T. (2017). Mechanisms of antibacterial resistance. In J. Cohen, W. G. Powderly, & S. M. Opal, Infectious diseases (4th ed., Vol. 2, pp. 1181–1196). *Elsevier*.

Rozwandwicz M., Brouwer M., Fischer J., Wagnaar J., Gonzalez–zorn B., Guerra B., *et al.* (2018). Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *J Antimicrob Chemother*..1: 73 (5):1121–1137.

Saito K, Warrier T, Somersan-Karakaya S, Kaminski L, Mi J, Jiang X, *et al*.(2017). Rifamycin action on RNA polymerase in antibiotictolerant Mycobacterium tuberculosis results in differentially detectable populations. *Proc Natl Acad Sci U S A*;114:E4832–40, http://dx.doi.org/10.1073/pnas.1705385114.

Salah, F.D.; Soubeiga, S.T.; Ouattara, A.K.; Sadji, A.Y.; Metuor-Dabire, A.; Obiri-Yeboah, D.; Banla-Kere, A.; Karou, S.; Simpore, J.(**2019)**. Distribution of quinolone resistance gene (qnr) in ESBL-producing *Escherichia coli* and Klebsiella spp. in Lomé, Togo. *Antimicrob. Resist. Infect. Control* , *8*, 104.

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning—Sambrook & Russel—Vol. 1, 2, 3 (Internet).

Sarkar P, Yarlagadda V, Ghosh C, Haldar J. A review on cell wall synthesis inhibitors with an emphasis on glycopeptide antibiotics. Medchemcomm. 2017 Jan 26;8(3):516-533. doi: 10.1039/c6md00585c. PMID: 30108769; PMCID: PMC6072328.

Sarowska, J., Futoma-Koloch, B., Jama-Kmiecik, A., Frej-Madrzak, M., Ksiazczyk, M., Bugla-PLoskonska, G.(2019). Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: Recent reports. Gut Pathog , 11, 10.

Sato, T.; Yokota, S.-I.; Uchida, I.; Okubo, T.; Usui, M.; Kusumoto, M.; Akiba, M.; Fujii, N.; Tamura, Y.(**2013)**. Fluoroquinolone resistance mechanisms in an *Escherichia coli* isolate, HUE1, without quinolone resistance-determining region mutations. *Front. Microbiol.* , *4*, 125.

Shahab S ,Shafi I and Ahmed N (2017).Indigenous oil Degarding Bacteria Isolation ,Screening and characteriztion ,National Journal of Health Sciences 2,100-165

Shakya B,Shaeestha S. R and Silvanus V.(2021). Bacteriological examination of water supply and drinking water at houshold level in makalbari area by the most probable number method *Nepal Med Coll J*; 23 (2): 146-52

Shetty, S.; Deekshit, V.K.; Jazeela, K.; Vittal, R.; Rohit, A.; Chakraborty, A.; Karunasagar, I.(**2019)**. Plasmid-mediated fluoroquinolone resistance associated with extra-intestinal *Escherichia coli* isolates from hospital samples. *Indian J. Med. Res.* , *149*, 192. (**Google Scholar**) (**CrossRef**).

Singh AK, Kaur R, Verma S and Singh S (2022). Antimicrobials and Antibiotic Resistance Genes in Water Bodies: Pollution, Risk, and Control. *Front. Environ. Sci.* 10:830861.

Singh NS, Singhal N, Kumar M, Virdi JS.(2022). Public health implications of plasmid-mediated quinolone and aminoglycoside resistance genes in *Escherichia coli* inhabiting a major anthropogenic river of India. *Epidemiol Infect*. 28; 150:1-21.

Singh, A. K., Das, S., Singh, S., Gajamer, V. R., Pradhan, N., Lepcha, Y. D., *et al.* (2018). Prevalence of antibiotic resistance in commensal Escherichia coli among the children in rural hill communities of northeast India. *PLoS One* 13:e0199179.

Singh, RK, Avanish P., Rahul Pandey, and SP Tiwari.(2010). "Microbial evaluation of water bodies from Jaunpur, UP, India." EM Int no. 29:365-70.

Sivaraman GK, Rajan V, Vijayan A, Elangovan R, Prendiville A, Bachmann TT.(2021) Antibiotic Resistance Profiles and Molecular Characteristics of Extended-Spectrum Beta-Lactamase (ESBL)-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolated From Shrimp Aquaculture Farms in Kerala, *India*. *Front Microbiol*. 19;12:622891.

Sobsey D.(2014). Antimicrobial Resistance and Water, Sanitation and Hygiene. Geneva: WHO.

Somorin Y M ;Wir N-J M,Pattison S H ,Crockard M A ;Hughes C ;M; tunney M M and Gipin (2022). Antimicrobial resistance in urinary pathogens and culture-independent detection of trimethoprim resistance in urine from patient with urinary tract infection *BMC microbiology* 22 Artical number 144

Sudheendra KR, Basavaraj PV.(2018). Analysis of antibiotic sensitivity profile of biofilm-forming uropathogenic Escherichia coli. *J Nat Sc Biol Med*;9:175-9.

Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F. (2016). Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic E. coli isolated from clinical samples in Iran. *Antimicrob Resist Infect Control*. Apr 1;5:11.

Thanawastien A, Cartee RT, Griffin TJ, Killeen KP, Mekalanos JJ(2015). Conjugate-like immunogens produced as protein capsular matrix vaccines. *Proc Natl Acad Sci U S A*;112:E1143–51, http://dx.doi.org/10.1073/pnas.1425005112.

Thornber, K., Verner-Jeffreys, D., Hinchliffe, S., Rahman, M. M., Bass, D., and Tyler, C. R. (2020). Evaluating antimicrobial resistance in the global shrimp industry. *Rev. Aquac*. 12, 966–986. doi: 10.1111/raq.12367

Tumbarello, M.; Sanguinetti, M.; Montuori, E.; Trecarichi, E.M.; Posteraro, B.; Fiori, B.; Citton, R.; D'Inzeo, T.; Fadda, G.; Cauda, R.; et al(2007). Predictors of Mortality in Patients with Bloodstream Infections Caused by Extended-Spectrum-Beta-Lactamase-Producing Enterobacteriaceae: Importance of Inadequate Initial Antimicrobial Treatment. *Antimicrob. Agents Chemother*., 51, 1987–1994. (CrossRef).

United States Geological Survey (USGS), (2018). Bacteria and E. coli in Water. Available at: https: //www.usgs.gov/special-topic/water-science-school/science/bacteria-and-e-coli-water?qt science_center_objects - science_center_objects (Accessed on 31 May 2021).

Ur Rahman, S.; Ali, T.; Ali, I.; Khan, N.A.; Han, B.; Gao, J. (2018). The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. *BioMed Res. Int.* , 9519718. (**Google Scholar**) (**CrossRef**) (**PubMed**).

Vardakas, K.Z., Tansarli, G.S., Rafailidis, P.I., Falagas, M.E. (2012).Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to

Enterobacteriaceae producing extended-spectrum _-lactamases: A systematic review and meta-analysis. *J. Antimicrob. Chemother*. 67, 2793–2803.

Vigi C ,Gaurav S , Naveen C and R. K R(2016). High prevalence of multiple drug resistance among pediatric Escherichia Coli infections *International Journal of Medical Research & Health Sciences,* 5, 10:166-169.

Von Wintersdorff, C.J.; Penders, J.; van Niekerk, J.M.; Mills, N.D.; Majumder, S.; van Alphen, L.B.; Savelkoul, P.H.; Wolffs, P.F(2016). Dissemination of Antimicrobial Resistance in Microbial Ecosystems through Horizontal Gene Transfer. *Front. Microbiol*., 7, 173. (CrossRef)

Waites, M. J., Morgan, N. L., Rockey, J. S., & Higton, G. (2008). Industrial Microbiology an Introduction London

Wall ,S.(2019). Prevention of antibiotic resistance – an epidemiological scoping review to identify research categories and knowledge gaps. Glob Health Action;12(1):1756191.

Watkins RR, Bonomo RA(2016). Overview: global and local impact of antibiotic resistance. *Infect Dis Clin North Am*;30:313–22, http://dx.doi.org/10.1016/j. idc.02.001.

Watts, J. E., Schreier, H. J., Lanska, L., and Hale, M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. Mar. *Drugs* 15:158. doi: 10.3390/md15060 158.

Winn, W.C.; Allen, S.D.; Janda, W.M.; Koneman, E.W.; Procop, G.W.; Schreckenberger, P.C. and Woods, G.L. (2006). Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. *Lippincott Williams and Wilkins*, USA. 234-241. Green, M. R.

World Health Organisation, WHO(2017). WHO Publishes List of Bacteria for which New Antibiotics are Urgently Needed. 27 February, News Release, Geneva.

Available online: https://www.who.int/news-room/ detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (accessed on 25 October 2018).

World Health Organization (2011). Guidelines for drinking-water quality. *WHO Chron* 38: 104–108. (PubMed) (Google Scholar).

Wróbel A, Maliszewski D, Baradyn M, Drozdowska D(2020). Trimethoprim: an old antibacterial drug as a template to search for new targets. Synthesis, biological activity and molecular modeling study of novel trimethoprim analogs. *Molecules;*25, http://dx.doi.org/10.3390/molecules25010116.

Yandai F H , Ndoutamia G , Nadlaou B and BarroN.(2019) Prevalence and resistance profile of Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections in N'Djamena, Tchad. *J. Biol. Chem. Sci*. 13(4): 2065-2073

Zhao M , Qi S , SunY and ZhengX (2024) Comparison of polymerase chain reaction and next-generation sequencing with conventional urine culture for the diagnosis of urinary tract infections: *A meta-analysis* Volume 19 Issue 1.

Zhou Y, Zhou Z, Zheng L, Gong Z, Li Y, Jin Y, Huang Y, Chi M.(2023) Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options. *Int J Mol Sci*. 23;24(13):10537

Zhou, K.; Yu, W.; Shen, P.; Lu, H.; Wang, B.; Rossen, J.W.A.; Xiao, Y.(**2017)**. A novel Tn1696-like composite transposon (Tn6404) harboring bla $_{IMP-4}$ in a Klebsiella pneumoniae isolate carrying a rare ESBL gene bla $_{SFO-1}$. *Sci. Rep.* , *7*, 17321.

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الخلاصة:

تعد التهابات المسالك البولية عند الأطفال من الأمراض الشائعة، ويؤدي سوء استخدام المضادات الحيوية إلى ظهور مقاومة من قبل البكتيريا المسببة للمرض. تم جمع مائة وخمسين عينة من الأطفال الذين تتراوح أعمارهم بين 1-10 سنوات الذين يعانون من التهابات المسالك البولية والذين حضروا إلى مستشفى الأطفال التعليمي في مدينة كربلاء. استمرت الدراسة الحالية من أواخر يوليو 2023 حتى فبراير 2024. تم جمع العينات في زجاجات معقمة. بالإضافة إلى ذلك، تم جمع عينات المياه في زجاجة معقمة بسعة 100 مل من ثلاث محطات تقع على نهر الحسينية موزعة على (العطيشي وفندق البارون والقنطرة البيضاء) والتي كانت 15 عينة لكل موسم. تم تطبيق طريقة العدد الأكثر احتمالا للكشف عن البكتيريا القولونية. تم جمع عينات المياه لشهري أغسطس وديسمبر 2023 لفصلي الصيف والشتاء على التوالي.

تمت زراعة جميع عينات البول والماء على بيئات مناسبة وتم الحصول على مستعمرات نقية بعد فترة الحضانة. تم إجراء الاختبارات الظاهرية والكيميائية الحيوية تم الحصول على عشرين عزلة من البكتيريا القولونية من عينات البول (14 عزلة من البكتريا الاشريشيا القولونية 5 عزلات من الكلبسيلا نيموني وعزلة واحدة من جنس الانتيروبكتر).

فيما تم الحصول على 30 عزلة من البكتريا القولونية من عينات ماء النهر (5 عزلات من الاشريشيا القولونية, 5 عزلات من الكلبسيلا نيموني و 5 عزلات من جنس الانتيروبكتر لكل فصل من السنة).

علاوة على ذلك تم استخدام نظام الفايتك 2 لاختبار نشاط مقاومة المضادات الحيوية لهذه العزلات ضد حوالي 23 نوعا من المضادات الحيوية الشائعة.

تم اجراء تفاعل البلمرة المتسلسل باستخدام بادي محدد لجينات التضخيم للجينات التالية: (CTX , TEM, SHV, qnr, aac) المشفرة لنشاط مقاومة المضادة الحيوية.

واخيرا تم اختيار بعض العزلات التي اظهرت مقاومة للمضادات الحيوية للتحقيق في قدرتها على تكوين الاغشية الحيوية بطريقة الانبوب.

أظهرت النتائج وجود فروق معنوية بين موسمين في كل محطة وبين المحطات فيما يتعلق بعدد البكتيريا القولونية. وكانت هذه الأعداد أعلى بشكل معنوي في محطتي فندق البارون و العطيشي خلال موسم الصيف مقارنة بأعدادها خلال مواسم الشتاء في نفس المحطات ($P<0.05$).

جميع عزلات البكتيريا القولونية الاشريشيا القولونية المعزولة من البول مقاومة للمضادات الحيوية التالية سيفترياكسون، سيفوروكسيم، سيفوروكسيم-أكسيتيل، إرتابينيم، حمض أموكسيسيلين، نتروفورانتوين، وفوسفوميسين. من ناحية أخرى، أظهرت جميع البكتيريا المختبرة مقاومة لما يلي في أعداد مختلفة من عزلاتها. هي سيفتازيديم، سيفيبيم، سيبروفلوكساسين، إيميبينيم، بايبيراسيللين، ميروبينيم، أمبيسيلين، سيفازولين، سيفيازيديم، سيفتازيديم/أفيباكتام، إيميبينيم، أميكاسين. جنتاميسين سلفاميثوكسازول وسيفازولين. تريميثوبريم.

لم تظهر جميع عزلات البكتيريا القولونية خلال فصل الصيف نشاطًا مقاومًا للمضادات الحيوية، وعلى النقيض من ذلك، أظهرت ثلاث عزلات من البكتيريا القولونية المعزولة خلال فصل الشتاء مقاومة للمضادات الحيوية للسيفوتاكسيم والسيبروفلوكساسين والتريميثوبريم.

أظهرت نتائج تفاعل البلمرة المتسلسل لعزلات البول أن 5 عزلات من إجمالي 5 عزلات من الاشريشيا القولونية تحمل جينات مقاومة بما في ذلك CTX-M وTEM وSHV واثنتان من عزلات الكليبسيلا نيموني تحملان جينات SHV وTEM وعزلة واحدة من جنس الانتيروبكترتحمل جين CTX-M فقط.

من ناحية اخرى اظهرت نتائج تفاعل البلمرة المتسلسل لعزلات الماء ان 3 من عزلات الاشريشيا القولونية من اصل 5 عزلات كانت حاملة لجينات المقاومة (CTX-M, TEM, SHV, qnr , aac). خلال فصل الشتاء فقط.

أظهرت قدرة البكتيريا القولونية المعزولة من البول على إنتاج الأغشية الحيوية أن جميع عزلات الاشريشيا القولونية كانت قوية ومعتدلة في تكونين الاغشية الحيوية في حين كانت جميع عزلات كليبسيلا الرئوية قوية في تكوين الاغشية الحيوية. أظهرت جميع عزلات البكتيريا القولونية نشاطًا حيويًا خلال موسمين، سواء كان قويًا أو معتدلا.

يمكننا أن نستنتج أن البكتيريا القولونية هي العوامل المسببة لالتهاب المسالك البولية وبالذات الإشريشيا القولونية مع نطاق من مقاومة المضادات الحيوية وتكوين الأغشية الحيوية. يشير عزل البكتيريا القولونية من المياه إلى أن مياه النهر ملوثة بالبكتيريا القولونية، والتي ربما انتقلت إلى الناس بطريقة ما.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء, كلية العلوم

قسم علوم الحياة

التوصيف الجزيئي لبعض محددات مقاومة المضادات الحياتية في بكتيريا القولون المعزولة من اصابات الجهاز البولي عند الاطفال ومياه نهر الحسينية

رسالة ماجستير
مقدمة الى مجلس كلية العلوم جامعة كربلاء وهي جزء من متطلبات نيل شهادة الماجستير في علوم الحياة.

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2025م 1446ه