Molecular detection of Uropathogenic *Escherichia coli* and their inhibition by probiotics

A Thesis

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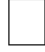

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DEDICATION

To:

- ❖ My mother and my spouse, for their overwhelming support, efforts and love, are the reason behind my success; without them, I would never be the person I am today. Thank you all so much for everything.
- ❖ The memory of my father.

- ❖ My cousin, Dr. Bayar Abdullah Ahmed, taught me to be resilient, keep moving forward, and never stop for any obstacles in life.

- ❖ My brother and sister never stop giving of themselves in countless ways.

- ❖ To all those who have supported me even with a single word, scientific advice or supplication, that was very helpful.

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SUMMARY

Urinary tract infections are the most popular type of diagnosed bacterial illness, and the most frequent bacteria responsible for Urinary tract infections are *Escherichia coli*. The most frequent resistance for gram-negative bacteria to β-lactam antibiotics, especially in *Escherichia coli,* are β-lactamases. The number of patients infected by extended-spectrum β-lactamases producing *Escherichia coli* is rising and regarded as a major health problem globally. This study aimed to evaluate *in vitro* antimicrobial activity of specific lactic acid bacteria against Uropathogenic *Escherichia coli* to prevent and treat Urinary tract infections.

Our research comprised 54 positive midstream urine samples from patients with symptomatic Urinary tract infections, in all age groups, from outpatient departments in Erbil hospitals from 1st October 2021 to 1st April 2022 for isolation and identification of *Escherichia coli*. The distribution of isolates among genders clarified that the females 33 (61.11%) have a higher percentage of Urinary tract infections than 21 males (38.89%).

When the antibiotic susceptibility of *Escherichia coli* isolates was tested using the VITEK 2 panel, all isolates from different regions expressed multiple antibiotic resistance; in this findings, these isolates showed the highest percentage of resistance to Ceftazidime (77.78%), Cefepime (75.93%), Ciprofloxacin (70.37%), Piperacillin (68.52%), while isolates have the highest rate of sensitive to Meropenem (88.89%), Imipenem (85.19%), Amikacin (83.33%), Netilmicin (77.78%). Additionally, this findings showed that certain antibiotic resistance in *Escherichia coli* has grown.

Three types of β-lactamases include ESβLs genes (bla_{SHV}, bla_{TEM}, bla_{CTX}) by the use of PCR assay. All Extended-spectrum β-lactamase -producing *Escherichia coli* isolates samples. The bla_{SHV} was detected in 41 isolates

(75.9%) of the Uropathogenic *Escherichia coli* isolates, while for the *bla*$_{TEM}$ gene the result showed that in 42 detected isolates (77.7%), there is 26 isolates (48.15%) which indicates the prevalence of *bla*$_{CTX}$ gene.

Antagonistic activity of four types of probiotics bacteria against (54) isolates Escheriachia coli were performed. Results showed that most probiotics types have inhibition activity against *Escherichia coli* isolates and the diameter of inhibition zone was highest (17.2 mm) when probiotic *Lactobacillus casei* used, while *Streptococcus thermophiles* showed less inhibitory activity against *Escherichia coli* isolates with (12.1 mm) of inhibition zone.

List of Contents

Title	Page
Title	Cover
Declaration	I
Supervisor Certificate	II
Examining Committee Certification	III
Dedication	IV
Acknowledgments	V
Summary	VI
List of Contents	VIII
List of Figures	XIII
List of Tables	XV
List of Abbreviations	XVI
List of Appendices	XIX
Chapter One	
1. Introduction	1
1.1.1.1. The aims of this study	4
Chapter Two	
2. Literature Review	5
2.1. *Escherichia coli*	5
2.2. Urinary tract infections	5
2.3. Origin and Pathotypes Diversity of *E. coli*	7
2.4. Uropathogenic *Escherichia coli*	8
2.4.1. Uropathogenic *Escherichia coli* and related virulence	11
2.4.2. Host immunological reaction to Uropathogenic *E. coli* infections	12
2.5. Resistance to antibiotics and antimicrobial agents	15
2.5.1. Antibiotic resistant in Uropathogenic *Escherichia coli*	15

2.5.2. Mechanisms of antibiotic resistance in UPEC	16
2.5.2.1. Extended Spectrum β-Lactamase among Uropathogenic *Escherichia coli*	18
2.5.3. Probiotics and their potential activity	19
2.5.4. Lactic Acid Bacteria as potential probiotics	21
2.5.5. Mechanism of action of probiotics	23
2.5.6. General features of probiotics	25
2.5.7. Potential benefits of probiotics in reducing antibiotic resistance	27
2.5.8. Side Effects of Probiotics	29
Chapter Three	
3. Materials and Methods	35
3.1. Materials	35
3.1.1. Equipment and apparatus	35
3.1.2. Chemical materials and bacterial cultured media	36
3.2. Methods	37
3.2.1. Culture Media Preparation	37
3.2.1.1. MacConkey agar	37
3.2.1.2. Blood agar	37
3.2.1.3. Nutrient agar	37
3.2.1.4. Nutrient broth	38
3.2.1.5. LB (Luria-Bertani) broth	38
3.2.1.6. Mueller Hinton agar	38
3.2.1.7. MRS (De Man, Rogosa, and Sharpe) agar	38
3.2.1.8. MRS (De Man, Rogosa, and Sharpe) broth	39
3.2.2. Collection of samples	39
3.2.3. The preservation of bacterial isolates	39
3.2.4. Bacterial culturing	40

3.2.5. Phenotypic identification of the isolates	40
3.2.5.1. Morphology of the colony and microscopic examination	40
3.2.5.2. Identification of the isolates by VITEK 2 System	41
3.2.6. Molecular identification of the isolates	42
3.2.6.1.1. Genomic DNA extraction	42
3.2.6.1.1.1. Sample preparation	42
3.2.6.1.1.2. Detection of Extracted DNA	44
3.2.6.1.1.3. Determination of DNA concentration	44
3.2.6.1.2. Polymerase Chain Reaction (PCR)	44
3.2.6.1.2.1. Preparation of primers for PCR	44
3.2.6.1.2.2. Amplification of DNA	45
3.2.6.1.2.3. PCR method protocol	45
3.2.6.1.2.4. Procedure for the PCR technique	46
3.2.6.1.3. Detection of ESBL genes of *E. coli*	46
3.2.6.1.3.1 Agarose gel electophoresis	46
3.2.7. Antimicrobial Susceptibility tests	47
3.3. Source of probiotic	48
3.3.1. The cell-free supernatant (CFS) extraction	48
3.3.2. Inhibitory effect of LAB against pathogenic bacteria *in vitro*	48
3.4. Analysis of the data using statistics	49
Chapter Four	
4. Results	50
4.1. Isolation of *Escherichia coli*	50
4.2. Identification of Uropathogenic *Escherichia coli* isolates	51
4.2.1. Phenotypical identification of Uropathogenic *Escherichia coli*	51

4.2.2. Molecular characterization of uropathogenic *Escherichia coli* isolates	52
4.3. Screening for extended-spectrum β–Lactamase genes in Uropathogenic *Escherichia coli*	53
4.4. Antibiotic susceptibility profile of Uropathogenic *Escherichia coli* isolates	56
4.5. Inhibitory effect of lactic acid bacteria on Uropathogenic *Escherichia coli*	57
4.6. Antagonism activity of probiotics against Uropathogenic *Escherichia coli*	59
Chapter Five	
5. Discussion	61
5.1. Isolation and identification of *E. coli* isolates	63
5.2. Molecular characterization of *E. coli* isolates	66
5.3. Antibiotic susceptibility profile of Uropathogenic *E. coli* isolates	67
5.4. Screening for extended-spectrum β–Lactamase genes in Uropathogenic *E. coli*	69
5.5. Inhibitory effect of lactic acid bacteria on Uropathogenic *E. coli*	70
5.6. Antagonism activity of probiotics against Uropathogenic *E. coli*	70
Chapter Six	
6. Conclusions And Recommendations	73
6.1. Conclusions	73
6.2. Recommendations	74
Appendices	75
References	R78
Kurdish Summary	A-C

List of Figures

Figures and Titles	Pages
Figure 2.1. The urinary tract and sites of infection	6
Figure 2.2. Adhesions and harboring/motile structures of *E. coli*	11
Figure 2.3. The UPEC's resistance to antimicrobials used to treat UTIs	12
Figure 2.4. Requirements for the perfect probiotic microbe	17
Figure 2.5. Prebiotics and probiotics' mechanisms of action against the colonization of MDROs in the gastrointestinal tract	24
Figure 2.6. Probiotics' impact on the typical microbiota, immune regulation, and metabolic impacts	26
Figure 2.7. Probiotics' mechanisms of action. (A) Pathogenic bacteria are excluded through competition. (B) The creation of antibacterial agents. (C) Enhanced intestinal mucosal adhesion and enhanced epithelial barrier. (D) Immune system stimulation	28
Figure 4.1. Distribution of Uropathogenic *E. coli* isolates according to age and gender	50
Figure 4.2. Uropathogenic *E. coli* has grown on MacConkey agar medium, B. Uropathogenic *E. coli* has grown on Blood agar medium.	51
Figure 4.3. Agarose gel electrophoresis of the genomic DNA extract of Uropathogenic *E. coli* isolates has successfully extracted genomic DNA.	51
Figure 4.4. Aarose gel electrophoresis picture of 16S rRNA gene amplification (797 bps) for molecular detection of Uropathogenic *E. coli* isolates from clinical cases. M: DNA ladder (100 bp), 1-17: positive 16S rRNA gene samples.	52
Figure 4.5. PCR amplification of *blaCTX* gene of *E. coli* isolates, amplified product (517 bps) of Uropathogenic *E. coli* isolates represented by lanes (1, 2, 3, 6, 7, 8, 9, 10, 16, 17, 18,	53

19, 20 21). In contrast, the remain lanes (4, 5, 11, 12, 13, 14, 15) represent negative for the blaCTX gene, M: ladder 100 bp.	
Figure 4.6. PCR amplification of *blaTEM* gene of Uropathogenic *E. coli* isolates, amplified product (861 bps) of Uropathogenic *E. coli* isolates represented by lanes from 2 to 24, while lane 1 was negative for *blaTEM* gene. M: ladder 100 bp.	54
Figure 4.7. Amplification of *blaSHV* gene amplified using PCR test, amplified product (868 bps) of Uropathogenic *E. coli* isolates represented by lanes (2, 3, 4, 5, 7, 8, 9, 10, 13, 14, 15, 16, 17, 18, 21, 22, 23). In contrast, the negative for the *blaSHV* gene was represented in the following lanes (1, 6, 11, 12, 19, 20, 24), M: ladder 100 bp.	54
Figure 4.8. The inhibitory effect of cell-free culture supernatant of probiotics against Uropathogenic E. coli isolates.	59
Figure 4.9. Antagonistic activity of cell-free supernatant of probiotics toward Uropathogenic *E. coli*	60

List of Tables

Tables and Titles	Pages
Table 3.1. Apparatuses and equipment, company, and origin	34
Table 3.2. Chemical materials and culture media, company, and origin	35
Table 3.3. Commercial kits and reagents were employed for the molecular analysis	35
Table 3.4. The six primers utilized in this study	43
Table 3.5. PCR cocktail for the all genes (25 µL) used in this work.	44
Table 3.6. Amplified the ESBL genes in *E. coli* used in the PCR program	45
Table 4.1. The age and gender distribution of the 54 positive Uropathogenic *E. coli* patients, approved by PCR test in the present study	49
Table 4.2. Prevalence of 16S rRNA and ESBL genes among Uropathogenic *E. coli* isolates	55
Table 4.3. Antibiotic resistance patterns of Uropathogenic *E. coli*	56
Table 4.4. Antimicrobial properties of the spent culture supernatant (SCS) broth from lactic acid bacteria against Uropathogenic *E.coli* isolates	57

List of Abbreviations

Abbreviation	Word details
ABU	Asymptomatic bacteriuria
EB	Elution buffer
AGPs	Antibiotic Growth Promoters
AK	Amikacin
ABR	Antibiotic Resistance
CAZ	Ceftazidime
BECs	Bladder epithelial cells
BHI	Brain Heart Infusion
BB	Binding Buffer
blaCTX	Cefotaximases
blaSHV	Sulfhydryl Variable
blaTEM	Temoneira
bp	Base Pair
BW	Washing Buffer
CAZ	Piperacillin\Tazobactam
CDC	Centers for Disease Control and Prevention
CFS	Cell-Free Supernatant
CFU	Colony-Forming Units
CIP	Ciprofloxacin
CL	Cell Lysis Buffer
CLSI	Clinical Laboratory Standard Institution
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
EFSA	European Food Safety Authority
ESBLs	Extended Spectrum β-Lactamases
ExPEC	Extra-Intestinal Pathogenic *E. coli*
FAO	Food and Agricultural Organization
FDA	Food and Drug Adminstration
FEP	Cefepime
g	Gram
GEN	Gentamicin
GIs	Genomic Islands

GIT	Gastro-Intestinal Tract
GN	Gram-Negative Card
GRAS	Generally Recognized as Safe
H	Flagella Antigens
HGT	Horizontal Gene Transfer
hlyA	α-hemolysin
IBCs	Intracellular Bacterial Communities
IMVIC	Indole, Methyl Red, Vogues Proskauer, Citrate
IPM	Imipenem
K	Capsular Antigens
KTP	Kidney Transplant Patient
L	Liter
L. acidophilus	*Lactobacillus acidophilus*
L. coagulans	*Lactobacillus coagulans*
L. reuteri	*Lactobacillus reuteri*
L. rhamnosus	*Lactobacillus rhamnosus*
LAB	Lactic Acid Bacteria
LB	Luria-Bertani Broth
LEV	Levofloxacin
LPS	Lipopolysaccharide
MDR	Multi-Drug Resistant
MEM	Meropenem
mg	Milligram
MGE	Mobile Genetic Elements
µL	Microliter
MICs	Minimum Inhibitory Concentrations
mL	Milliliter
MDRGN	Multidrug-Resistant Gram-Negative Bacteria
MRS	De Man, Rogosa, and Sharpe
MRS agar	De Man, Rogosa, and Sharpe agar
MRS broth	De Man, Rogosa, and Sharpe broth
MSU	Mid-Stream Urine
NET	Netilmicin
OD	Optical Density
OMPs	Outer Membrane Proteins

PAIs	Pathogenicity Islands
PCR	Polymerase Chain Reaction
PIP	Piperacillin
QIRs	Quiescent Intracellular Reservoirs
QPS	Qualified Presumption of Safety
RCTs	Randomized Controlled Trials
SCFAs	Short-Chain Fatty Acids
TAE	Tris-Acetate-EDTA Buffer
Tcps	Secreted Toxin
TIG	Tigecycline
TLRs	Toll-Like Receptors
TSB	Tryptic Soy Broth
TW	Twice Wash Buffer
UPEC	Uropathogenic *E. coli*
UTI	Urinary Tract Infection
VFs	Virulence Factors
WHO	World Health Organization

List of Appendices

Appendices and Titles	Pages
Appendix 1. Laboratory report for *Escherichia coli* identification using VITEK 2 system GN card	A76
Appendix 2. Laboratory report of antimicrobial susceptibility test using VITEK 2 system AST-N326 card	A77
Appendix 3. Acceptance letter of the manuscript in *Diyala Journal of Medicine*	A78

1. INTRODUCTION

Urinary tract infection (UTI) is one of the big health issues, especially in the female gender, which frequently happens with *Escherichia coli* (*E. coli*) infection. About 150,000,000 cases are widely detected with UTI (Mahdavi *et al*., 2018b). Nearly 40% of women may have a UTI at least once in their lifetime (Micali *et al*., 2014). And almost (11%) of women over 18 years of age have at least one attack of UTI annually (Foxman, 2003, Foxman, 2014).

Urinary tract infection can be associated with urological issues; for example, it can lead to hypertension in case of long-term renal failure (Stamm and Norrby, 2001). The UTI is defined by the appearance of the absolute amount of bacterial number in the urine (> 10^5/mL) and according to the severity of symptoms of the urinary tract; UTIs generally are usually divided into three categories: cystitis (lower UTI, or bacterial infections of the bladder), pyelonephritis (upper UTI, or infection in the kidney) and urosepsis syndrome (Smelov *et al*., 2016).

Uropathogenic *E. coli* (UPEC) are kinds of *E. coli* bacteria that frequently lead to UTI, and they are usually derived from fecal colonization at the periurethral area or normal intestinal flora. In addition, the bacteria extent in water or food considered as a contamination of stool which causes disease in the alimentary tract and UTI like (urethra and bladder) (Al-Dulaimi, 2016).

UTIs are a serious public health concern in developing countries. Usually, UTI is produced by the type of pathogenic known as "UPEC" that are the most frequent causative organism; *E. coli* is responsible for nearly 90% of acquired in the community UTIs (Maheswari *et al*., 2013).

The type of *E. coli* named UPEC strongly attaches to the urinary tract by a certain factor which empowers them to survive. The pathogenesis of UPEC was regulated and defined by the creative variety of virulence factors (VFs) (Firoozeh *et al*., 2014).

Due to having more specialized virulence genes than commensal *E. coli* strains, UPEC may colonize the urinary tract and persist in the face of an extremely efficient defense by the host (Forsyth *et al*., 2018), these VFs, which are found on mobile genetic elements (MGE) known as pathogenicity islands (PAIs), raise the capacity to produce symptomatic UTIs (Lin *et al*., 2008).

Adhesins, toxins, hemolysin, and cytotoxic necrotizing factor, siderophores, the aerobactin system, and polysaccharide coatings are some of the VFs connected to UPEC (Schneider *et al*., 2011).

A major global public health concern is antibiotic resistance (ABR), which is the term used to describe bacterial infections that are resistant to antibiotics. In 2015, there were more than 30,000 deaths attributed to resistant bacterial infections just in Europe (Chan *et al*., 2023). ABR also results in higher morbidity, longer hospital stays, and raising medical expenses (Naylor *et al*., 2018).

Multidrug-resistant gram-negative bacteria (MDRGN), as well as notably extended-spectrum β-lactamases (ESBL) producing Enterobacterales, were of exactly increasing medical problem (Giske *et al*., 2008, Exner *et al*., 2017) including *E. coli*.

Monobactams, cephalosporin and penicillins are examples of β-lactam antimicrobials that, a class of antibiotics mostly utilized for human health management which is usually most develop resistances for ESBL-producing Enterobacterales (ECfD, 2019).

ESBLs are enzymes that break 3rd generation cephalosporin, penicillins and monobactams (Rupp and Fey, 2003). This is the cause of such a limited selection of antibiotics available for treating ESBL-producing organisms. The most effective treatment for severe illnesses brought on by ESBL-producing microorganisms is carbapenems (Paterson, 2000). Among ESBL-producing Uropathogenic bacteria, *E. coli* has been reported to be the major cause of public health issues worldwide. Although ESBL genes are most commonly

acquired from *bla*$_{TEM}$ or *bla*$_{SHV}$ parents, since 1998, *bla*$_{CTX}$ types has risen rapidly in most of the globe (Kawamura *et al*., 2014).

Treatment of UTI cases is often started empirically based on the antimicrobial-resistant pattern of the urinary pathogens from existing surveillance reports (Sharma *et al*., 2016). The treatment method will increase; as a result, causing the management of UTIs to become more complex (Abou Heidar *et al*., 2019).

Antibiotic-free treatments have grown in favor in the latest years as an outcome of the appearance and spread of new isolates that are resistant to antibiotics. Probiotics are a useful substitute for these treatment methods for managing UTIs (Landers *et al*., 2012). Lactic acid bacteria (LAB) are the most frequently utilized microorganisms as probiotics. LAB "lactobacilli species" were the most commonly used class of bacteria because of their potential probiotic benefits. Bacteria with a negative effect on pathogenic microorganisms, like LAB. Various bacteria can be managed and inhibited through multiple processes, such as competition for nutrients, the creation of inhibitory chemicals, immunostimulation, and competition for binding sites. The most significant of these processes is the synthesis of organic acids (like lactic acid), which reduces pH. Moreover, some strains can create bioactive compounds with antibacterial activity, including ethanol, formic acid, fatty acids, hydrogen peroxide and bacteriocins (De Vuyst and Leroy, 2007).

According to some studies, probiotics can treat and prevent various infectious illnesses. The effect of probiotics and dietary fibers on infectious diseases. At the moment, antibiotics are frequently used to treat infectious diseases. Antibiotic overuse, however, may negatively affect patients, such as adverse drug reactions and public health, like choosing bacteria resistant to multiple drugs (Yang *et al*., 2019, Yang *et al*., 2020, Silva *et al*., 2020a)

Probiotics are being studied as non-pharmaceutical, extremely safe options to treat and prevent various diseases, including UTIs (Fraga *et al*.,

2005). New species of the *Lactobacillus* family are provided to diets as probiotics. Members of this genus are most frequently accorded the safe or generally recognized as safe (GRAS) category (Jeevaratnam *et al*., 2005).

Live microorganisms are referred to as probiotics because they can benefit their host's health when given in sufficient quantities (Quigley, 2019).

1.1. The aims of this study

1. Isolation and identification of E.coli from patients with urinary tract infections.
2. In vitro evaluation of the antagonism activity of different probiotics on E. coli isolates.
3. Determination the bacterial susceptibility of E.coli isolates to different antibiotics.
4. Using a PCR assay to reidentify the ESBL genes, bla_{CTX}, bla_{TEM} and bla_{SHV} from the Uropathogenic *E. coli*.

2. LITERATURE REVIEW

2.1. *Escherichia coli*

E. coli a typical resident of the intestine tracts of animals and humans. It was one of the first enteric bacilli to be characterized and grown. *E. coli* strains of biological importance to people could be generally characterized from both a genetic and clinical standpoint as (1) commensal strains, (2) intestine pathogenic strains, and (3) extraintestinal pathogenes *E. coli* (ExPEC) strains. UPEC strains are the ExPEC typically related to human illness (Nontongana, 2014).

This type of bacteria accounts for a significant fraction of nosocomial UTIs (50%) and the majority of UTIs acquired in the community (70–95%), accounting for considerable morbidity and costs for healthcare globally. Owing to specialized virulence-associated genes situated on movable genetically and components called "Pathogenicity Islands," UPEC isolated display an elevated level of genetic variability. Most UPEC isolates have certain adhesions (like as category I fimbriae and P), strategies for avoiding either undermining defenses of the host mechanisms (such as a capsule or lipopolysaccharide), mechanisms for acquiring nutrients (such as siderophore), and also toxic substances (such as cytotoxic necrotizing factor 1 and hemolysin) (Sarowska *et al*., 2019).

The association between phylogroup and distribution of VFs among UPEC strains obtained from India is not well understood. So, the current investigation was carried out to phylogroup the UPEC strains, connect the presence of particular virulence markers with distinct phylogroup and also connect the results with patient clinical outcomes (Pakbin *et al*., 2021).

2.2. Urinary tract infections

A significant fraction of the human population is afflicted with urinary tract infections (UTIs), which are common. Each year, around 150 million people experience UTI, which has significant social costs. According to estimates, 11% of women over the age of 18 experience a UTI episode each year, and 40% of women are predicted to experience at least one UTI in their lifetime. There are around 11 million instances recorded in the United States alone each year, and the expenditures are estimated to be $5 billion (Figure 2.1) (Terlizzi *et al*., 2017b).

Infections of the urinary tract have frequent bacterial illnesses linked to high morbidity and money spent on medical care. They continue to be one of the most prevalent, but also one of the most complex, infectious disorders observed in diagnostic techniques. Their clinical severity ranges widely, from non-symptomatic bacteria in the urine to multi-organ systemic dysfunction with a condition known as septic shock, cystitis, and pyelonephritis. UTIs are particularly troublesome for women; 20–50% will experience repeated episodes, and 50–80% will experience a minimum of a single UTI in their lifespan. *E. coli* is the most prevalent uropathogen (between 70% to 90 %), isolating it acute acquired in the community simple diseases in anatomical typical, unblocked UTIs. This additionally is the cause of 85% of asymptomatic bacteriuria (ABU) and more than 60% of recurring cystitis (Mansour *et al*., 2009). Certain *E. coli* strains depart from their commensal role as a component of the intestinal flora in animals and adopt a more pathogenic phenotype, capable of causing illness inside and external to the gut. Such pathogen species are frequently classed as extraintestinal pathogenic *E. coli* or ExPEC.

ExPEC can remain in the gut unharmed, but they can also spread and colonize other host locations, such as the blood, central nervous system, and urinary tract, where they can cause illness. UPEC are subsets of fecal *E. coli*

that can invade the urinary tract, colonize the periurethral region, and cause illness. The existence of particular virulence-associated genes, typically transmitted by various kinds of PAIs, plasmids, bacterial phage, and transposons, causes this variability among distinct *E. coli* pathotypes. Understanding the molecular bases of these variations and the colonization, commensalism, and virulent development abilities is crucial, providing flexibility in gene content as well as the potential for the transfer of genes among various ecotypes or pathotypes throughout the intestine tract (Kaper *et al*., 2004).

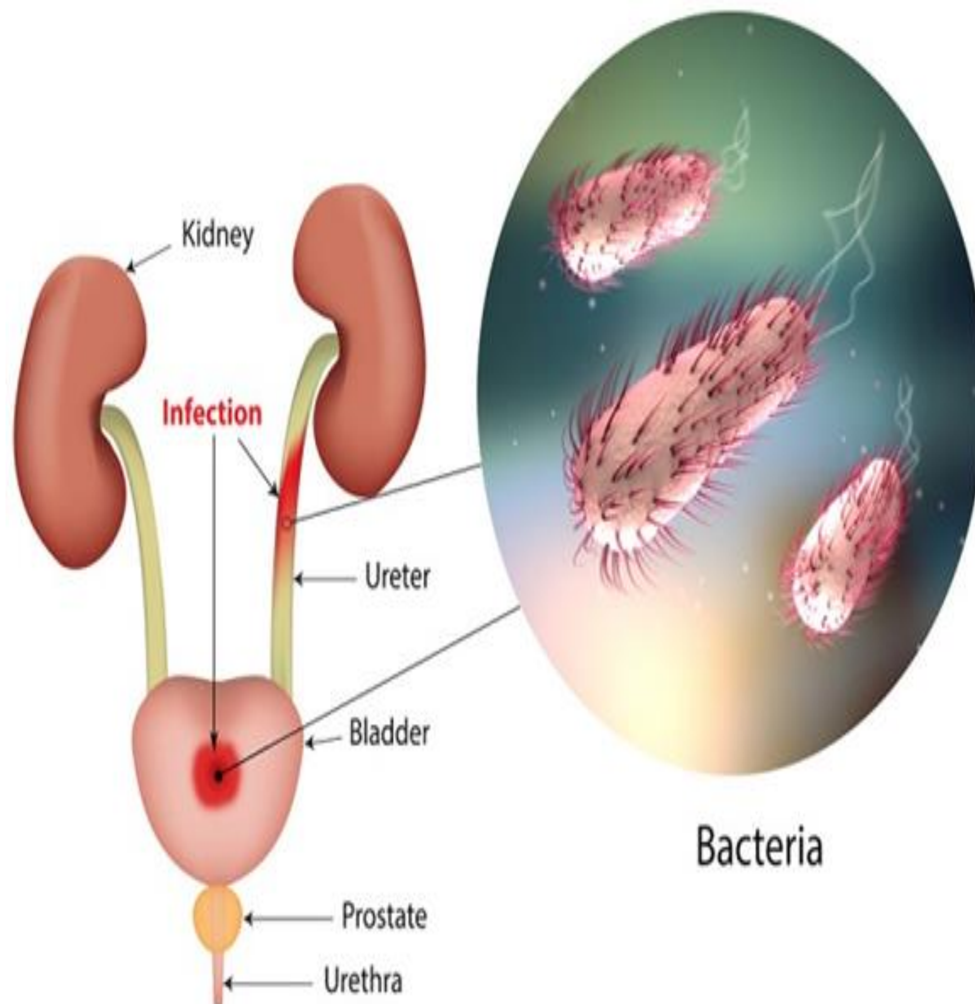


Figure 2.1. The urinary tract and sites of infection (Terlizzi *et al*., 2017a).

2.3. Origin and Pathotypes Diversity of *Escherichia coli*

The presence of different and varying "genomic islands" (GIs) within the conserved "chromosomal backbone" in several bacterial lineages of E. coli is supported by comparative genomics data as being the result of the major role played by horizontal gene transfer (HGT). If a newly acquired DNA is advantageous, it may be permanently incorporated into the genomic through the method of natural choice, resulting in the evolution of bacteria. This extra DNA typically resides in areas where tRNA and tmRNA have been reported to undergo insertion. Chromosome-captured plasmids, bacterial phage genomics, typical GIs, and numerous mosaic and defective components are examples of foreign DNA segments (Brzuszkiewicz *et al*., 2009). ExPEC may stably colonize the human intestine, which makes them more common in ~20% of healthy people than diarrheagenic *E. coli*. However, simple gut colonization by ExPEC was inadequate to produce illness since they must also penetrate another intestinal region in the host. Extraintestinal virulence factors, which are shared by some commensal strains and thus contribute to their diversity, are thought to have evolved to improve survival in the gut and transmission between hosts following the colonization of places external to the gut is not likely to supply any transmission benefit (Kaper *et al*., 2004). As a result, the *E. coli* genome comprises a core genome plus a flexible gene pool that confers fitness features particular to a given pathotype or ecotype. The potential for pathogenicity of strains is determined by their capacity to "accumulate" such fitness features. The 5 PAIs that comprise UPEC strain 536's urovirulence have all been thoroughly analyzed, and the results show that every island improves the strain's adaptation and fitness inside the urinary system. According to an in *vivo* transcriptome study of UPEC, the bladder environment promotes virulence by regulating key virulence components (Hagan *et al*., 2010).

2.4. Uropathogenic *Escherichia coli*

The UPEC strains are part of a small group of clones with certain lipopolysaccharide (LPS; O), capsular (K), and flagellar (H) antigens (O: K: H serotype) expressed. For the first time in the late 1970s, it was shown that *E. coli* strains that cause UTIs often agglutinate human erythrocytes even when mannose is present, with fimbriae playing a major role in this process. Studies have shown that UPEC expresses various surface features and produces unique protein molecules, including cytotoxic ones (Anderson *et al*., 2003).

Adhesion organelles facilitate UPEC entrance and attachment to host urinary tract epithelial cells (Bower *et al*., 2005). A UPEC strain was discovered to include the genes for two potential type IV pili, ten putative chaperone-usher pilus complexes, and at least seven suspected autotransporter proteins. Phase variation, a process driven by environmental signals, allows pilus operons within a bacterial cell to communicate and switch from one pilus kind to another (Holden *et al*., 2007). Due to the wide variety of pilus systems, UPEC has several attachment specificities and the capacity to colonize different locations throughout the urinary tract. In escalating models of UTI, flagella are crucial during UPEC ascent through the bladder to the renal and to drive bacteria through urine and mucus layers. One of the numerous possible virulence determinants that UPEC possesses is the ability to produce biofilm. It is a dynamic process that could result in various physiological occurrences, such as developing virulence factors, antibiotic tolerance, and enhanced resistance to host defensive systems (Schwan, 2008). According to investigations, isolates obtained from urine were better able to produce in vitro biofilms than feces. UpaH, a newly discovered auto-transporter protein expressed at the surface of the cell of UPEC strain CFT073, is demonstrated to aid in bladder colonization and biofilm development (Allsopp *et al*., 2010).

Several described outer membrane iron compound receptors, siderophore biosynthesis systems, and potential TonB-dependent receptors were identified

by the genomic investigation of the UPEC strain CFT073. Up to four distinct siderophores, including enterobactin, salmochelin, aerobatic, and *Yersinia* bactin, are produced by UPEC strains (Henderson *et al*., 2009).

The interaction between E. coli virulence features, phylogenetic background, and antibiotic resistance is a complex phenomena. B2, the most common UPEC biotype, appears to be the only biotype with a relationship between quinolone resistance (acquisition of the gyrA mutation, the most common way to acquire resistance) and low virulence due to a lack of PAIs, resulting from two distinct mechanisms that act simultaneously as proposed by (Piatti *et al.*, 2008). They further suggest that the frequency of group B2 among both uropathogenic and commensal E. coli isolates, regardless of VF carriage is owing to the higher adaptability and genetic 'plasticity' of the species. The absence of fimbrial antigens is irreversible, although it may be appropriate where bacteria, without causing specific damage and by avoiding host defenses, might colonize or cause illnesses and transmit antibiotic resistance, indicating bacterial evolution. In an individual with anatomical defects of the urinary system or a catheter, organisms of low pathogenicity can cause infection, and the above-mentioned properties of the bacteria are not required (Agarwal *et al*., 2012).

2.4.1. Uropathogenic *Escherichia coli* and related virulence

UPEC is responsible for approximately 80-90% of UTIs acquired within the community (Flores-Mireles *et al*., 2015). Based on the existence of genomic PAIs and the expression of virulence components like adhesions, toxins, surface polysaccharides, flagella, and iron-acquisition systems, four primary UPEC phylogroup (A, B1, B2, and D) have been determined. Typically, UPEC needs many virulence features to induce UTI (Flores-Mireles et al., 2015, Terlizzi et al., 2017a). The pathogenesis of UPEC during UTIs consists of the following steps: (a) colonization of the periurethral and vaginal regions with

the colonization of the urethra; (b) ascent into the bladder lumen and growth as planktonic cells in the urine; (c) adhesion to the surface and interaction with the bladder epithelium defense system; d) the development of biofilms; and e) bladder invasion and replication Quiescent intracellular reservoirs (QIRs) arise and reminder in the underlying urothelial in intracellular bacterial communities (IBCs); (f) colonization of kidney and host tissue destruction with a more danger for bacteremia/septicemia (Terlizzi *et al*., 2017b).

The bacterial replicating within the IBC can quickly increase to as high as 10^5 bacteria per cell. Additionally, the bacteria in the IBC experience alterations in their morphology, move out of the infected cell and proceed to infect adjacent cells (Spaulding and Hultgren, 2016). Most invasive bacteria are removed by flushing urine and exfoliated bladder epithelial cells (BECs) filled with UPEC (Terlizzi *et al*., 2017b).

Because UPEC uses several virulence factors to colonize the bladder, they are crucial in the pathogenesis of UTIs. There are also secreted toxins, secretion systems, and TonB-dependent iron-uptake receptors, such as siderophore receptors, as well as surface structural elements like LPS, polysaccharide capsules, flagella, outer-membrane vesicles, pili, curli, non-pilus adhesion’s, and outer-membrane proteins (OMPs) (Figure 2.2). Each element makes an appealing candidate for creating novel medications and vaccines (O'brien *et al*., 2017).

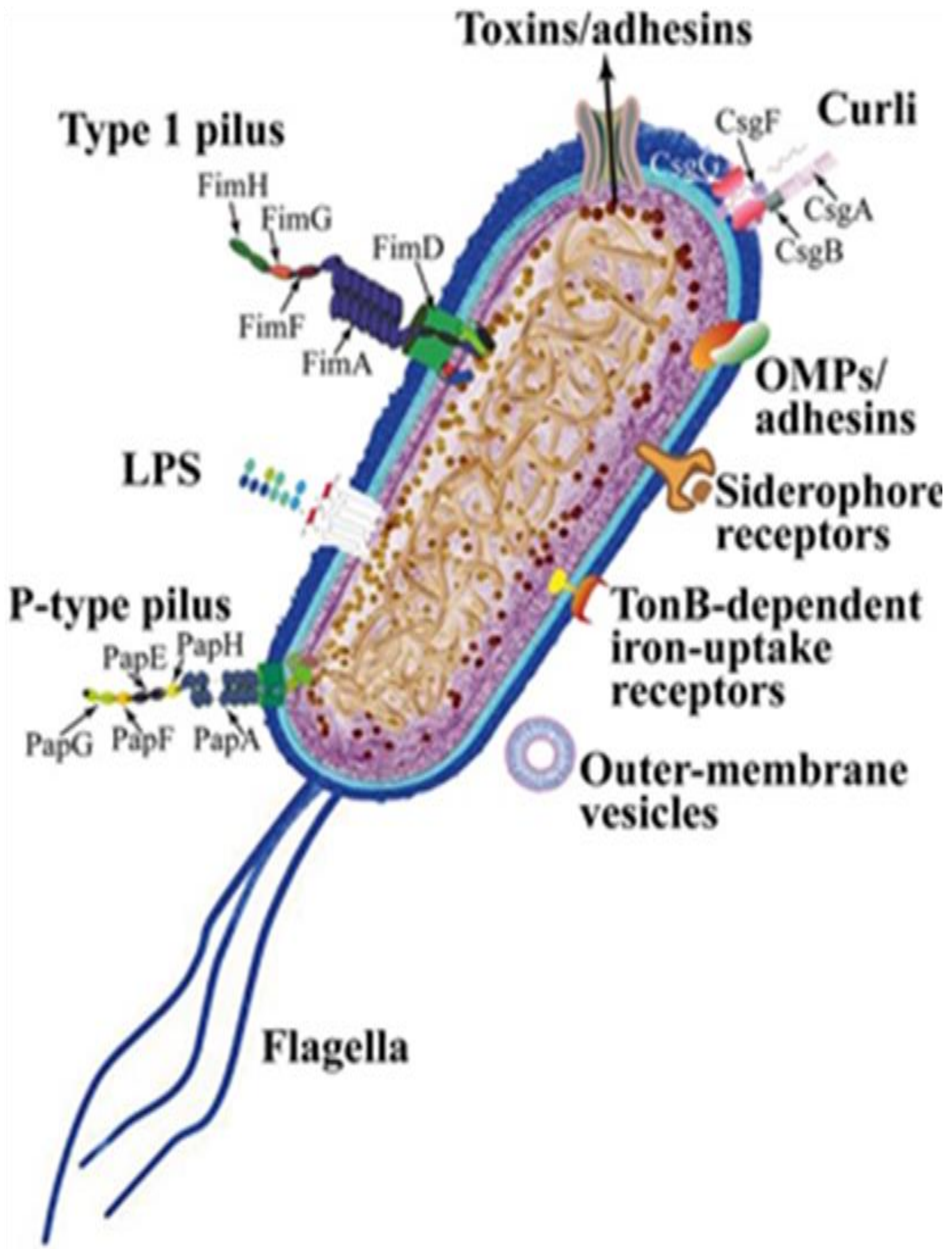


Figure 2.2. *E. coli* adhesins and harboring/motile structures (Terlizzi *et al.*, 2017a).

2.4.2. Host immunological reaction to Uropathogenic *E. coli* infections

UTIs are most usually seen in the bladder. The bladder epithelium has strong barriers and BECs have antibacterial properties. Despite their qualities, BECs and the bladder epithelium are frequently bypassed by UPEC (Wu *et al*., 2017). The urethra is contaminated by bacteria that gradually rise, as was previously mentioned, and this illness typically originates in the gut. Urine is the perfect growing medium for bacteria, allowing them to multiply quickly. Nevertheless, most invaders are flushed out of the body when urinating. On the other hand, bacterial strains can utilize fimbriae organelles to adhere firmly to BECs lining the bladder (Chahales and Thanassi, 2015).

The multi-layered bladder epithelium also referred to as "transitional epithelium," is made up of 3 layers: a basal cells layer (5–10 µm in diameter), intermediate cells in cell layer (20 µm in diameter), a superficial apical layer with huge cells that are hexagonal (diameters of 25–250 µm), which are likewise referred to as "umbrella cells,". Under the basal epithelium, there is a basement membrane.

The umbrella cells exhibit various characteristics, such as specific membrane lipids, asymmetrical unit membrane particulates, and plasma lemma with rigid plaques, and they play a significant function in preserving a barrier against the majority of chemicals found in urine. These plaques can cover up to 90% of the urothelial cell surface, and each plaque is made up of over 1,000 subunits. These components are formed by proteins (uroplakins, UPs), which act as the primary receptors for UPEC adhesion to the host cell and are found in plaques on the apical membranes of mature umbrella cells. There is a link between glycosylation alterations in UPs and various urothelial pathologies such as UTI and interstitial cystitis (Habuka *et al*., 2015).

The activation of the host immune response, which leads to the effector phase involved in bacterial clearance, is a critical stage in the pathogenesis of UTI. In response to UPEC's entry into the typically sterile urinary tract, the

host's innate immune response is activated. It is a quick and powerful process that frequently involves Toll-like receptors (TLRs), which identify diverse microbial products and initiate signaling pathways that finally lead to pathogen clearance from the host and the establishment of a memory response for future attacks (Creagh and O'Neill, 2006).

Cirl *et al*. (2008) have discovered a secreted toxin (Tcps) in the UPEC CFT073 strain that has a Toll/IL1-like receptor domain and suppresses MyD88 (a necessary TLR signaling adaptor)-dependent cytokine secretion in Uroepithelial cultured cells. As a result, human UTIs become more severe. This inhibits innate immune responses. The synthesis of cytokines, exfoliation of infection bladder epithelial cells, residual inflammatory cells, and clearance of bacteria and inflammatory cells are all necessary for the effector phase.

The effector phase also relies on neutrophils' CXCL8/CXCR1-directed migration. UPEC reduces uroepithelial cells' innate immune response because it avoids being recognized by "pattern recognition receptors." As a result, the NF-kB pathway, which controls the production of cytokines, is inhibited and is pro-inflammatory and pro-survival (Mulvey *et al*., 2000).

Reactive nitrogen and oxygen species produced throughout the illness also help to limit the infection. A NO-detoxifying enzyme, flavohaemoglobin generated by UPEC, aids in protecting it from nitrosative stress and may make an appealing target for more current UTI therapy approaches (Svensson *et al*., 2010).

Li *et al*. (2010). found that because epithelial cells produce adequate quantities of DNA damaging agents, a component of the DNA damage repair regulon, SulA, is necessary for UPEC pathogenicity in a mouse model for human UTI. For the repair of DNA damage in UPEC, RecA and LexA coordinate many operons.

Although little is known about adaptive immune responses from UPEC urinary tract infections, they occur. The enhanced susceptibility to UPEC

infection in mice with severe combined immunodeficiency suggests that T- and B-cell-mediated immunity significantly removes bacterial pathogens (Sivick and Mobley, 2010).

2.5. Resistance to antibiotics and antimicrobial agents

The main issue with treating UTIs is that common antibiotic resistance can develop through various processes, including altered efflux pumps, increased permeability of outer membranes, target alteration, and drug inactivation (Mahdavi and Isazadeh, 2019a). In earlier research, it was discovered that *E. coli* isolated from individuals with UTI had greater levels of drug-resistant, particularly to fluoroquinolones and beta-lactam medicines(Albiger *et al*., 2015, Mahdavi *et al*., 2019). Owing to the advent of multi-drug resistant strains and the global rise in antibiotic resistance, numerous investigations have been done to develop natural chemicals as antibacterial agents (Mahdavi *et al*., 2019).

2.5.1. Antibiotic resistant in Uropathogenic *Escherichia coli*

Over the past few decades, there has been an increase in reports of bacteria that cause UTIs that exhibit antibiotic resistance, which has raised serious global health concerns (Terlizzi *et al*., 2017b). *E. coli* is the most frequent cause of UTIs worldwide (Raeispour and Ranjbar, 2018). One of the most frequent ExPEC strains seen is UPEC. MGE such as plasmids, insertion sequences, transposons, and gene cassettes/integrons are the most common MGE by which *E. coli* obtains ABR genes. Sharing several mobile elements, particularly plasmids, that encode resistance among various Enterobacteriaceae members facilitates the dissemination of resistant genes (Conlan *et al*., 2014). MGE also can encode virulent factors, and virulence and antibiotic-resistant may interact (Bunduki *et al*., 2021).

Particularly when findings from the empirical antibiotic treatments of recurrent UTIs, UTIs are related to greater antibiotic utilization that has ramifications for bacterial ecology and propagation of antimicrobial resistance. A clinical issue, particularly in women who experience repeated UTIs, is the development of MDR UPEC and antibiotic resistance in UPEC. MDR UPEC is becoming more prevalent, especially in poor nations. As a result, broad-spectrum antibiotics, including fluoroquinolones, cephalosporins, and aminoglycosides, are used excessively, raising the expense of therapy and hospitalizations (Sanchez *et al*., 2016). Antibiotic resistance among UPEC is rising in various nations, demonstrating the variability of time and place (Figure 2.3) (Kot, 2019b).

The vast majority of the enzymes that can degrade penicillin, third-generation cephalosporins, and monobactams are ESBLs. Antimicrobials were useless as a treatment because they dissolved the β-lactam ring and were covalently attached to their carbonyl moiety (Toussaint and Gallagher, 2015). A bacterium that produces ESBL can only be treated with a very limited number of antibiotics. Carbapenem is the treatment of choice for severe infections brought on by ESBL-producing microorganisms. Studies noted isolates resistant to carbapenem occur (Paterson and Bonomo, 2005).

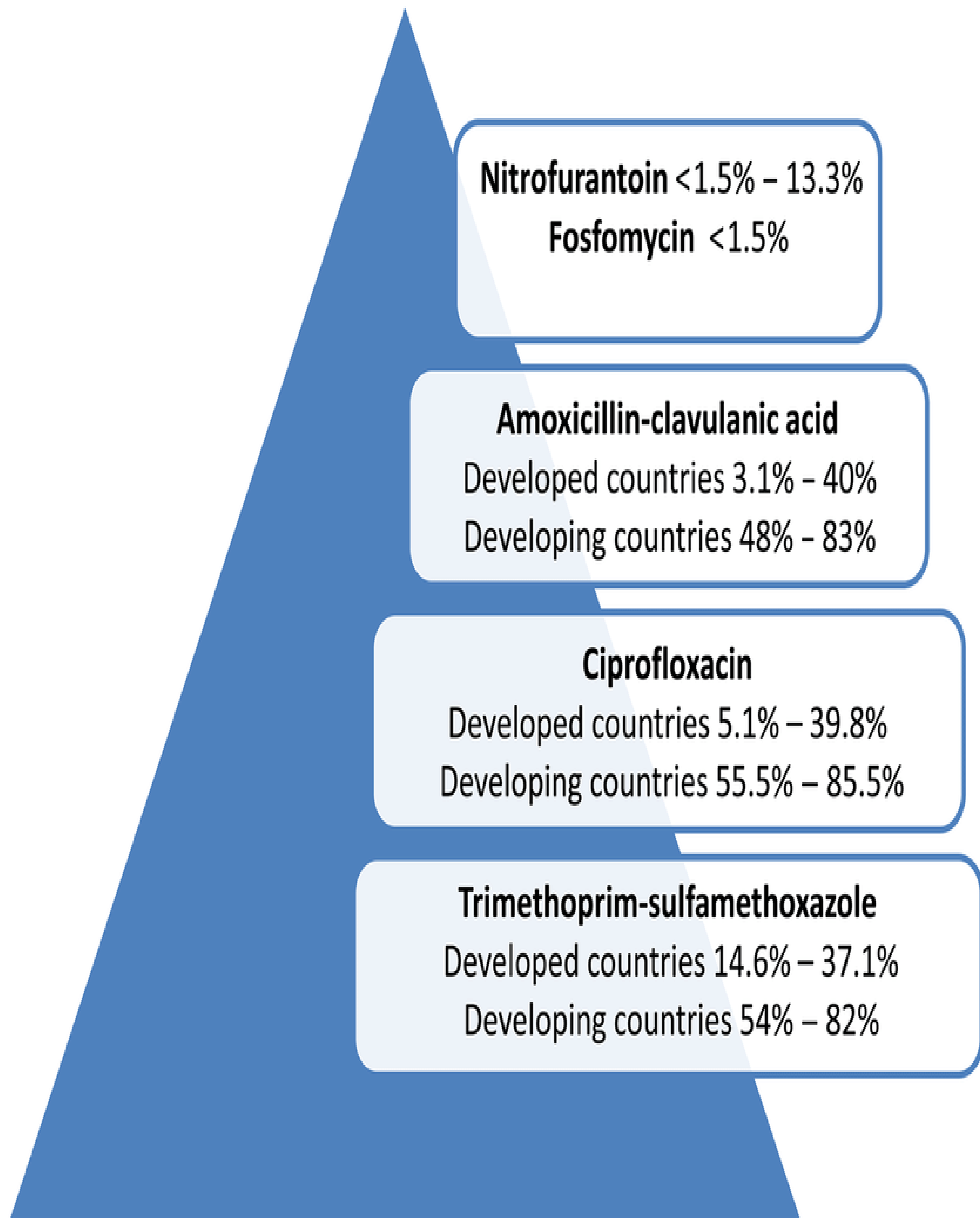


Figure 2.3. The UPEC's resistance to antimicrobials used to treat UTIs (Kot, 2019a)

2.5.2. Mechanisms of antibiotic resistance in UPEC

Synthesis of several β-lactamase enzyme subtypes is associated with resistance to β-lactams. Several forms of β-lactamases (*bla* genes) are frequently found on plasmids (Adamus-Białek *et al*., 2018). The amide bond of the four-membered-β-lactam ring of β-lactam antibiotics (penicillin, cephalosporin, monobactams, and carbapenems) is hydrolyzed by β-lactamases. Except for carbapenems, cephamycins, and β-lactamase inhibitors, ESBLs are enzymes that impart resistance to -lactam antibiotics (all penicillins, cephalosporins, and monobactams) (Kot, 2019b). Because of changes in the genes encoding for the ancestral enzymes $bla_{\text{TEM-1}}$, bla_{TEM}, and bla_{SHV}, ESBL are the main cause of Enterobacteriaceae resistance to 3rd and 4th generation cephalosporins. Among the ESBL produced by UPEC were three classes of β-lactamases, including TEM and SHV, and since 2000, a new category of ESBL, $bla_{\text{CTX-M}}$ (Shahbazi *et al*., 2018). According to genetic analysis, nearly all of the examined strains of UPEC from hospitalized patients in various hospital wards had bla_{TEM} present (Adamus-Białek *et al*., 2018). Ceftazidime is less active than cefotaxime and ceftriaxone in the presence of the $bla_{\text{CTX-M}}$ enzymes (Bhat *et al*., 2012). In underdeveloped nations (Iran - 37.1%, Nepal - 38.9%, Pakistan - 40%, and Jordan - roughly 50%), UPEC-generating ESBL are more frequently found (Ali *et al*., 2016, Parajuli *et al*., 2017, Shakhatreh *et al*., 2019).

Prasada *et al.* (2019) found that the percentage of India's UPEC that produced ESBLs rose from 45.2 to 59.6% over five years. Several regions of the globe, and occasionally even different hospitals inside the same nation, have varying rates of *E. coli* isolates that produce ESBLs.

ESBL-producing *E. coli* isolates resist antimicrobial substances and β-lactam antibiotics, including aminoglycosides, Tetracycline, and Trimethoprim/Sulfamethoxazole (Rezai *et al*., 2015).

Shahbazi *et al*. (2018) have discovered that a greater proportion of UPEC isolates that produce ESBL are resistant to aminoglycosides and quinolones than UPEC strains that do not.

The best choice for treating UTIs brought on by ESBL-producing bacteria is to utilize carbapenems (imipenem and meropenem) (Idil *et al*., 2016). When used with β-lactamase inhibitors, cephalosporins, penicillin, and monobactams are recommended (Bartoletti *et al*., 2016).

2.5.2.1. Extended Spectrum β-Lactamase among Uropathogenic *Escherichia coli*

UTI is one of the most common types of infections, affecting almost half of all adults at some point in their lives. The widespread use of b-lactam antibiotic drugs to treat UTI patients has resulted in the establishment of resistant strains all over the world. ESBL genes, which are typically encoded by plasmids, mediate beta-lactam resistance (Topaloglu *et al*., 2010). The genes encoding ESBLs, a group of enzymes with significant heterogeneity, have been identified. These genes are responsible for conferring resistance to the 3rd and 4th generations of cephalosporins and monobactams. However, β-lactamase inhibitors can successfully inhibit them (Fernandes *et al*., 2014).

The four types of ESBL enzymes are A, B, C, and D. Class A ESBLs include the enzymes Cefotaximase (CTX-M), Temoneira (TEM), and Sulfhydryl variable (SHV). *E. coli* is a significant cause of UTIs and the most common bacteria to create various ESBL types (Topaloglu *et al*., 2010, Fernandes *et al*., 2014). Researchers say 6 and 88% of ESBLs have been found in various healthcare settings, particularly in *Klebsiella pneumoniae* (*K. pneumoniae*) and *E. coli* (Shahid *et al*., 2011). Yet, ESBL-producing *E. coli* is becoming more common, and they are also becoming more resistant, which is regrettably a new trend. Since their discovery, bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ groups have produced more than 400 ESBLs, with 183, 134, and 103 variations,

respectively. These ESBLs are mainly obtained via point mutation (Barguigua *et al*., 2011).

Although TEM and SHV variants were the most common types of ESBLs produced by *Klebsiella* spp., *Enterobacter* spp., and *E. coli*, the nature of ESBL dissemination has changed in years, and *E. coli* strains expressing CTX-M have now replaced TEM and SHV as the most common types of ESBLs (Oteo *et al*., 2010). Bla_{SHV} β-lactamases confer a high degree of resistance to Ceftazidime but not by Cefotaxime and Cefazolin, and $bla_{\mathrm{CTX\text{-}M}}$ β-lactamases are more effective against those antibiotics than Ceftazidime (Shahid *et al.*, 2011). The $bla_{\mathrm{CTX\text{-}M}}$ family was initially introduced in 1992 (Bauernfeind *et al*., 1992). The resemblance between bla_{TEM} and bla_{SHV} is only 40%. Five separate forms of $bla_{\mathrm{CTX\text{-}M}}$ were categorized based on similarity in amino acids (Topaloglu *et al*., 2010, Fernandes *et al*., 2014) to be known. According to geography and the country under study, different $bla_{\mathrm{CTX\text{-}M}}$ subtypes are more or less prevalent in other areas. The most pervasive $bla_{\mathrm{CTX\text{-}M}}$ genotype at the moment is $bla_{\mathrm{CTX\text{-}M\text{-}15}}$ in particular. As previously indicated, the prevalence of $bla_{\mathrm{CTX\text{-}M}}$ type enzyme in recent years has led to a remarkable rise in the epidemiology of ESBLs since 1995 in the majority of the world (Topaloglu *et al.*, 2010). The clinical application of 3rd generation cephalosporins for the therapy of serious infections is extremely threatened by the rising incidence of TEM, SHV, and CTX-M type ESBLs, which also restricts the options for efficient antibacterial medications to carbapenems or colistin (Bali *et al*., 2010).

UPEC strains have developed significant antimicrobial resistance to first-line medicines such as Trimethoprim-Sulfamethoxazole and Ciprofloxacin over the last two decades. Several surveillance studies conducted in Europe, North America, and South America during the 2000s revealed that resistance to these antibiotics was identified in as many as 20-45% of UPEC isolates (Croxall *et al*., 2011).

UPEC has also been linked to an increased amount of ESBL gene carriage. Many plasmid-mediated variants of ESBLs exist, including TEM, OXA, and SHV. Since the year 2000, a novel class of ESBLs known as CTX-M has appeare (Peirano and Pitout, 2010). Since that time, *bla*$_{CTX-M}$ β-lactamases have dominated the ESBL world market. Today's most common *bla*$_{CTX-M}$ genotype is *bla*$_{CTX-M-15}$, which belongs to the *bla*$_{CTX-M}$ family. This class of ESBLs has been linked to a widespread antibiotic resistance pattern to numerous β-lactam drugs, especially penicillin, cephalosporins, monobactams, and carbapenems (Accogli *et al*., 2014). Moreover, strains of *E. coli* that produce *bla*$_{CTX-M}$ are frequently resistant to other significant antibiotic families, like aminoglycosides and fluoroquinolones (Rogers *et al*., 2011). The rising risk of the establishment of pandrug resistance in UPEC is highlighted by the increasing level of antimicrobial resistance in this organism, which is of significant worry since it can restrict the therapeutic options utilized to treat common bacterial illnesses like UTIs (Alqasim *et al*., 2018).

2.5.3. Probiotics and their potential activity

From the Greek word meaning "for life," the word "probiotic" derives. Ferdinand Vergin originally used the term "probiotika" in 1954 to refer to something beneficial to the gut flora (Vergin, 1954). Lilly and Stillwell defined this phrase as "a substance produced by one microbe encouraging the growth of another microorganism" in 1965 (Lilly and Stillwell, 1965). Fuller later described the phrase as "non-pathogenic microbes which, when swallowed, exert a beneficial influence on host's health or physiology" in 1989 (Fuller, 1989).

Moreover, Sperti introduced the word in 1971 to refer to tissue extracts that promote microbial development (Sotillo *et al*., 1971). Parker was the one who first introduced the term "probiotic" to refer to live microorganisms or the products they produce that promote gut microbial equilibrium (Parker, 1974).

Probiotics are described by the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) as live microorganisms that, when taken in sufficient amounts, can have positive health benefits on the host body. Due to their antibacterial qualities, some isolates of LAB are utilized as food preservatives (Kaur *et al*., 2002).

Probiotics are living bacteria or fungi that are taken directly and give the host health benefits. Short-chain fatty acids (SCFAs), which probiotics produce from metabolic precursors, have the same downstream impacts as immune regulation and improved mucosal barrier functioning (Patel and DuPont, 2015).

The most prevalent microbial strains include *Bifidobacterium*, *Lactobacillus coagulans*, *Lactobacillus reuteri*, *Lactobacillus acidophilus* ,*Lactobacillus rhamnosus* and *yeasts* such as *saccharomyces* (Figure 2.4). A single strain or a mixture of two or more strains may be present in probiotic products (Chapman *et al*., 2011). According to the FAO, WHO and European Food Safety Authority (EFSA) guidelines on selecting probiotic strains, the probiotic strains must satisfy safety requirements, functioning standards, and technological usefulness. While functional features of the strain include its survival in the gastrointestinal system and its immunomodulatory qualities, its safety is defined by its origin, lack of connection with infections, and resistance to antibiotics. The utility of technology, on the other hand, is associated with its manufacturing and survival and upkeep of characteristics throughout storage and distribution. Additionally, the Food and Drug Adminstration (FDA) requires that microorganisms utilized for human consumption in the USA have a "Generally Recognized as Safe" (GRAS) status. In contrast, the EFSA introduced the "Qualified Presumption of Safety" (QPS) standard in Europe, which includes additional safety requirements for bacterial supplements (Gaggìa *et al*., 2010).

2.5.4. Lactic Acid Bacteria as potential probiotics

Probiotic LAB is a non-spore-forming, gram-positive, and non-respiratory microorganism that uses the fermentation of carbohydrates to make lactic acid. A major class of microorganisms called *Lactobacillus* is recognized as a probiotic and has lately been investigated as an antibacterial agent for treating human infections. Probiotics are widely known for their antibacterial effects on pathogenic microbes. The synthesis of organic acids (which antagonize pathogens), attachment to pathogens, and decrease in bacterial adherents give probiotics antimicrobial properties (Mahdavi *et al*., 2018a). Since many LABs show probiotic traits, they are considered a major source of potential probiotics. Probiotic LAB is nonpathogenic and keeps the equilibrium of bacteria in the gut (Pringsulaka *et al*., 2015). Tolerance to conditions in the gastrointestinal tract (GIT), survival at low pH, and the health advantages of their ingestion are among the main probiotic characteristics of LAB (Evivie *et al*., 2017). LAB was said to have anti-microbes, anti-cancer, anti-inflammation, anti-overweight, and anti-diabetes impact (Tsai *et al*., 2014, Evivie *et al*., 2017, Wang *et al*., 2019). LAB are utilized in creating fermented food products, and they are crucial to maintaining the organoleptic quality and preservation of food (Sáez *et al*., 2018). It has been possible to isolate and identify probiotic LAB from a variety of sources, including dairy products, fermented foods, fruits, and vegetables (Tamang *et al*., 2016, Abushelaibi *et al*., 2017, Fessard and Remize, 2019).

Instead of being employed as a beginning culture to create aromatic flavoring agents, LAB is starting culture in the food industry that acidifies food with lactic acid (de Souza Motta and Gomes, 2015). Because to their probiotic properties, fermentation ability, and potential for preservation, LAB serve as an useful food ingredient (Fessard and Remize, 2019). For farm animals, probiotic LAB-based feed has been suggested as an alternative to antimicrobials (Vieco-Saiz *et al*., 2019). Safety assessment and probiotic

characterization for commercial uses of LAB as probiotics should be assessed by *in vitro* and *in vivo* screening studies (Koirala and Anal, 2021). LAB can be utilized in creating various fermented food products because it is the most well-known source of probiotics (Anal, 2019).

Furthermore, earlier research revealed that the Lactobacilli bacteria create a number of bactericidal substances. The results revealed that the primary causes of Lactobacillus strains' antimicrobial activity are their synthesis of organic acids and bacteriocins (Mahdavi and Isazadeh, 2019b).

They can employ these microorganisms to manage human infections. Gram-positive, rod-shaped, flagellate-free, non-spores-free, and anaerobic *Lactobacillus* are microaerophilic (Salvetti *et al*., 2012). The majority of LAB species that can turn lactose and other carbohydrates into lactic acid are found in the genus *Lactobacillus*. Both homofermentative and heterofermentative *Lactobacillus* species exist. Homofermentative species primarily ferment carbohydrates into lactic acid more than (90%), but they don't release gas. The opposite is true for heterofermentative organisms, which also create CO_2 and lactic acid from the fermentation of sugar (glucose) (Allen *et al*., 2014). The amount of lactic acid that each species of *Lactobacillus* produces varies, which is a distinguishing characteristic. Interestingly, many Lactobacillus species can create several kinds of lactic acid, including L or D lactate or a combination of the two (Darvishi *et al*., 2021).

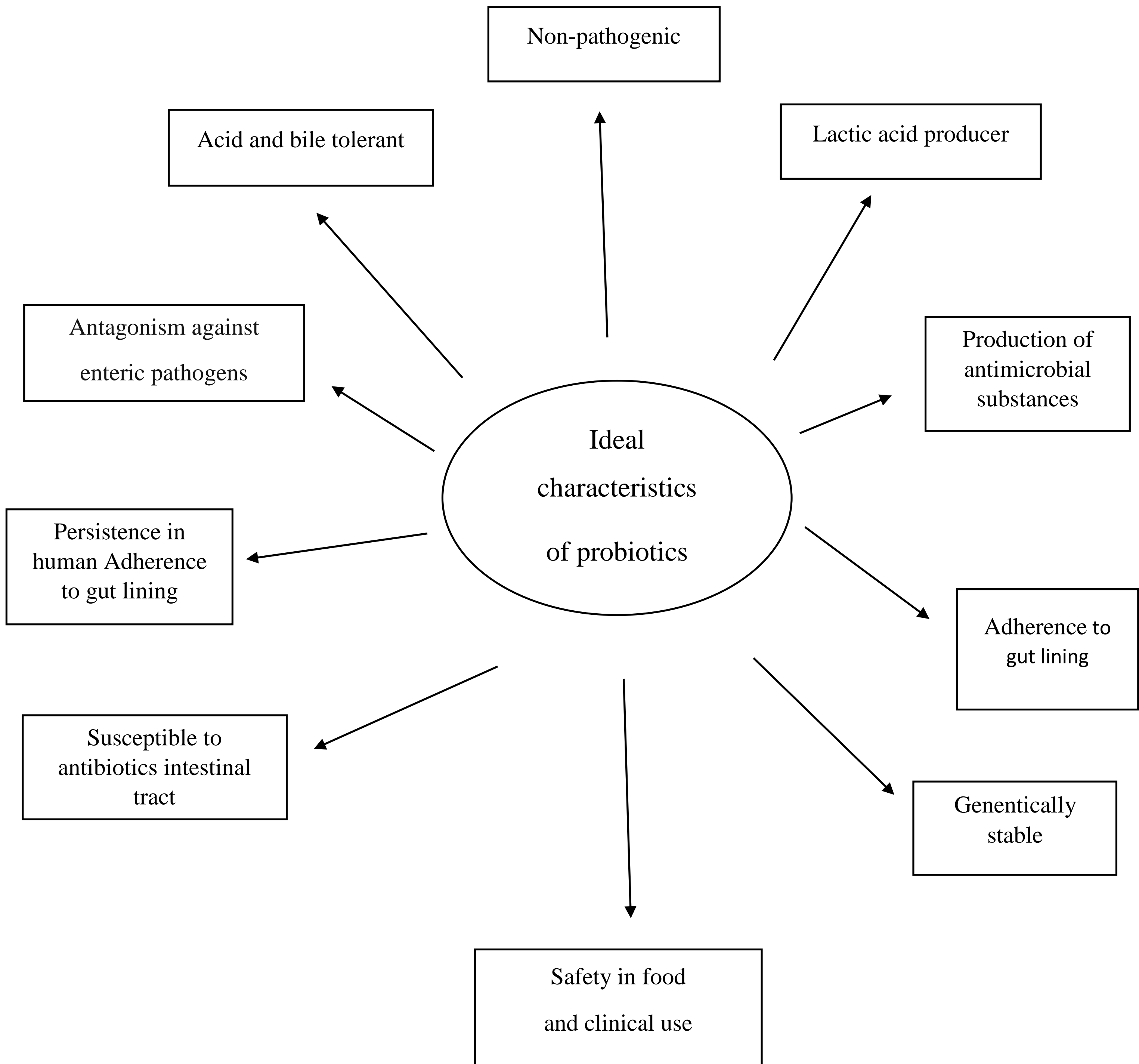


Figure 2.4. Requirements for the perfect probiotic microbe (Goel and Kumar, 2021).

2.5.5. Mechanism of action of probiotics

Probiotics are significant for the growth of the microbiota in the host and for maintaining the equilibrium between bacteria and pathogenic that is required for the host to function normally. B-group vitamins are naturally produced by a small number of microbes, including *L. plantarum*, *L. reuteri*, *Bifidobacterium adolescentis*, and *B. pseudocatenulatum* (Li and Gu, 2016); immunomodulatory enhance vitamin and mineral absorption and permit the creation of organic and amino acids (Nova *et al*., 2007). In addition, some probiotic microorganisms are identified to produce coenzymes (A, Q, NAD, NADP) and enzymes like esterase and lipase. These byproducts of bacterial metabolism also have anticancer genic, immunosuppressive, and antibiotic activities. Based on molecular and genetic studies, probiotic microbes have four main processes that are beneficial to human health: (i) opposition caused by the production of antimicrobial agents; (ii) competition with infections for nutrition and adherence to the epithelium; (iii) host immunomodulation; and (iv) blocking the production of toxins. By preventing pathogens from adhering to epithelial cells, probiotic bacteria assist in stopping the spread of infectious illness. This triggers the signaling cascade or releases some of the substances that trigger the activation of immune cells. Furthermore, increased immunoglobulin production, lymphocyte and macrophage production, and γ-interferon production can all contribute to immunomodulation (Oelschlaeger, 2010, Markowiak and Śliżewska, 2017, Panesar *et al*., 2022).

As well as physically filling the epithelial niche and preventing other pathogens from colonizing the intestinal microbiome, probiotics may also benefit from creating their antibacterial chemicals (Figure 2.5). Probiotics have a more immediate impact on the microbiome than prebiotics, which have an indirect effect through metabolic pathways and the proliferation of commensal microbes. Probiotics are naturally present in fermented foods, including yogurt,

cheese, kimchi, sauerkraut, and commercial goods (Newman and Arshad, 2020).

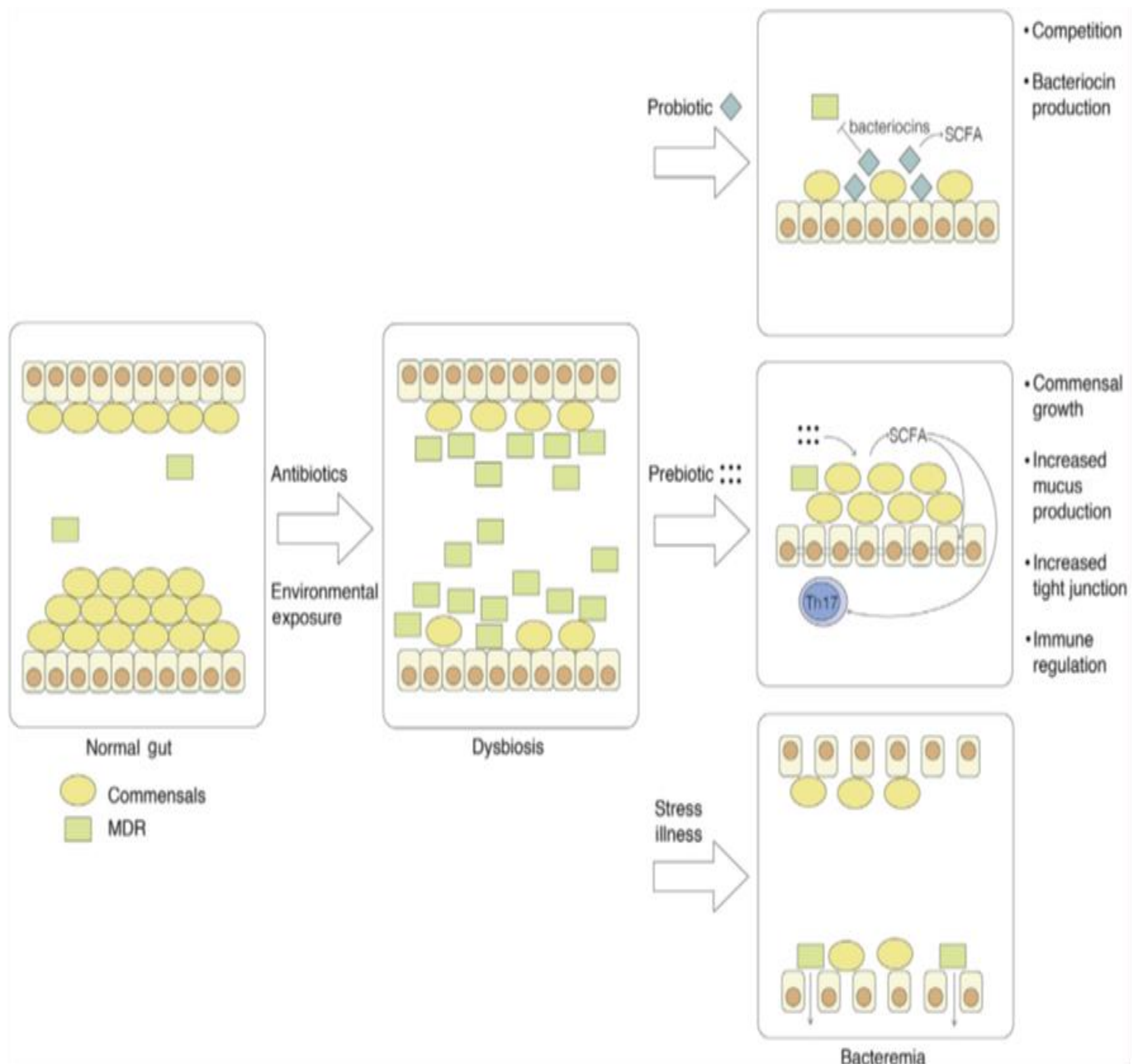


Figure 2.5. Prebiotics and probiotics' mechanisms of action against the colonization of MDROs in the gastrointestinal tract.

The picture shows the process of developing dysbiosis and MDRO colonization, with subsequent figures on the far right detailing the action of probiotics (far right, top) and prebiotics (far right, middle). Without intervention, further stress and illness can allow translocation of MDRO pathogens into the bloodstream (far right, bottom). Th17 ¼ T helper 17 cell (Newman and Arshad, 2020).

2.5.6. General features of probiotics

Probiotics' core function is to promote "balance" in the development and bioactivity of the good bacteria that live in our intestines while minimizing the bad ones. The primary objectives of probiotics are to strengthen their hosts' immunological and digestive systems, but strain-specific health advantages such as the generation of bio-actives are also important (Gibson *et al.*, 2017). During passing through the Gastrointestinal system, a probiotic should be taken at a dosage of approximately (10^8–$10^{9)}$ colony-forming units (CFU) per day to be active. Probiotics' key issue is the strain's capacity to survive through the various producing phases. The first-generation probiotics' vitality ranged from 7 to 30% because they were only available as living and lyophilized bacteria cells and had no additional protection. The release of the probiotic strain was the main problem in having the biological activity; thus, the lyophilized beneficial bacteria were more encapsulated to raise their longevity to 80%. These were commonly known as 2nd generation probiotics. As a result, the method was enhanced to produce 3rd-generation probiotics, in which the encapsulated probiotic strains are liberated during the metabolically active phase when the microcapsules are damaged. Fourth-generation probiotics were created by further refining these first-generation probiotics. In these probiotics, bacterial cells are found in biofilms, which increase their survival and vitality. Many bacteria are employed as probiotics today because they positively impact human health. These bacterial communities are mostly LAB (Mohan *et al.*, 2017).

Investigations on distinct probiotic strains have produced varying conclusions, such as the need for particular traits in the strains employed for positive effects. They are not pathogenic in this sense and, under normal circumstances, fall within the GRAS group. Probiotic bacteria's major traits include a) surviving in environments where bile salts and stomach acids are present, b) the ability to attach to the gastrointestinal tract's mucosal surface

and prevent leaking from Smokey bowel movements, c) the production of antibacterial compounds, having an antagonistic impact on a particular pathogen, etc., d) drug resistance and e) the capacity to activate the immune response without inflaming the body (Fijan, 2014).

The probiotics' capacity to modify the intestinal microbiota to combat pathogenic microorganisms is one of their most well-studied effects. The probiotic microorganisms produce a number of metabolites, including the short-chain fatty acids (lactic, acetic, formic, butyric and propionic acids), that are known to prevent diseases like *Salmonella enterica serovar Typhimurium* and *Clostridium difficile* (Bermudez-Brito *et al.*, 2015). The colonic lumen's pH is said to be healthy by SCFAs, which considerably raise the digestion of foreign substances and carcinogenic residuals in the gut. Additionally, probiotics create a variety of pathogen-reducing inhibitory substances, including bacteriocins, peptides, diacetyl, organic acids, and peroxide of hydrogen. Most probiotic metabolome constituents are in metabolism processes that control cellular pathways for proliferation, distinction, and apoptosis. Moreover, probiotics stimulate the host's defense mechanisms against pathogens, including releasing chemicals that reduce inflammation (Figure 2.6) (Goldenberg *et al.*, 2017).

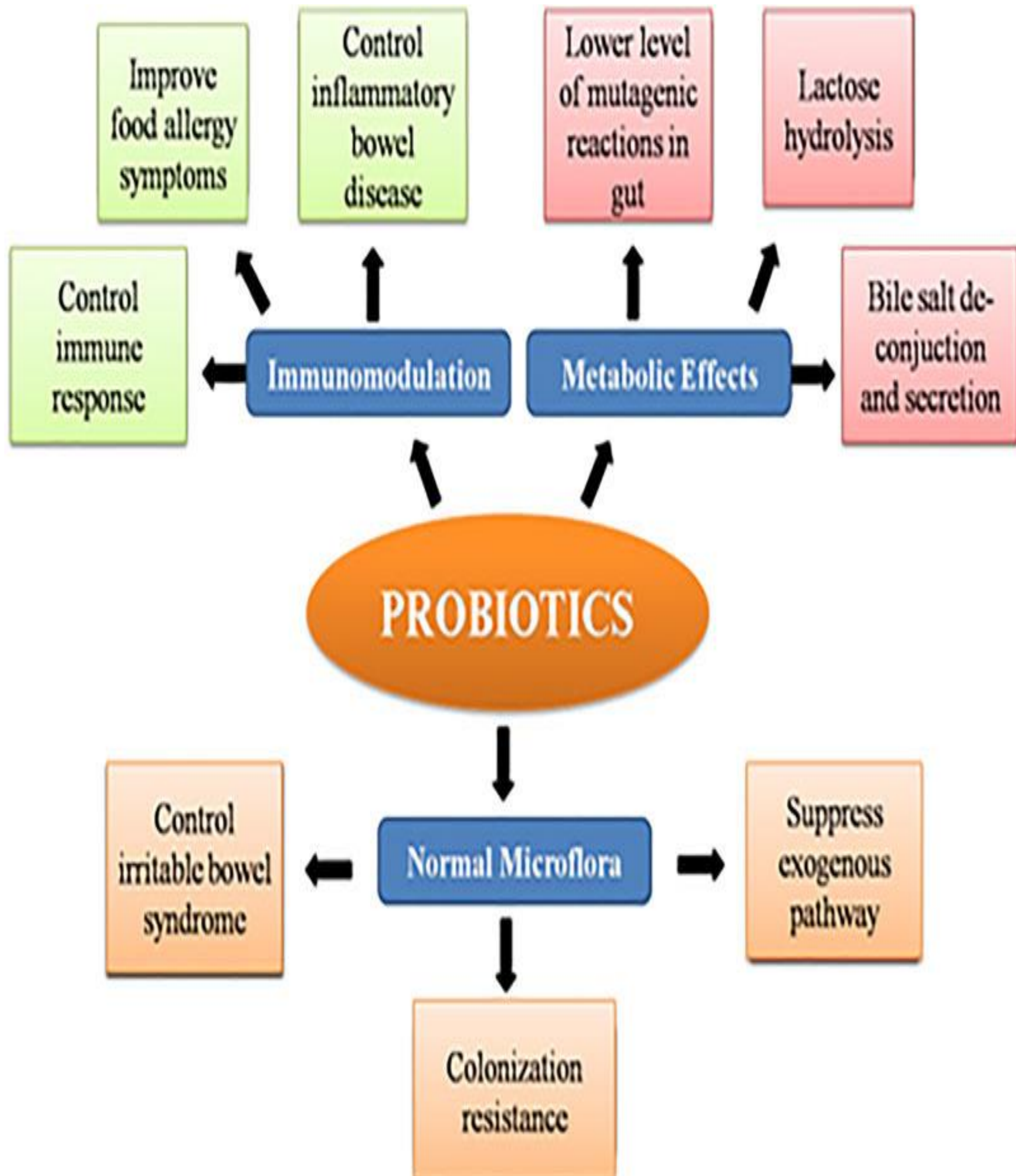


Figure 2.6. Probiotics' impact on the typical microbiota, immune regulation, and metabolic impacts (Yadav *et al*., 2020).

2.5.7. Potential benefits of probiotics in reducing antibiotic resistance

To enhance the effectiveness of antibiotics, it may be possible to leverage probiotics' synthesis of bacteriocins to reduce the number of pathogenic bacteria at mucosal locations and to destroy biofilms (Lin *et al*., 2015).

The generation of antibacterial substances like bacteriocins, competitive elimination of pathogens, improvement of the role of the intestinal barrier in resisting pathogenic bacteria, and enhancement of the host immune response are all examples of the probiotics' antibacterial or antagonistic activity, which is a crucial characteristic (Fijan, 2016).

Additionally, probiotics could enhance mucosa, the body's defense which helps to eliminate pathogen organisms at the mucosal location. Although the majority of bacteriocins activity has been recorded *in vitro*, there is still a lack of evidence that bacteriocins are also efficient *in vivo*, especially in humans where doubling blind randomized controlled trials remain rare, particularly regarding in-situated production of efficient bacteriocins by probiotics (Lin *et al*., 2015).

Probiotics have not yet been shown to be effective in stopping resistance to drug illnesses in people. Although some investigations have demonstrated an advantage, the Centers for Disease Control and Prevention (CDC) is actively studying the matter because the data is not yet final enough to issue particular suggestions for improvement. Probiotics could thus assist in curing resistance to multiple UTIs by acting as an adjuvant or partial substitute to therapy with antibiotics. Most research on resistant antibiotics has focused on harmful bacteria in nosocomial conditions, including nursing homes and hospitals. However, drug resistance is a much larger issue, so it's critical to research antimicrobial resistance in both aquaculture and food animal manufacturing. As mobile genetic elements frequently contain antimicrobial resistance genes, it is crucial to understand how these elements migrate throughout the entire food ecosystem. Even though antibiotic growth promoters (AGPs) were

outlawed in Europe in 2006, they are still employed in other regions of the globe. Moreover, antimicrobial agents are still often utilized in European veterinary medicine to treat sickness in livestock. More focused actions are required to lessen the selection pressure on the emergence, evolution, and dissemination of antibiotic resistance because we need to understand the ecology of resistance. The utilization of probiotics in the feed may help to improve animal health and slow the emergence of resistance to antibiotics (Ouwehand *et al*., 2016).

2.5.8. Side Effects of Probiotics

Prebiotics carry less risk than probiotics do. Probiotics are live organisms that are directly injected into a host; thus, there is a chance that these colonies could change from beneficial commensals to overt pathogens (Cohen, 2018). Probiotic bacteria also have a protective effect on the gut flora when taken orally. Probiotics have been proven to have numerous positive benefits on the host body throughout the diseases produced by the gut microflora after many researches and investigations, but their medical application is yet unknown. Probiotics could shield the hosts from various intestinal disorders, significantly increasing the number of good bacteria in the gut. Even though probiotics have a wide range of advantages, some precautions must be taken while using the correct doses of probiotics based on the person's age and health problems to avoid the hazards related to probiotic utilization (Acharjee *et al*., 2022).

Probiotics' biological effectiveness is supported by growing research; however, caution should be taken when considering whether to utilize these microbes in clinical settings. The FAO and WHO 2002 released guidelines for assessing Probiotics in Food. These recommendations created safety and efficacy requirements by systematizing the discovery and selection of probiotics. As a result, the FAO and WHO recommendations indicate a number of factors that should be considered when choosing probiotics, including

resilience to unfavorable conditions in the human body, the capacity to adhere to epithelial tissues, antibacterial activity, and safety. A probiotic strain's origin, absence of connection with pathogenic cultures, and antimicrobial resistance profile all contribute to its protection (Silva *et al*., 2020b). Enhanced epithelial barrier, increased adherence to the intestinal mucosa, inhibition of microbial adhesion, competitive exclusion of pathogenic bacteria, generation of antibiotic compounds, and immune system regulation are among the main biological mechanisms of action of probiotics. The schematic diagram in (Figure 2.7) depicts how these mechanisms work in the intestinal mucosa (Bermudez-Brito *et al*., 2012).

Probiotics have long been used in a safe manner. Little elevated hazards of negative consequences have been discovered by meta-analysis. Abdominal pain, nausea, lack of appetite, headaches, and flu-like signs are a few side effects documented in Randomized Controlled Trials (RCTs) investigating probiotics' use in antibacterial-associated diarrhea therapy. The signs, however, are most typically the result of underlying infections or the adverse effects of medication. Potential dangers have been shown by data from clinical studies, trials, and experimental models (Doron and Snydman, 2015).

Probiotic strains may vary in their safety profiles and clinical effects. This is an essential fact to remember. The additional ingredients of that product, in addition to the probiotic organism employed in the commercial preparations, affect how safely that product should be used. Systemic infections, colon ischemia, excessive inflammation, gene transfer between probiotic bacteria and microbiome bacteria, and gastrointestinal adverse effects, including cramping and nausea, can all be brought on by probiotics. Despite the rarity of these findings, it is advised that researchers who perform probiotic clinical studies actively monitor for side effects and identify any particularly vulnerable patients (Kopacz and Phadtare, 2022).

Because probiotics are widely used, probiotic safety needs more consideration. Probiotics have been shown to impact the health of the host negatively, most notably by (i) pathogenicity and infectivity: probiotics could translocate from the digestive system to the intestine or extra-intestinal tissues like lymph nodes in the local area, livers, and spleens, causing systemic illnesses if the host intestine is damaged and suppression of the immune system occured; (ii) hazardous metabolic processes: 4070 nmol/mL of histamine is excessively high in MRS broth lead to the production of amino acid decarboxylase by a few strains of *Lactobacillus* and *Enterococcus*, which converts tyrosine and histidine into tyramine and histamine. Histamine or tyramine consumption at specific quantities can result in heart failure, urticarial, hypertension, migraines, brain hemorrhage, and cramping in susceptible people; (iii) probiotics have reportedly been linked to an elevated danger of allergic reactions such as atopic sensitization, wheezing bronchitis, rhinitis, or asthma; (iv) overactive immunological response: *L. casei*, a probiotic, can balance the (Th1/Th2) response as well as promote the (Th1) responding, and overstimulation of the Th1 response can worsen autoimmune illnesses in the body; (v) gene transfer that causes resistant to antibiotics: in animals such as mice, *Lactobacillus* and *E. faecalis* could transfer plasmid-based vancomycin genes that are resistant. In the intestines of mice, *E. faecalis* can transmit the plasmids containing kanamycin-resistant genes and the transposons with tetracycline and erythromycin-resistant genes to *E. coli* and *Listeria monocytogenes*. The name "probiotic" in and of it self, it is claimed, corresponds to a health claim; however, the EFSA does not recognize it because the effect claimed cannot be measured (Li *et al*., 2020).

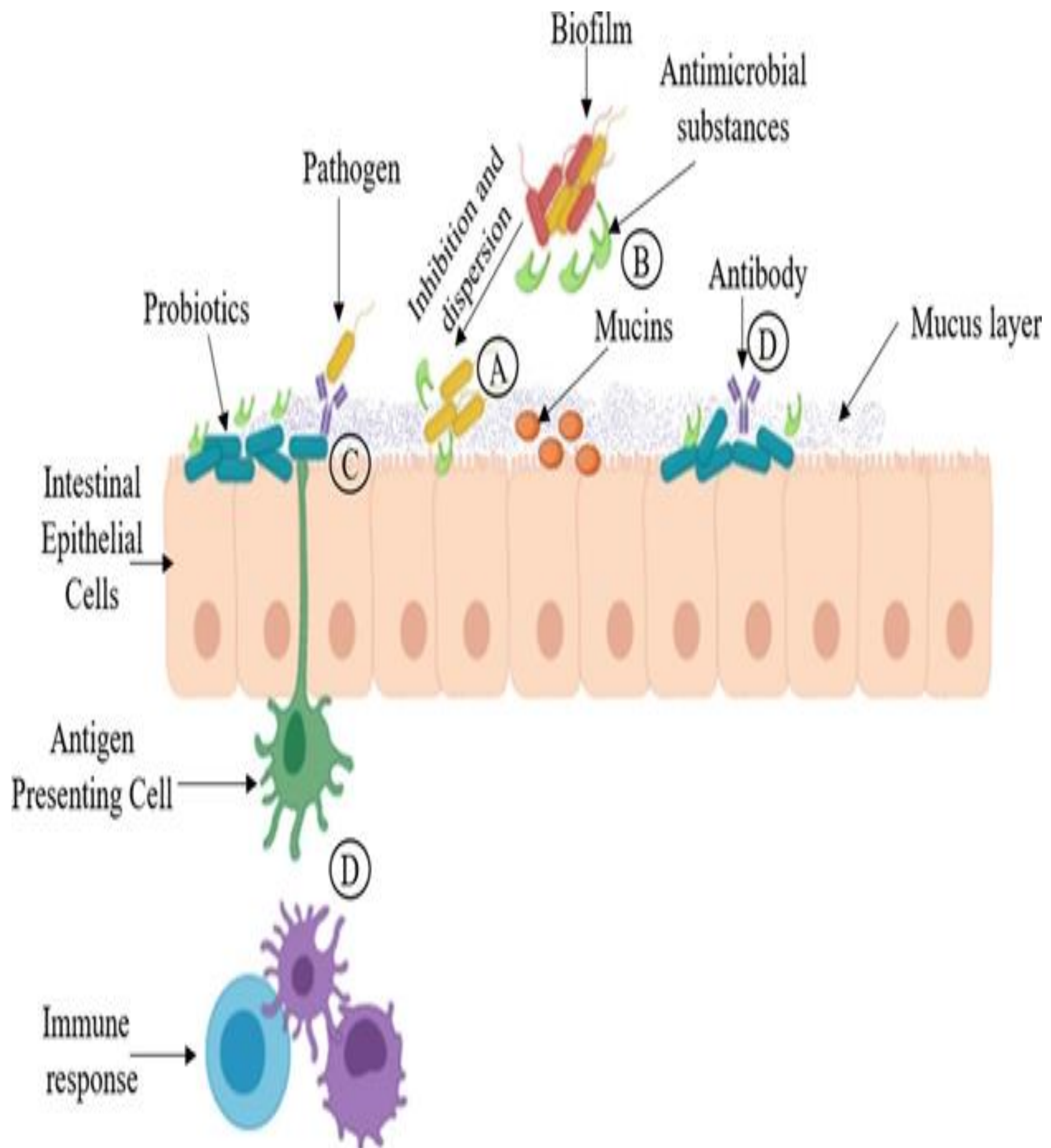


Figure 2.7. Probiotics' mechanisms of action. (A) Pathogenic bacteria are excluded through competition. (B) The creation of antibacterial agents. (C) Enhanced intestinal mucosal adhesion and enhanced epithelial barrier. (D) Immune system stimulation (Silva *et al*., 2020a).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. Equipment and apparatus

The equipment, instruments, and manufacturer lists utilized in this study have been condensed to (Table 3.1).

Table 3.1 Apparatuses and equipment, company, and origination.

Apparatuses	Company	Origin
Autoclave	Memmert	Germany
Centrifuge	Eppendorf	Germany
Bunsen burner	WLD-TEC	England
Hood	BioTech	USA
Thermal cycler PCR machine	Alpha max	UK
Vortex	Memmert	Germany
Mini spin	Memmert	Germany
Micropipettes	Eppendorf	Germany
Water bath	BioTech	Germany
Gel electrophoresis system	BioTech	Germany
Light Microscope	OPTIKA	Italy
Disposable Petri dishes	BIOZEK	Netherlands
VITEK system, glass, test tubes, Gram-negative ID Card	BioMerieux	France
Rack	Solar bio	life sciences
Refrigerator	LG	Korea
Sterilized cotton swabs	Indiamart	India
Incubator	Fisher Scientific	USA
Rack PCR (0.2ml-1.5ml)	Solarbio	life sciences
Icebox	Tank	Egypt
Eppendorf tubes tips	Sterelin Ltd	UK
Urine container	BIOZEK	Netherlands
Plane tubes	BIOZEK	Netherlands
Sensitive balance	Sartorius	Germany

3.1.2. Chemical materials and bacterial cultured media

A list of the chemicals, culture media and molecular kits with reagents used in the studies and those that their manufacturers declared can be stated in (Table 3.2) and (Table 3.3).

Table 3.2 Chemical materials and culture media, company, and origination.

Chemical materials and culture media	Company	Origin
Gram stain set	ATOM SCIENTIFIC	UK
Absolute alcohol	Sharlau	Spanish
Agarose	Geneon	Germany
Distilled water	Natural	Turkey
MacConkey agar	Lab M Neogen	UK
Blood agar base	Oxoid	England
Nutrient agar	Oxoid	England
Nutrient broth	Oxoid	England
LB broth	Lab M Neogen	UK
Mueller Hinton agar	Oxoid	England
MRS agar	Oxoid	England
MRS broth	Oxoid	England

Table 3.3 Commercial kits and reagents were employed for the molecular analysis.

Molecular kits and reagents	Company	Origin
DNA Extraction kit	GeneAll® ExgeneTM	South Korea
Safe stain	Biolabs	USA
Primers	Macrogen	South Korea
Free nuclease water	Thermo Fisher Scientific	USA
Buffer TAE (Tris-acetate-EDTA Buffer)	GeNet Bio	South Korea
DNA ladder (Marker)	GeNet Bio	South Korea

3.2. Methods

3.2.1. Culture Media Preparation

The instructions provided by the manufacturer were followed to prepare the culture media. Subsequently, the media were autoclaved at a temperature of 121°C for 15 minutes, as detailed below:

3.2.1.1. MacConkey agar

To isolate the majority of gram-negative bacteria and distinguish lactose fermenters from non-lactose fermenters, use MacConkey agar without Crystal Violet (De la Maza *et al.*, 2013). This medium was made by suspending 52.0 g of the substance in 1000 mL of distilled water, heating it while agitating frequently, boiling it for one minute to dissolve it completely, and then autoclaving it for 15 minutes at 121°C.

3.2.1.2. Blood agar

Blood agar base was an enhanced medium used to isolate, identify, and discriminate hemolytic bacteria. It was made by dissolving 40.0 g of blood agar base in 1000 mL of sterile distilled water, autoclaving it, and then adding 5% blood after cooling to 50°C. Blood agar was added to the plates and inoculated with bacteria, which were then incubated at 37°C for 24 hrs. and evaluated for hemolysis zones surrounding the colonies (Jorgensen and Turnidge, 2015).

3.2.1.3. Nutrient agar

To purify bacteria, keep pure slant medium, and perform a conventional platform process, dissolve 28.0 g in 1000 mL of sterilized distilled water and autoclave (Rees *et al*., 2009).

3.2.1.4. Nutrient broth

This medium was used to cultivate and stimulate the development of bacteria. The preparation involved melting 8.0 g of the substance in one liter of sterile distillate water, followed by autoclaving (Rees *et al*., 2009).

3.2.1.5. LB (Luria-Bertani) broth

In a lab setting, LB broth is utilized in molecular genetic research. To isolate pure recombinant strains, this nutrient-rich broth was created. To prepare the media, 20.0 g of the substance was dissolved in one liter of sterile distillate water and thoroughly mixed, as well as then the pH was corrected to 7.2 after autoclaving at 121°C for 15 minutes to sterilize it (Asitok *et al*., 2022).

3.2.1.6. Mueller Hinton agar

The suggested medium for antibacterial susceptibility testing is Mueller-Hinton agar. Dissolve 38.0 g of the substance in one liter of sterilized distilled water to prepare it. After sterilization, it is heated to a boil to dissolve the media completely and Cooled to 45–50°C. Stir thoroughly, then transfer to sterile Petri dishes. Note: This batch's performance was evaluated and standardized in compliance with the most recent clinical laboratory standard institution (CLSI) document (Rees *et al*., 2009).

3.2.1.7. MRS (De Man, Rogosa, and Sharpe) agar

MRS agar is applied to identify and enumerate Lactobacillus in various substances, such as dairy products, other food items, and goods intended for animal feed (Metras *et al*., 2020) and suspended 52.0 g in one liter of distilled water at 60°C. Mix until thoroughly dissolving, dispense into final containers, and sterilize using an autoclave at 121°C for 15 minutes.

3.2.1.8. MRS (De Man, Rogosa, and Sharpe) broth

To aid in the growth of lactobacilli and other organisms, MRS broth includes sources of carbon, vitamins, and nitrogen (Ayad *et al*., 2020). Suspended 52.0 g in one liter of distilled water at 60°C. Mix until thoroughly dissolving, dispense into final containers, and sterilize using an autoclave at 121°C for 15 minutes.

3.2.2. Collection of samples

We prospectively analyzed and recorded 54 positive samples of urine taken from cases ages 1-65 with symptomatic UTI. Samples were collected from the outpatient department in Erbil hospitals (Erbil Teaching Hospital, Rzgari Hospital, Raparin Hospital, and Bio center in Erbil City) from 1st October 2021 to 1st April 2022 for collection and isolation of *E. coli* samples.

After instructing the patients to wipe the perineum and avoid touching between urine and skin, a mid-stream urine (MSU) sample was collected (30 mL of urine) in sterilized screw-capped tubes. Urine specimens were obtained and brought to the lab within 30 minutes for microbiological analysis (Forbes *et al*., 2007). The samples were refrigerated at 4 °C in case of delays (Diks *et al*., 2019).

3.2.3. The preservation of bacterial isolates

Following incubating the single bacterial colony on a plate of nutrient-agar at 37°C, the visible growth was collected and transferred to 1.5 mL Eppendorf tubes. These tubes contained 1.0 mL of 20% glycerol for sterilization. The tubes, together with the slants, were preserved at -20°C. Each plate received one mL of nutrient broth applied to its surface (Sultana and Hossain, 2022).

3.2.4. Bacterial culturing

The calibrated loop was placed vertically after the urine sample had been properly mixed, and the urine specimens were then cultivated on MacConkey agar and blood agar base (for first isolation of Enterobacteriaceae members) and subsequently incubated at 37°C for 24 hours (Al-Hadithi, 2018).

3.2.5. Phenotypic identification of the isolates

Based on culture, morphological, and biochemical features, phenotypic identification of the isolated from pure colonies was carried out (Tille, 2015), and confirming VITEK 2 system employing GN cards (ID).

3.2.5.1. Morphology of the colony and microscopic examination

The primary characteristics of colony morphology (color, shape, edges, size, texture, etc.), as seen on the surface of MacConkey plates, were used to perform the initial identification of isolated bacteria. The colonies were also investigated by Gram staining to detect the isolates' Gram reaction and morphology.

Cultures were stained using the Gram method after 18 to 24 hours of incubation. A loopful of overnight cultures was air-dried before being heated and fixed on a glass slide. Applying a dye of crystal violet 0.3% and allowing it is sitting for a min was sufficient at that point. A moderate stream of water was used for rinsing to remove any remaining visible stains. After that, an iodine solution with a concentration of (0.4% in g) was utilized and permitted to sit for thirty seconds before being rinsed. Following an ethanol (95%) rinse, the dye was stained for 1 min with safranin (0.4%), a counterstain. The excess staining was then removed by washing this for five seconds with water. Under a microscope, the bacteria's color revealed whether it was Gram-negative or Gram-positive. A magnified lens would reveal pink if the cell were Gram-

negative bacteria. Later, isolated single pink colonies that were lactose fermenters were selected off MacConkey plates and transferred to fresh MacConkey agar plates for further purifying using dilution streaks to isolate single colonies, which were then utilized for additional diagnostics. *E. coli* isolates were detected utilizing conventional techniques and biochemical analysis (such as catalase, oxidase, the indole test, Vogues Proskauer, methyl red, citrate utilization, etc.), and their results were compared to the identifying system proposed by Mahon *et al*. (2022).

3.2.5.2. Identification of isolates by VITEK 2 System

Biochemical methods were also used in the VITEK system to identify isolates (Doğruöz-Güngör *et al*., 2019). Per the supplier's guidelines, identification with the VITEK 2 scheme was made using ID-GN cards. The ID-GN cards include biochemical tests, decarboxylase tests, sugar fermentation, sugar assimilation, carbon source utilization and enzyme activities. The cards were inoculated using a vacuum machine with 0.5 McFarland standard suspensions of the organism made from an 18–20 hours-old MacConkey agar plate, and they were, after that, automatically sealed and manually processed placed within the VITEK 2 reader inoculator module. Fluorescence was measured every 15 minutes, and the results of identification were interpreted by Vitek 2 software, and final results were acquired and printed automatically.

3.2.6. Molecular identification of the isolates

All UPEC isolates were submitted to molecular analysis to establish the further identities of isolates. The unique 16S rRNA genes were used for this purpose and three genes were found in each isolate and confirmed a bacterial diagnostic feature utilizing PCR assay.

3.2.6.1.1. Genomic DNA extraction

3.2.6.1.1.1. Sample preparation

The Clinic Cell SV Small Kit (Songpa-gu, Seoul, KOREA) is a product manufactured and has been used for Genomic DNA extraction from pure cultures through the GeneAll® Exgene™. By incubating the culture sample at 37°C for 12-24 hours and shaking it dynamically till these cells reach the log phase, It's accepted for bacteria cells to grow. The cells of bacteria that were obtained could be utilized immediately or stored at (-20°C or -80°C) for future utilization.

1. A 1.5 mL Eppendorf tube should be centrifuged for 1 minute at the highest quick-to-harvest cells (up to 2.0×10^9 CFU/mL). Eliminate the supernatant. Maybe corresponding to $1\sim2\times10^9$ cells is 1~2 mL of an overnight culture of bacteria (A_{600}=1).
2. The cell pellet must completely dissolve with 200 µL of buffer CL.
3. Place a previously used Eppendorf tube with 20 µL of Proteinase K solution (20 mg/mL). To ensure thorough mixing, vortex the mixture quickly. Followed by incubation for 15 minutes at 56°C. After the lysis steps are finished, the mixture will turn transparent from clouds. If the lysate is still cloudy or turbid after this period, it must be incubated until it is clear and free of particulates.
4. To remove any droplets that may have accumulated on the interior of the lid, gently spin the tube in a downward motion.
5. The mixture was added with 200 µL of Buffer BL, vortexed to mix thoroughly.
6. The tubes were allowed to incubate for 10 minutes at 70°C. Spin downward to remove any drips from the lid's interior for a brief period.
7. Two hundred µL of absolute ethanol was added to the tube, pulse vortex to mix the sample thoroughly, and spin down temporarily to eliminate any

drops from inside of the lid. Transfer all the mixture to the SV column carefully, centrifuge for 1 min at >8,000 rpm, and replace the collection tube with new one. If the mixture has not passed completely through the membrane, centrifuge again at full speed (>14,500 rpm) until all the solution has passed through.

8. After adding 600 µL of Buffer BW, the collecting tube was replaced and centrifuged for one minute at speed greater than 8,000 rpm. Repeat this process if there are colorful deposits on the SV column after centrifugation until the deposits are gone.
9. Seven hundred µL of Buffer TW was added. Centrifuge for 1 min at >8,000 rpm. Remove the pass-through and re-insert the SV column into the collection tube, following the manufacturer's instructions. Using full-speed centrifugation will not have any effect on DNA extraction.
10. Centrifuge at 14,500 rpm for 1 min to eliminate remaining wash buffer. Place the SV column into a fresh 1.5 mL Eppendorf tube. Centrifugation necessity be performed at full speed (14,000 ~ 20,000 rpm).
11. One hundred µL of Buffer AE was added and incubated at room temperature for one minute and centrifuged at 14,500 rpm for one minute.

3.2.6.1.1.2. Detection of Extracted DNA

Agarose gel electrophoresis was used to evaluate the extracted genomic matter in *E. coli* before the PCR run. 0.8% Agarose gel was used and ran on 85 V for 45 minutes (Bakr *et al*., 2021).

3.2.6.1.1.3. Determination of DNA concentration

Once the instrument has been calibrated and the initial blank has been adjusted to zero absorption, the concentration and purity of DNA can be measured, The Nano Drop spectrophotometer was ready to obtain the Optical

density (OD) of the DNA samples, and an assessment of the concentration and purity of the extracted genomic DNA was carried out using one µL of the sample. When the ratio is between 1.8 and 2.0, it suggests the DNA specimen is pure, and when it is higher than 2.0, it contains RNA. With the genomic DNA extracted, a ratio of less than 1.8 suggests the presence of protein.

3.2.6.1.2. Polymerase Chain Reaction (PCR)

3.2.6.1.2.1. Preparation of primers for PCR

Required from Macrogen (Korea), the primers mentioned in Table (3.4) and utilized in the present study were produced by adding the suggested volume of free nuclease water indicated in the datasheet to lyophilized primers to make 100 µM (stock solution). As an appropriate solution for the PCR process, a 10 µM concentration was then created. Every primer aliquot was stored at -20°C.

Table 3.4 The six primers utilized in this study

Target genes	The sequence of the primers (5' to 3')	Size of the product by base pairs	Reference
16S rRNA	F- AGT TTG ATC MTG GCT CAG R- GGA CTA CHA GGG TAT CTA AT	797	(Ugbo et al., 2020)
bla$_{TEM}$	F- ATGAGTATTCAACATTTCCGTGT R- TTACCAATGCTTAATCAGTGAGG	861	
bla$_{CTX-M}$	F- AACGCACAGACGCTCTACC R- GGGTAGCCCAGCCTGAAT	517	
bla$_{SHV}$	F- TCGTTATGCGTTATATTCGCC R- GGTTAGCGTTGCCAGTGCT	868	

3.2.6.1.2.2. Amplification of DNA

It's an improved ready-to-use 2× PCR mixture made up of DNA polymerase called Taq, gel-loading dyes, deoxynucleotide triphosphates, PCR buffer, and Novel green dyes that generate a fluorescent dye which could be seen right away after the DNA electrophoresis utilizing a blue light trans illuminator or ultraviolet light. With an exclusion of the primers and DNA templates, Master Mix includes all of the components necessary for PCR.

3.2.6.1.2.3. PCR method protocol

For each gene, the PCR was carried out in a 25 µL response volume. Master mix tube has a (12.5 µL) volume, both reverse and forward primers, each requiring 1 µL, DNA template 2 µL, followed by 8.5 µL of sterilized PCR grade water, as shown in table (3.5).

Table 3.5 PCR cocktail for the all genes (25 µL) used in this work.

Components	Volume (µL)
PCR master mix	12.5
Primer F	1
Primer R	1
Genomic DNA template	2
PCR grade water	8.5

3.2.6.1.2.4. Procedure for the PCR technique

Table (3.6) shows the amplicon size and PCR program conditions for every gene under analysis. The 16S rRNA gene was amplified by utilizing DNA in the thermal cycler at 94°C for 5 minutes to completely denaturize the DNA templates before being used to identify *E. coli*. The following program was then used to continue the PCR: initial denaturation at 95°C for 5 min, followed by denaturation at 94°C for 30 seconds, annealing at 55°C for 1

minute and extension at 72°C for 1 minute, and these steps were repeated 35 cycles. With an final extension of 10 minutes at 72°C. Eventually, PCR tubes were kept at -20°C till the additional analysis (Ugbo *et al*., 2020).

3.2.6.1.3. Detection of ESBL genes of *E. coli*

Three ESBL genes (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX}$) were among those that were detected by PCR during the screening of all isolates used in this study, and the PCR programs for all three of these genes are described in Table (3.6).

Table 3.6 Amplified the ESBL genes in *E. coli* used in the PCR program.

Genes	Stages						
	Cycle No.	Denaturation		Annealing		Extension	
		Temperature	Time	Temperature	Time	Temperature	Time
bla$_{TEM}$	35	94°C	30 sec	50°C	30 sec	72°C	40 sec
bla$_{SHV}$	40	95°C	30 sec	50°C	30 sec	72°C	45 sec
bla$_{CTX}$	40	95°C	30 sec	58°C	30 sec	72°C	45 sec

3.2.6.1.4. Agarose gel electrophoresis

The following steps were taken to create agarose gel: 1.2 g of agarose was added to a 250 mL conical flask. The flask already included 100 mL of 1× TAE buffer. The mixture was then swirled to ensure thorough mixing. In a microwave, the mixture melted. Red-safe dye was added at 10 µL per 100 mL of agarose gel after it cooled to between 50 _55°C. Place the correct comb into the tray, then pour Agarose into the tank until it reaches approximately one centimeter deep. Allow the gel to set up at the temperature of the room. At the temperature of the room, the gel was permitted to solidify. Once the comb has

been gently eliminated, place the gel in the electrophoresis tank with the wells nearest the cathode end. A 1× TAE running buffer was used to cover the gel, ensuring it was submerged. The requested DNA specimens were loaded and pipetted up (0.2) volume of loading dye; after filling and emptying the pipette, a little time was performed for mixing the sample and loading dye. The prepared mixture is loaded into a well. The gel tank closed, the power supply turned on, and the gel ran at 5 Volts/cm agarose gel. After, the voltage was also raised to (75-100) voltages, and the electrophoresis probably proceeded for a sufficiently period. The gel motion is measured by reference to a marker loading stain. When the bromophenol blue ran 3/4 for the duration of the gel, the gel running stopped. The electrical current was switched off, UV at (240-366 nm) wavelength on a UV trans illuminator used for visualizing DNA bands, and the gel photographed with a Polaroid photo documentation camera (Hamasalih and Abdulrahman, 2020).

3.2.7. Antimicrobial Susceptibility tests

According to the producer's recommendations, susceptibility tests were run on the VITEK 2 compact system using AST-N326 cards. For illustration, the AST-N326 (BioMerieux) card had the following antibiotics: Piperacillin (PIP), Aztreonam (ATM), Ceftazidime (CAZ), Levofloxacin (LEV), Cefepime (CPM) Trimethoprim\Sulfamethoxazole (SXT), Ciprofloxacin (CIP), Tazobactam/piperacillin (TZP), Netilmicin (NET), Gentamicin (GEN), Amikacin (AK), Imipenem (IPM), Meropenem (MEM), Tigecycline (TIG), Tobramycin (TOB) and Tetracycline (TE). After being sealed and read, the cards were inserted with an inoculum containing 1.0×10^8 CFU/mL (made from the 0.5 McFarland suspension utilized with the identity cards). The VITEK 2 scheme automatically processes the antibiotic susceptibility cards until the MICs are acquired. End findings were calculated automatically after the results were interpreted utilizing VITEK 2 software.

3.3. Source of probiotic

The tested LAB strains, including *L. plantarum*, *L. casei*, *L. acidophilus*, and *S. thermophiles* have been taken gradually from the Ph.D. student (Midea Azeez) which were previously isolated from food for her projects and identified by molecular methods, 16S rRNA gene sequences of the strains submitted in GenBank with accession numbers *L. plantarum* OQ117233, *L. casei* OQ117232, *L. acidophilus* OQ130182 and *S. thermophiles* OQ130181 respectively.

3.3.1. The cell-free supernatant (CFS) extraction

Cell free extract of all LAB which utilized in this study was prepared according to (Azat *et al*., 2016) as follows: LAB was inoculated in MRS broth at 2% of broth volume and incubated in an anaerobic condition jar at 37°C for 48-72 hrs. After incubation, CFS was obtained by centrifuging the bacterial culture at 6000 rpm for 15 minutes.

3.3.2. Inhibitory effect of LAB against pathogenic bacteria *in vitro*

According to this method of (Vaughan *et al*., 1994), the antibacterial activity of the cell-free culture supernatant of isolated (against the *E. coli* isolates) was assessed using the agar well diffusion assay. In nutrient broth, an overnight culture of *E. coli* isolates was created. By streaking the swab over the surface of Muller Hinton agar, these bacteria (about 1.5x108 bacteria/ml, turbidity compared against McFarland), were inoculated. Wells sized (6 mm) were cut into the agar plate, and 50 µl of each cell-free culture supernatant (CFS) was poured into each well. The plates were incubated for 24 h at 37c° by utilizing candle Jar and inhibition of growth was examined by clear zone surrounding each well. The diameter of the growth inhibition zones was measured and recorded in millimeter (mm).

3.4. Analysis of the data using statistics

$Chi - square$ (Graph pad version 9.0.1) test was utilized to analyze the results of our study with (p-value<0.05) considered significant differences. Frequencies (number of isolates) and relative frequencies were employed to describe the data (percentages) statistically. Microsoft Excel 2016 was used for all statistical assessments (Microsoft Corporation, New York, USA).

4. RESULTS

4.1. Isolation of *Escherichia coli*

The total number of the analyzed cases was 54 isolates of *E. coli*, taken from patients who admitted to the laboratory with symptomatic UTIs and assembled for the studies. The mean age of cases was (29.86±0.932), ranging from 1 to 68 years old.

The majority of the sample was taken from females 33 (61.11%), and 21 (38.89%) specimens were collected from male cases. Depending on the age of the patients, 40 (74.07%) of the sample size are below 40 years, and the rest 14 patients (25.93%) are above 40 years old age, as shown in (Table 4.1).

Table 4.1. The age and gender distribution of the 54 positive Uropathogenic *E. coli* patients were approved by PCR test in the present study.

Characteristics of samples	Number (n)	Percentage %
Gender		
Female	33	61.11
Male	21	38.89
Age (years)		
<40	40	74.07
≥40	14	25.93

Regarding distribution according to age and gender, most of the female cases, 28 (70%), are in the group aged < 40 years, and in patients aged ≥ 40, most of them are males 9 (64.29%), statistica analysis using *Chi square* test regarding the gender of the positive cases approved by PCR test showed significand difference (p-value <0.0209), which is more detailed in (Figure 4.1).

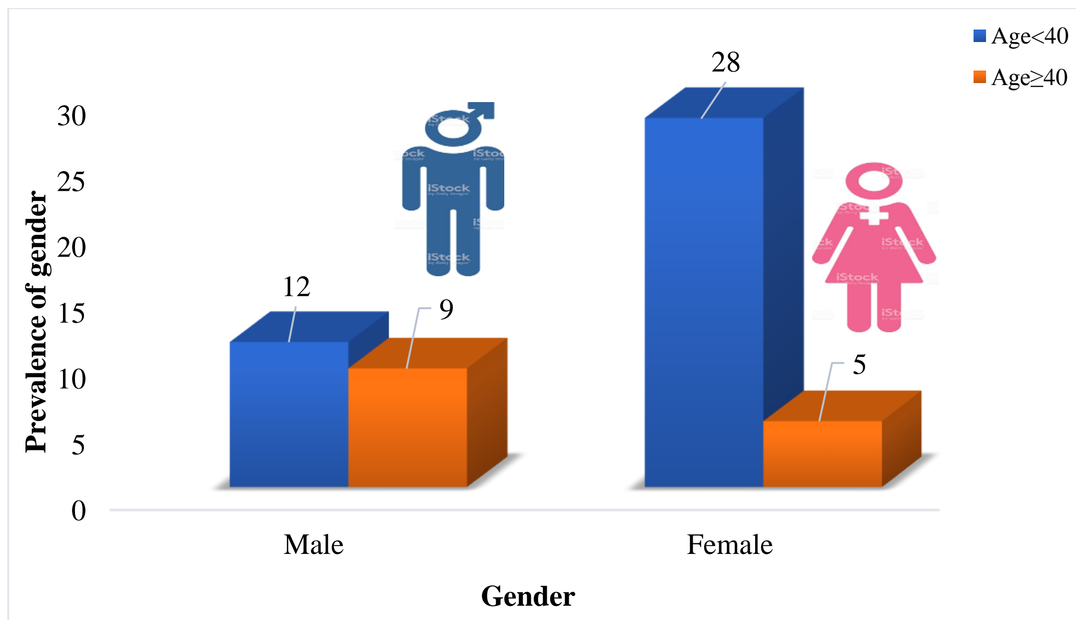
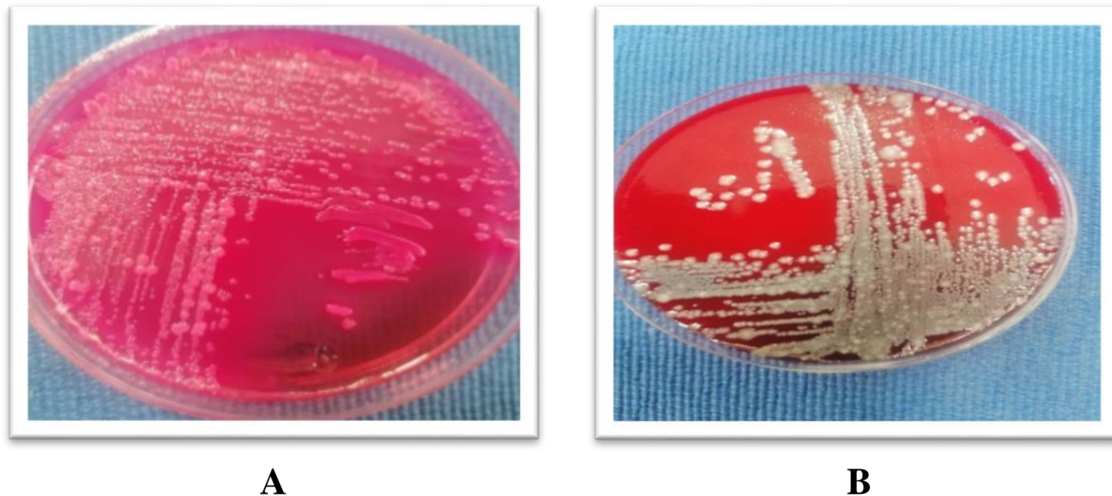


Figure 4.1. Distribution of Uropathogenic *E. coli* isolates according to age and gender.

4.2. Identification of Uropathogenic *E. coli* isolates

4.2.1. Phenotypical identification of Uropathogenic *E. coli*

Using morphological characteristics on MacConkey agar and Blood agar, all *E. coli* isolates on MacConkey agar medium were reidentified; microscopic analysis was used (gram stain, shape and arrangement of the cells), catalase, oxidase, IMVIC, and other biochemical features were also used to identify *E. coli* further. When the bacteria were stained with Gram stain under a microscope, they were observed as rod-shaped, gram-negative organisms (Figure 4.2). The biochemical tests analysis was performed to identify Uropathogenic *E. coli,* including positive tests for catalase, indole, and methyl red, and negative tests for Vogues Proskauer, citrate utilization and oxidase. For more confirmation of the identification of *E. coli* isolates used in this work, VITEK 2 system (Appendix 1) was used. All 54 isolates were confirmed and reidentified as *E. coli* bacterium.



A B

Figure 4.2. A. Uropathogenic *E. coli* has grown on MacConkey agar medium, B. Uropathogenic *E. coli* has grown on Blood agar medium.

4.2.2. Molecular characterization of Uropathogenic *Escherichia coli* isolates

In the present investigation, genomic DNA was extracted from the entire bacterial cells using the Clinic Cell SV small kit . Extracted genomic DNA was electrophoresed on 0.8% agarose gel to confirm the integrity of the isolated DNA (Figure 4.3).

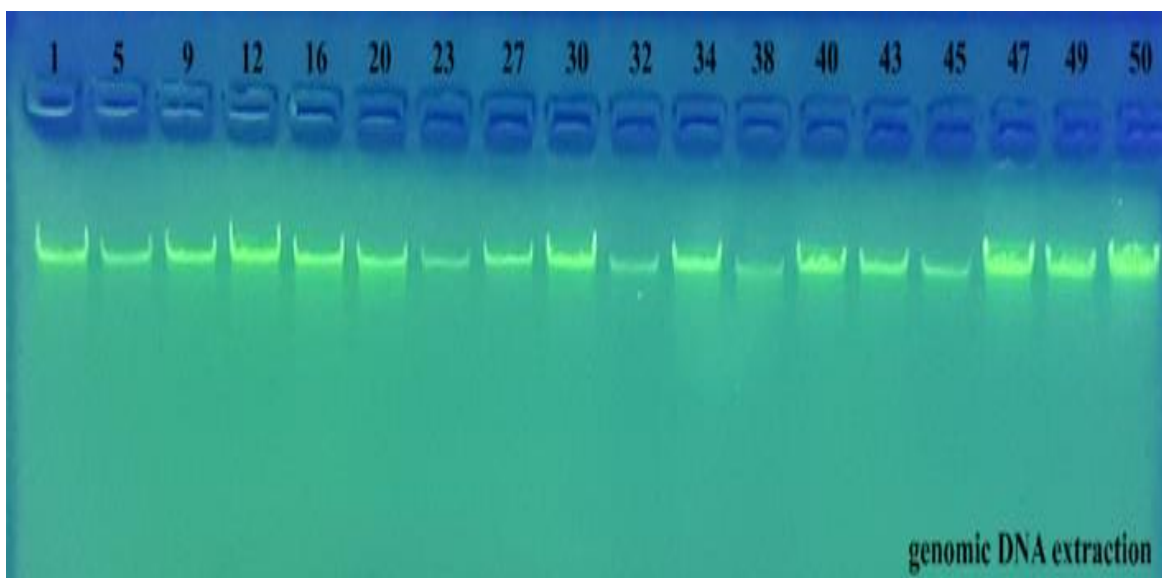


Figure 4.3. Agarose gel electrophoresis of the genomic DNA extract of Uropathogenic *E. coli* isolates has successfully extracted genomic DNA.

A PCR experiment was performed for every isolate to further identity of all isolates of *E. coli*, and precisely designed primers were utilized to detection between each strain. Regarding the result of the PCR test using 16S rRNA (797 bps) for determining the existence of the *E. coli*, and among isolates, 54 samples showed 100% positive for the present bands at 797 bps (Table 4.2). Regarding results of the PCR test using 16S rRNA for detecting the presence of the *E. coli* genome among 54 urine samples showed 54 (100%) as positive, as shown in (Figure 4.4).

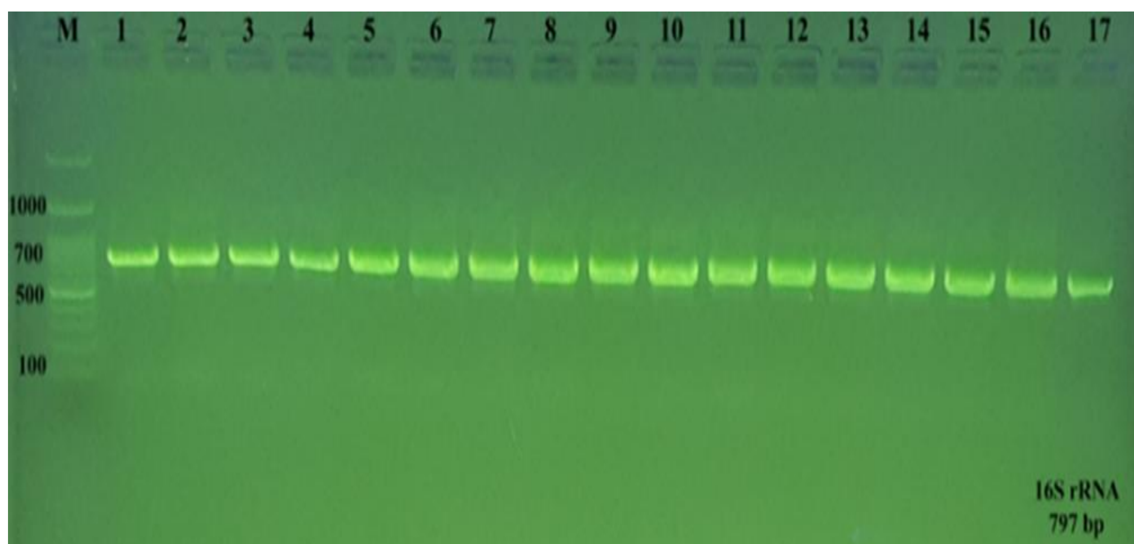


Figure 4.4. Agarose gel electrophoresis picture of 16S rRNA gene amplification (797 bps) for molecular detection of Uropathogenic *E. coli* isolates from clinical cases. M: DNA ladder (100 bp), 1-17: positive 16S rRNA gene samples.

4.3. Screening for extended-spectrum β–Lactamase genes in Uropathogenic *E. coli*

Detection of the β-lactamase genes was performed for all *E. coli* isolated. For this purpose, the bla_{CTX}, bla_{TEM} and bla_{SHV} genes were used to determine the ESBL genes among all isolated Uropathogenic *E. coli*.

Molecular detection of bla_{CTX} gene among Uropathogenic *E. coli* isolates

The findings of molecular detection were revealed in Figure (4.5) demonstrated that 26 isolates (48.15% positive) among 54 samples of Uropathogenic *E. coli* isolates were positive for the existence of bla_{CTX} gene at 517 bps (Table 4.2), these isolates were undergone a run-on gel electrophores.

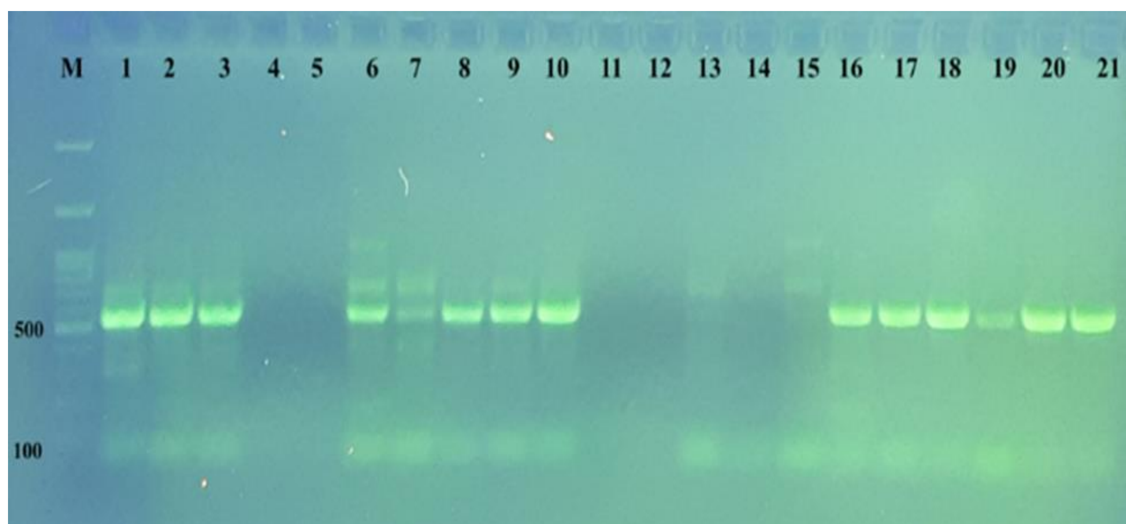


Figure 4.5. PCR amplification of bla_{CTX} gene of *E. coli* isolates, amplified product (517 bps) of Uropathogenic *E. coli* isolates represented by lanes (1, 2, 3, 6, 7, 8, 9, 10, 16, 17, 18, 19, 20 21). In contrast, the remain lanes (4, 5, 11, 12, 13, 14, 15) represent negative for the bla_{CTX} gene, M: ladder 100 bp.

Detection of bla_{TEM} gene among Uropathogenic *E. coli* isolates using PCR Technique

Consequences of molecular detection of the bla_{TEM} gene, as shown in Figure (4.6) indicated that 77.7% (42/54) of all isolates of Uropathogenic *E. coli* isolates were positive for the existence of the bla_{TEM} gene at 861 bps, these isolates were undergone run on gel electrophoresis.

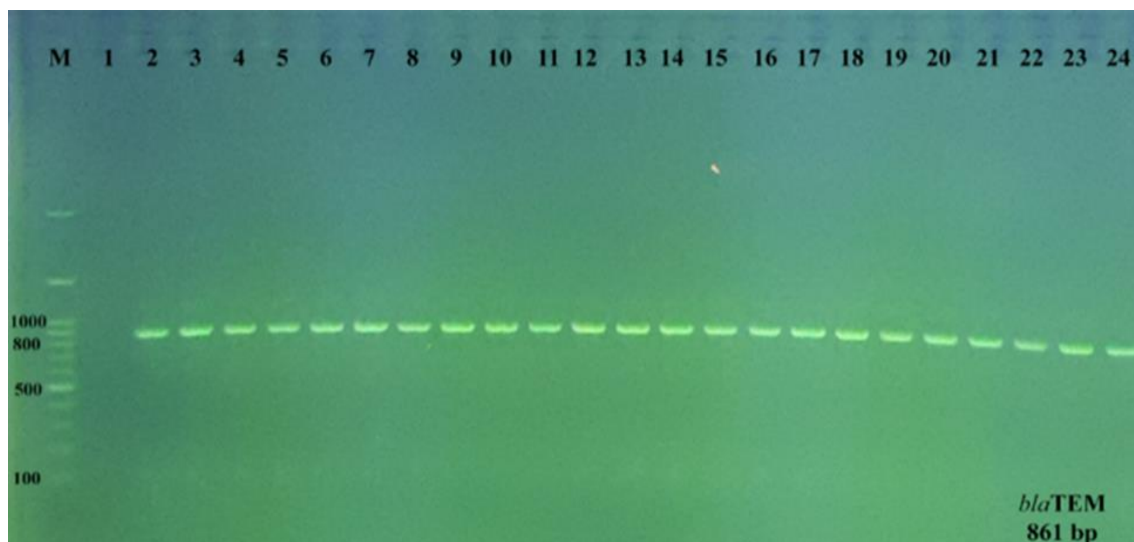


Figure 4.6. PCR amplification of *bla*$_{TEM}$ gene of Uropathogenic *E. coli* isolates, amplified product (861 bps) of Uropathogenic *E. coli* isolates represented by lanes from 2 to 24, while lane 1 was negative for *bla*$_{TEM}$ gene. M: ladder 100 bp.

The molecular detection of the *bla*$_{SHV}$ gene among Uropathogenic *E. coli* isolates

The consequences of molecular detection revealed in Figure (4.7) shows that 75.9% (41/54) of samples of Uropathogenic *E. coli* isolates were positive PCR products on gel electrophoresis for the *bla*$_{SHV}$ gene at 868 bps.

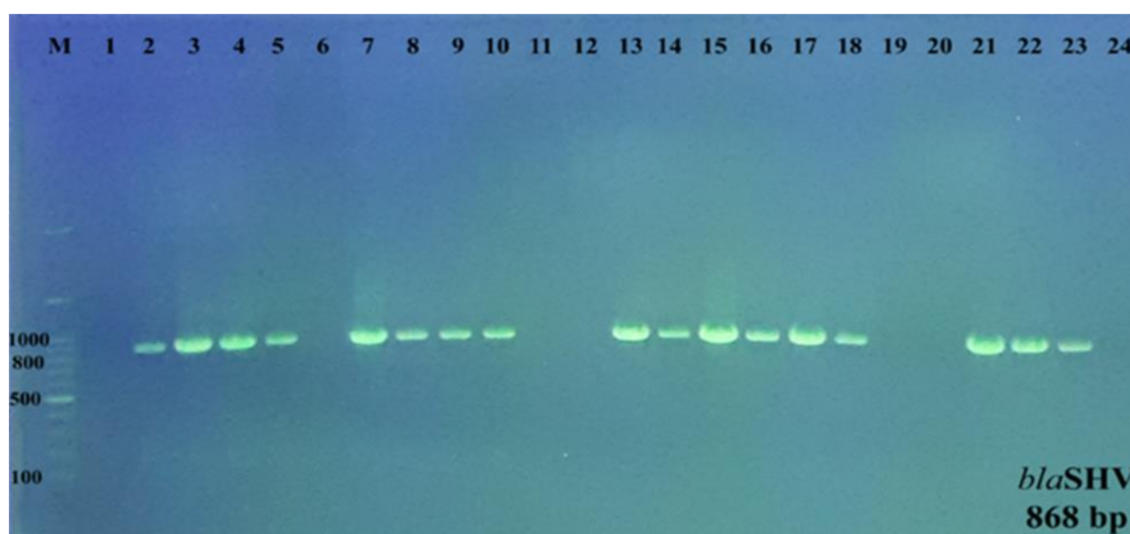


Figure 4.7. Amplification of *bla*$_{SHV}$ gene amplified using PCR test, amplified product (868 bps) of Uropathogenic *E. coli* isolates represented by lanes (2, 3, 4, 5, 7, 8, 9, 10, 13, 14, 15, 16, 17, 18, 21, 22, 23). In contrast, the negative for the *bla*$_{SHV}$ gene was represented in the following lanes (1, 6, 11, 12, 19, 20, 24), M: ladder 100 bp.

Results of the PCR approach using a specific primer to detect the *bla*$_{SHV}$ gene among 54 positive *E. coli* showed 41 (75.9%) positive, as shown in Figure (4.7). PCR assay results for the *bla*$_{TEM}$ gene showed 42 (77.7%) positive results, as shown in (Figure 4.6). And finally, the PCR test for the *bla*$_{CTX}$ gene showed 26 (48.15%) positive results, as shown in Figure (4.5).

Table 4.2. Prevalence of 16S rRNA and ESBL genes among Uropathogenic *E. coli* isolates.

Genes result	Studied genes				p-value
	16S rRNA	*bla*$_{CTX}$	*bla*$_{TEM}$	*bla*$_{SHV}$	
Positive bands	54 (100%)	26 (48.15%)	42 (77.7%)	41 (75.9%)	< 0.0001 (H.S)
Negative bands	0 (0%)	28 (51.85%)	12 (22.3%)	13 (24.07%)	

4.4. Antibiotic susceptibility profile of Uropathogenic *E. coli* isolates

All isolates demonstrated significant distinct resistant patterns to specific antimicrobials. Susceptibility of antimicrobials was carried out for 15 antimicrobial agents through the run on the VITEK 2 system using AST-N326 cards (Table 4.3). The highest resistant percentage of the isolates was recorded toward the Ceftazidime at 77.78% (*n*=42), followed by Cefepime (75.93%), Ciprofloxacin (70.37%), Piperacillin (68.52%), Tetracycline (64.81%), Aztreonam (62.96%), Trimethoprim\Sulfamethoxazole (53.70%), Gentamicin, Levofloxacin, and Tobramycin (37.04%), Piperacillin/Tazobactam (20.37%), Imipenem and Netilmicin (14.81%), and Meropenem (11.11%). The lowest resistant percentage (1.85%) was recorded against Amikacin antibiotic.

Table 4.3. Antibiotic resistance patterns of Uropathogenic *E. coli*.

Antibiotic	No. (%) of resistant strains	No. (%) of intermediate strains	No. (%) of sensitive strains
Amikacin	**1 (1.85)**	8 (14.81)	45 (83.33)
Aztreonam	34 (62.96)	0 (0.00)	20 (37.04)
Cefepime	41 (75.93)	1 (1.85)	12 (22.22)
Ceftazidime	**42 (77.78)**	0 (0.00)	12 (22.22)
Ciprofloxacin	38 (70.37)	3 (5.56)	13 (24.07)
Gentamicin	20 (37.04)	0 (0.00)	34 (62.96)
Imipenem	8 (14.81)	0 (0.00)	46 (85.19)
Levofloxacin	20 (37.04)	5 (9.26)	29 (53.70)
Meropenem	6 (11.11)	0 (0.00)	48 (88.89)
Netilmicin	8 (14.81)	4 (7.41)	42 (77.78)
Piperacillin	37 (68.52)	4 (7.41)	13 (24.07)
Piperacillin/Tazobactam	11 (20.37)	2 (3.70)	41 (75.93)
Tetracycline	35 (64.81)	0 (0.00)	19 (35.19)
Tobramycin	20 (37.04)	8 (14.81)	26 (48.15)
Trimethoprim\Sulfamethoxazole	29 (53.70)	0 (0.00)	25 (46.30)

4.5. Inhibitory effect of lactic acid bacteria on Uropathogenic *E. coli*

To assess the effect of specific LAB strains to inhibit the growth of Uropathogenic *E. coli*. After sub culturing the LABs between 48 and 72 hrs., on a medium containing MRS broth with inoculated (1%, v/v) cells from bacteria (OD_{600}=2.0) growing in MRS broth at 37°C incubated in anaerobic condition jar, LAB strain derived CFS was generated. To determine the influence of the CFS on Uropathogenic *E. coli* growth, *in vitro* culture batch was conducted by applying Nutrient broth which has a proven effect used to cultivate UPEC.

Four LAB strains had inhibition zones with diameters of all >16 mm, which significantly impeded the growth of most Uropathogenic *E. coli* strains. These four LAB strains (Table 4.4) were identified through 16S rRNA gene

using PCR assay as *L. plantarum*, *L. casei*, *L. acidophilus*, and *S. thermophilus*. Nevertheless, only four isolates of UPEC were not affected by these four LAB probiotics, and these Uropathogenic *E. coli* include isolate number 31, 39, 42 and 48, which haven't inhibited the growth of Uropathogenic *E. coli* strains (inhibition zones were zero mm in diameter) (Table 4.4). The results demonstrated that the tested LAB strains and the probiotic products have antibacterial activity against growing UPEC strains.

Table 4.4. Antimicrobial properties of the spent culture supernatant (SCS) broth from lactic acid bacteria against Uropathogenic *E. coli* isolates.

No. of isolates	*L. plantarum*	*L. casei*	*S. thermophilus*	*L. acidophilus*
1	++	+++	+	+++
2	+++	+++	++	+++
3	+++	+++	++	++
4	+++	+++	+++	+++
5	+++	+++	+++	+++
6	++	+++	++	+++
7	+++	+++	++	++
8	++	+++	++	+++
9	++	++	++	++
10	++	+++	++	+++
11	++	+++	++	++
12	++	++	++	++
13	++	+++	-	++
14	+++	+++	+++	+++
15	+++	+++	++	+++
16	+++	+++	+++	+++
17	++	+++	-	-
18	++	+++	-	-
19	++	+++	++	++
20	++	+++	++	++
21	++	+++	-	+
22	++	++	+++	+++
23	+++	+++	+++	++
24	+++	+++	++	++
25	++	++	-	-

26	+++	+++	+++	+++
27	++	+++	+++	+++
28	+++	+++	+++	+++
29	++	+++	++	++
30	+++	+++	+++	+++
31	**-**	**-**	**-**	**-**
32	++	+++	++	++
33	++	+++	++	++
34	++	+++	-	-
35	+++	+++	++	+++
36	++	+++	++	++
37	++	+++	++	+++
38	+++	+++	++	+++
39	**-**	**-**	**-**	**-**
40	++	+++	++	++
41	+++	+++	+++	+++
42	**-**	**-**	**-**	**-**
43	++	+++	++	++
44	+++	+++	+++	+++
45	+++	+++	+++	+++
46	+++	+++	+++	+++
47	+++	+++	+++	+++
48	**-**	**-**	**-**	**-**
49	+++	+++	+++	+++
50	+++	+++	+++	+++
51	+++	+++	+++	++
52	+++	+++	+++	+++
53	+++	+++	++	++
54	+++	+++	+++	+++

***: Sign denotes the degree of inhibition: Symbols represent zones of inhibition in mm: (-): no inhibition; (+): weak inhibition (≤7); (++) moderate inhibition (8-15); and (+++): strong inhibition (≥ 16**) (Sirichokchatchawan *et al*., 2018).

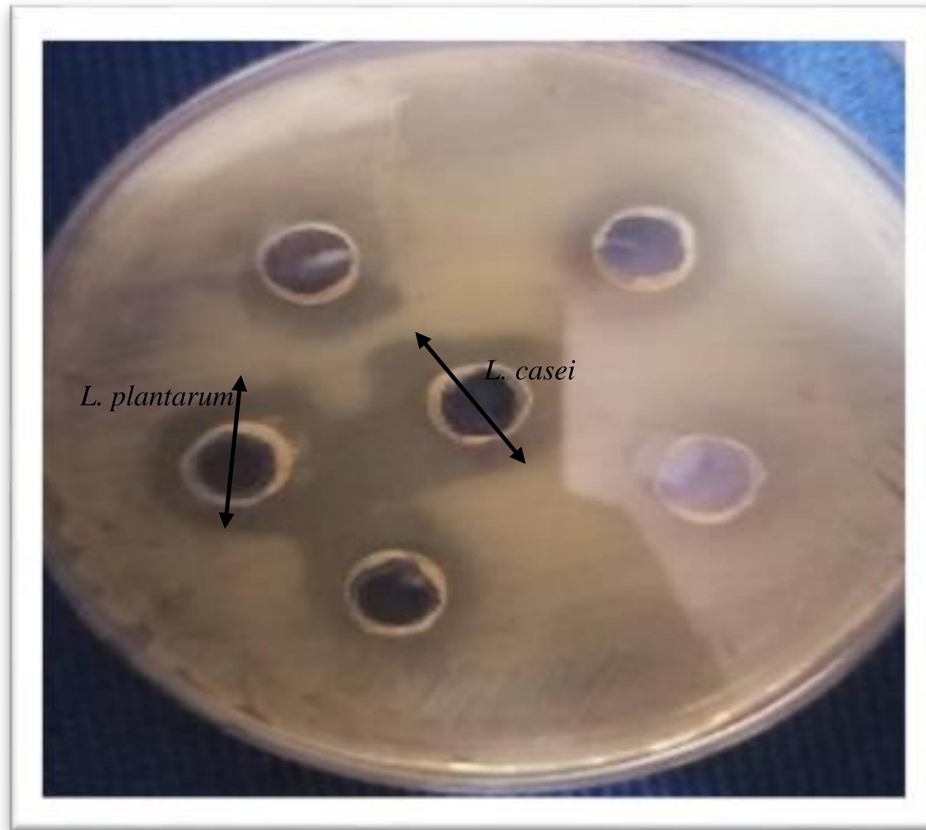


Figure 4.8. The inhibitory effect of cell-free culture supernatant of probiotics against Uropathogenic *E. coli* isolates.

4.6. Antagonism activity of probiotics against Uropathogenic *E. coli*

The antagonism activity of CFS of probiotics against UPEC isolates was evaluated utilizing the well diffusion method. The results of the inhibition zone on UPEC are stated in (Figure 4.9). The range of inhibition done diameter was 12.1-17.2 mm. The highest diameter of the inhibition zone was documented at 17.2 mm for *L. casei* against UPEC, and the lowest inhibition zone diameter (mm) was noted for *S. thermophilus* (12.1 mm).

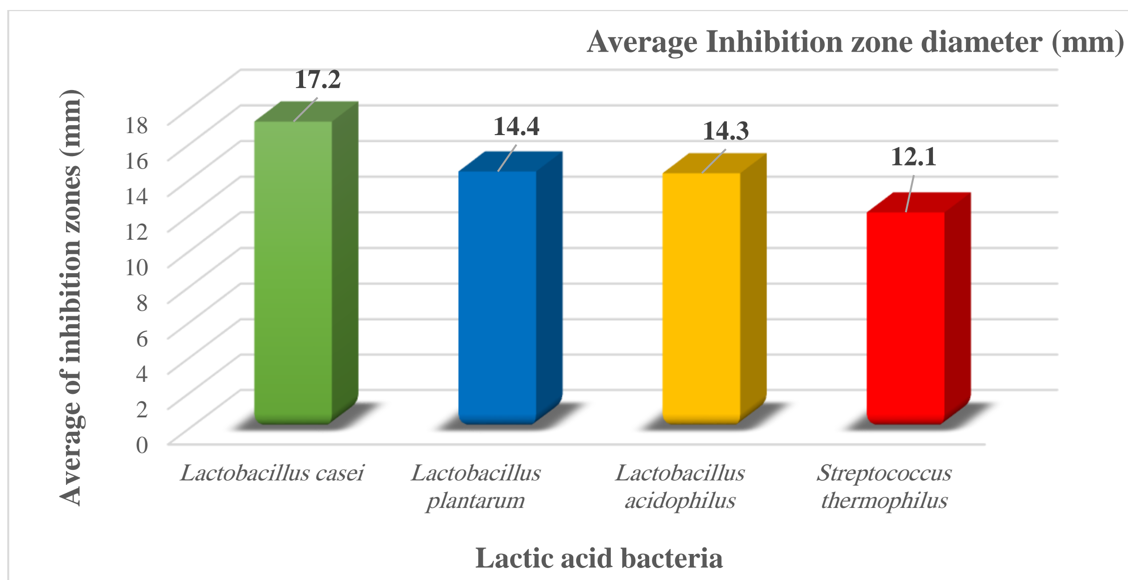


Figure 4.9. Antagonistic activity of cell-free supernatant of probiotics toward isolates of Uropathogenic *E.coli*.

5. DISCUSSION

UPEC is a class of pathogens that cause various illnesses and affect people at all ages worldwide. The rising prevalence of sickness caused by UPEC, the related expenditures, and the escalating challenges connected with the formation and dissemination of resistant to multiple drugs UPEC strains indicate an immediate need for an effective strategy against UPEC infection.

E. coli are members of the *Enterobacteriaceae* family; this is a common commensal microflora in humans and numerous other animals and is a gram-negative bacterium (Jena *et al*., 2017). This study considered *E. coli* because it's one of the most prevalent opportunistic bacteria. These were predominantly facultative anaerobes and commensal microbiota, and they have been linked to various types of infectious illnesses, which includes hemorrhage colitis, hemolytic uremic disorder, thrombotic in thrombocytopenic purpura, pain in the abdomen, vomiting, and diarrhea (Abimiku *et al*., 2019). Bacteria known as gram negative bacteria like *E. coli*, which are a major contributor to nosocomial and community bacterial infections, are highly researched pathogens. *E. coli* isolates may have been derived from either pathogenic or commensal samples. This research didn't examine *E. coli's* pathogenicity. Our focus was solely on *E. coli* generating ESBL (Tanko *et al*., 2021).

UTIs are frequently occurred infections in hospital where (29.9%) were hospital acquired infections. The prevalence rate of UTIs in female had a higher prevalence rate of UTIs (55.3%) than male (44.7%) because of the close proximity between vagina and anus, cystitis, sexual behavior, vaginal infections, pregnancy, diabetes mellitus, obesity and genetic sensitivity in female (Flores-Mireles *et al*., 2015). The incidence rate of illness was also discovered to be greater in the age 60 years and older. From the above evidence, it was obvious that UTIs were found to be more prone to older ages rather than

younger ages. It's due to the hormonal alterations and the gradual decrease in immune system response that occurs with aging both contribute to UTIs (Shrestha *et al*., 2019).

The bla_{TEM} enzyme family was identified as the most prevalent in this investigation. Several studies have revealed varying levels of dominance at various times and locations. Nevertheless, Egwuatu et al. (2019) reported no bla_{TEM}-type ESBL generation in their research in Nigeria. Bajpai *et al*. (2017) was demonstrated that the most prevalent enzymatic family, have been identified as bla_{TEM}, followed by bla_{CTX} and bla_{SHV} in India. Contrary to our findings, considerable research conducted during the past ten years has demonstrated that MDR UTIs and bloodstream infections are increasingly caused by Enterobacteriaceae-producing bla_{CTX}-type ESBLs (Hijazi *et al*., 2016). In our investigation, bla_{SHV} gene was shown to be the second most frequent gene at 75.93%, but bla_{CTX} was identified as the dominant gene in other research (Zeynudin *et al*., 2018, Abrar *et al*., 2019).

5.1. Isolation and identification of *E. coli* isolates

The total number of the analyzed samples was 54 isolates of *E. coli*, taken from patients who attended the laboratory with symptomatic UTIs and assembled for the studies. The cases ranged in age between 1 to 68 years, with a mean age of (29.86) years.

Most samples were taken from females; 33 (61.11%) and 21 (38.89%) specimens were collected from male cases. Depending on the age of the patients, 40 (74.07%) of the sample size are below 40 years, and the rest, 14 patients (25.93%), are above 40 years old age, as shown in Table (4.1). In the investigation of Halaji *et al.* (2020), a total of 111 isolates confirmed with UPEC isolated, with 69.6% (32/46) and 30.4% (14/46) of the isolates coming from female and male KTP (kidney transplant patient) patients, respectively,

and 69.2% (45/65) and 30.8 (20/65) from the female and male control group, respectively.

Two strains were identified from uterine scrapings, and 98 strains were obtained from urine samples by Prakapaite *et al*. (2019).

Eighty seven % of *E. coli* strains were obtained from females, while 13% were isolated from males. In all study groups, *E. coli*-related symptoms were more prevalent in females than males (p-value<0.05), indicating a sex-specific susceptibility to infection.

Regarding distribution according to age and gender, most of the female cases 28 (70%) are in the group aged < 40 years, and in patient aged ≥ 40, most of them are males 9 (64.29%), which is more detailed in Figure (4.1). Also, in the study of Prakapaite *et al*. (2019), among 87 females, the age of the patients ranged from 2 to 90 years. Among 13 males, the age ranged from 2 to 89 years. The median age of females was 33 years, while the observed median age among males was 44 years.

Urine samples from 780 research participants with urologic symptoms of UTI were cultured by Dadi *et al.* (2020), and they identified 200 isolates (25.6%) of *E. coli* were detected by biochemical analysis. There were 265 (34% male) and 515 (66% female) male participants in the study.

Using morphological characteristics on MacConkey agar and blood agar, all *E. coli* isolates on MacConkey agar and blood agar media were reidentified; microscopic analysis was used (gram stain, shape and arrangement of the cells), catalase, oxidase, IMVIC, and other biochemical features were also used to further identification of *E. coli*. When the bacteria were stained with Gram stain under a microscope, they became rod-shaped, gram-negative organisms (Figure 4.2). The biochemical tests analysis was performed to identify Uropathogenic *E. coli,* and yhe our results for biochemical tests revealed that all 54 isolates were positive for catalase, indole, and methyl red, and negative for Vogues Proskauer, citrate utilization and oxidase. For more identification of *E. coli*

isolates used in this study, VITEK 2 system was used, and all 54 isolates were confirmed and reidentified as *E. coli*.

A total of 50 urine samples were analyzed by Zahera *et al*. (2011), and 15 isolates were verified to be *E. coli* by Gram's staining and biochemical analysis. Compared to males, females had a higher rate of *E. coli* isolation in urine samples. All 15 *E. coli* isolates were shown positive results for Indole, methyl-red, catalase, and lactose fermentation; however, they were negative for Voges-Proskauer, citrate, and urease, confirming that these organisms are *E. coli*. Throughout the analysis of Zahera *et al*. (2011), we discovered that the majority of urine samples contained *E. coli*. Hence, they are the leading cause of UTIs. The discovery of this harmful bacterium, therefore, requires phenotypic characterization. The colonies on the LB agar plates were Gram-negative because they had picked up pink dye (i.e., counter stain) and exhibited Gram-negative rods under the microscope. All *E. coli* isolates were positive for the indole, methyl-red, and catalase tests, confirming that these organisms are *E. coli*.

In the investigation of Aldamarchi *et al*. (2017), among 180 patients with acute UTIs, *E. coli* was the underlying bacterium in 137 patients, whereas other microorganisms caused UTIs in the other 43 individuals. The development of the culture and identification of *E. coli* revealed that the colonies were soft and small. Approximately 1-3 mm in size, surrounded by a hemolytic zone (β-hemolysis) on Blood agar and MacConkey agar, the colonies were shiny, pink in color, small and soft, and approximately 1-2 mm in size. Based on the biochemical analysis, *E. coli* isolates were tested positive for maltose, catalase, eosin methylene blue, indole test, methyl red, gas production test, mannitol, and lactose.

Due to lactose fermentation, the obtained isolates generated rose-colored colonies when cultivated on MacConkey agar. Biochemical reactions, including methyl red and indole positivity, urea and citrate negativity, and

Motility Indole Ornithine positivity, confirmed that they were *E. coli* bacteria. From the 80 urine samples, 45 isolates of *E. coli* were found, and all isolates were examined with nine antibiotics using the disc diffusion method (CRO, AMP, CN, F, CIP, CXM, AK, AMC, CAZ). Thirteen UPEC isolates resisted at least one antibiotic in three or more antimicrobial classes (Dawwam *et al*., 2022).

5.2. Molecular characterization of *E. coli* isolates

The PCR was utilized for reidentifying all strains of *E. coli*, especially utilizing the specific unique primers. Regarding the result of the PCR test using 16S rRNA 797 for detecting the presence of the *E. coli* genome, 54 samples showed 100% positive, as shown in Table (4.2) and (Figure 4.3).

Tshabuse *et al*. (2022) were used a VITEK 2 compact technology for the identification of *E. coli* isolates; 135 isolates of *E. coli* were positively detected in all examined samples, which isolated from various clinical specimens.

The antimicrobial sensitivity pattern of was performed by Mohamed *et al*. (2020) for 128 isolates of *E. coli* from outpatients with urinary tract infections on five regularly administered antibiotic discs at Shaafi hospital. The majority of *E. coli* isolates were susceptible to Ciprofloxacin (67.6%) and Nitrofurantoin (85.3%), but all *E. coli* isolates exhibited strong resistance to Cephalosporins (Ceftriaxone (97.1%) and Cefixime (61.8%). Alarmedly, 88.2% of *E. coli* 30 isolates displayed resistance to two or more medications, indicating a significant likelihood of MDR *E. coli* in clinical isolates.

The susceptibility of *E. coli* strains toward ten antibiotics and the minimum inhibitory concentrations (MICs) of a hundred isolates of *E. coli* were performed by Wu *et al.* (2021). According to the CLSI standard, amoxicillin had the greatest rate of resistance of *E. coli* (85%), then Cefuroxime (65%) and Ceftriaxone (60%). In addition, 6% of the total isolated had been resistant to Amoxicillin/Clavulanic acid, and 5% were resistant to

Cefoperazone/Sulbactam. The incidence of resistance to Ceftazidime, Gentamicin, Ciprofloxacin, and Sulfonamides were correspondingly 31%, 33%, 33%, and 47%. All isolates were Meropenem-susceptible.

5.3. Antibiotic susceptibility profile of Uropathogenic *E. coli* isolates

All isolates demonstrated significant distinct resistant patterns to different antimicrobials. Antimicrobial susceptibility was carried out for 15 antimicrobials by running the VITEK 2 system using AST-N326 cards Table (4.3). The highest resistant percent of the isolates was recorded toward the Ceftazidime at 77.78% (n=42), followed by Cefepime (75.93%), Ciprofloxacin (70.37%), Piperacillin (68.52%), Tetracycline (64.81%), Aztreonam (62.96%), Trimethoprim\Sulfamethoxazole (53.70%), Gentamicin, Levofloxacin, and Tobramycin (37.04%), Piperacillin/Tazobactam (20.37%), Imipenem and Netilmicin (14.81%), and Meropenem (11.11%). The lowest resistant percentage (1.85%) was recorded against each Amikacin.

E. coli was found to be the most prevalent cause of UTI and was found to be extremely resistant to Norfloxacin 99 (62.3%), Cotrimoxazole 90 (56.6%), and Cefotaxime 82 (51.6%), with resistance patterns comparable to those identified in a previous study (Arsalane *et al*., 2015). There was a weak positive connection between biofilm generation and ESBL production (r = 0.157). 65.2%, 50%, and 54.3% of strong, moderate, and weak biofilm-producing E. coli, respectively, were ESBL producers. Tabasi *et al*. (2015) and Neupane *et al.* (2016) reported a positive link between biofilm and ESBL-producing *E. coli*. Research demonstrated that biofilm increases the transfer of the ESBL gene between *E. coli* and other microbes due to the matrix, which stabilizes and enhances the horizontal transferability of genetic elements and resists immune clearance (Maheshwari *et al*., 2016, Flemming *et al*., 2016).

The development of resistance in *E. coli* may be attributable to the hazards use of antibiotics, plasmid-mediated genes, such as $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{OXA},

etc., quorum sensing, etc. (Öztürk *et al*., 2015). The proliferation of MDR UPEC poses a grave danger to the management of UTIs, as well as an increase in treatment costs. The biofilm-forming microorganisms are responsive to co-therapy with macrolides, such as Erythromycin, Clarithromycin, and azithromycin, and other effective antibiotics as well as macrolides are regarded as dependable anti-biofilm agents (Jamal *et al*., 2018).

The findings of antibiotic susceptibility pattern were done by Halaji *et al*. (2020), that performed the disk diffusion method and demonstrated that more than 52% of UPEC strains were resistant to Trimethoprim/Sulfamethoxazole (84.8%), Nalidixic acid (78.3%), Cefixime (58.7%), Ciprofloxacin (54.3%), Norfloxacin (56.5%), and Ofloxacin (52.2%), while Carbapenems (100%), Amikacin (100%), and Nitrofurantoin (71.7%) were most effective in vitro antibiotics against UPEC isolates. The antimicrobial sensitivity profiles of 200 *E. coli* isolates were done by Dadi *et al*. (2020). Exposed to the same testing protocol revealed that ampicillin resistance was the strongest (86.5%), followed by Ceftazidime (84%), Ceftriaxone (80.5%), Tetracycline (80%), Trimethoprim-Sulfamethoxazole (68.5%), and Cefotaxime (66%).

5.4. Screening for extended-spectrum β–Lactamase genes in Uropathogenic *Escherichia coli*

Resistance to β-lactam antibiotics has grown in recent years among gram-negative bacteria isolated from clinical samples (Shayan and Bokaeian, 2015). The most common method of β-lactam resistance in Enterobacteriales, especially *E. coli*, is the development of ESBL or AmpC enzymes (Mehrabi, 2017). In this investigation, our data suggested that 67.083% of UPEC isolates from Erbil City produced ESBLs. Notwithstanding the variety of reported rates, our findings are consistent with the average of these studies' findings.

Detection of the β-lactamase genes was performed for all *E. coli* isolates. For this purpose, the bla_{CTX}, bla_{TEM} and bla_{SHV} genes were used to detect the

ESBL genes among all isolates of UPEC The findings of molecular detection illustrated in (Figure 4.4) indicated that 26 isolates (48.15% positive and 51.85% negative) among 54 samples of UPEC isolated had been positive for the existence of bla_{CTX} gene at 517 bps Table (4.2), and the results are shown in (Figure 4.4). Consequences of molecular detection of the bla_{TEM} gene shown in (Figure 4.5) indicated that 77.7% (42/54) of all isolates of UPEC isolates were positive for the presence of the bla_{TEM} gene at 861 bps. The findings of molecular detection illustrated in (Figure 4.6) show that 75.9% (41/54) of samples of Uropathogenic *E. coli* isolates were positive products of PCR for the bla_{SHV} gene at 868 bps.

Results of the PCR test using a specific primer to detect the bla_{SHV} gene among 54 positive *E. coli* showed 41 (75.9%) positive, as shown in (Figure 4.6). PCR test for the bla_{TEM} gene showed 42 (77.7%) positive results, as shown in Figure (4.5). And finally, the PCR test for the bla_{CTX} gene showed 26 (48.15%) positive results, as shown in (Figure 4.4).

Other nations with the same degree of heterogeneity were Brazil (7.1%), India (41.6%), Pakistan (40%), France (69.4%), and Nepal (91.7%) (Sadeghi *et al*., 2022). The difference observed in the prevalence of ESBL in UPEC isolates between Iraq and the rest of the world is likely attributable to differences in geographical distribution, infection control policies, sample source and size (Halaji *et al*., 2020). Comparing the results of this enzyme is hampered by the absence of a consistent phenotypic approach for identifying bacteria that produce AmpC (Sadeghi *et al*., 2022).

Through our investigation, the investigated ESBL genes were determined with nearly equal frequencies. Naziri *et al*. (2020) reported, among UPEC isolates, a high prevalence of bla_{SHV} (47.4%), followed by bla_{CTX} (37.2%) and bla_{TEM} (15.2%). In another study conducted in Kermanshah, the bla_{CTX} gene was more prevalent than the bla_{TEM} and bla_{SHV} ESBL genes among UPEC isolates (Mohajeri *et al*., 2014). In addition, a number of international studies,

including those conducted in Saudi Arabia, Vietnam, China, and Mexico, identified the *bla*$_{CTX}$, *bla*$_{SHV}$, and *bla*$_{TEM}$ genes as the most significant mechanisms of ESBL production in *E. coli* strains (Trang *et al*., 2013, Zhao *et al.,* 2015, Alyamani *et al.*, 2017, Ramírez-Castillo *et al.*, 2018)

The molecular analysis revealed that 53.2% (59/111), 45.0% (50/111), and 5.4% (6/111) of the isolates had the *bla*$_{CTX}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ genes, respectively. Moreover, three (2.7%) isolates contained all ESBL genes. Of 35 ESBL-positive isolates, (82.9%) included *bla*$_{CTX}$, followed by *bla*$_{TEM}$ (40%) and *bla*$_{SHV}$ (2.9%). *bla*$_{CTX}$ and *bla*$_{TEM}$ genes were significantly more prevalent in KTP than in the control group, while *bla*$_{CTX}$ was much more prevalent in ESBL-positive isolates than in ESBL-negative isolates. In addition, 29 (26.1%) isolates included both *bla*$_{CTX}$ and *bla*$_{TEM}$ (Halaji *et al*., 2020).

Twenty-three isolates were confirmed for ESBL-producing in UPEC isolates phenotypically by Catherine *et al*. (2022), and they recorded that all 23 isolates of Uropathogenic *E. coli* carried at least one ESBL gene. *bla*$_{TEM}$ and *bla*$_{CTX}$ were the most prevalent ESBL genes, found in 22 of 23 (95.6%) isolates, followed by *bla*$_{SHV}$ in 5 of 23 (21.7%) isolates. Five of 23 (21.7%) isolates included all three ESBL genes (*bla*$_{TEM}$, *bla*$_{CTX}$, and *bla*$_{SHV}$), whereas 16 of 23 (69.5%) isolates contained just the *bla*$_{CTX}$ and *bla*$_{TEM}$ genes. One of the 23 isolates encoded only *bla*$_{TEM}$, while another encoded only *bla*$_{CTX}$.

In the similar work of our findings, the main ESBL gene in the study of Tanko *et al*. (2021) was *bla*$_{TEM}$, found in 22 isolates (95.6%). The *bla*$_{SHV}$ gene was found in 9 isolates (39.1%), whereas *blaCTX* and *blaOXA* were found in 7 isolates each (30.4%).

5.5. Inhibitory effect of lactic acid bacteria on Uropathogenic *E. coli*

To assess the impact of selected LAB strains in terms of UPEC growth inhibition. LAB strain derived CFS was prepared after sub-culturing for 48-72 hrs., on MRS broth with inoculation (1%, v/v) of bacterial cells (OD600=2.0) grown in MRS broth at 37°C incubated in anaerobic condition jar. To investigate the impact of the CFS on uropathogenic *E. coli* growth, in vitro batch culture was performed using Nutrient broth proven to be effective in cultivating UPEC.

The diameters of the inhibition zones for four LAB strains were all greater than 16 mm, indicating that these LAB strains strongly inhibited the growth of most UPEC strains. These four strains of LAB in the (Table 4.4) were identified through 16S rRNA gene using PCR assay as *L. plantarum*, *L. casei*, *L. acidophilus*, and *S. thermophilus*. Nevertheless, they only four isolates of UPEC not affected by these four LAB probiotics and these UPEC include isolate number 31, 39, 42 and 48 which have not inhibited the growth of UPEC strains (inhibition zones were zero mm in diameter) Table (4.4). These results revealed that the screened LAB strains and the probiotic products possess certainly antimicrobial activity against the growth of UPEC strains.

Liu *et al*. (2016) investigated the antimicrobial activities of 366 LAB strains isolated from fermented foods. When they employed the UPEC strains BCRC 10675, 15585, and 15479 as indicators bacterial, 7 LAB strains (PM, PM68, PM20, PM78, PM206, RY2 and PM229) were more effective than other LAB strains at inhibiting the development of these bacteria. The zone of inhibition widths of five LAB strains was all greater than 23 mm, showing that those LAB strains prevented the growing of 3 pathogen UPEC strains. API 50CHL testing identified the seven organisms as *L. paracasei* (strain PM2), *L. salivarius* (strain PM78), *L. plantarum* (strains PM206 and PM229), *L. crispatus* (strain RY2), and *Pediococcus pentosaceus* (strains PM68 and

PM201). On the other hand, Zeynudin et al. (2018) used the LAB probiotics against ten isolates of *E. coli*. They found that eight of ten MDR *E. coli* isolates displayed sensitivity to *L. acidophilus*, while seven of ten isolates displayed sensitivity to *L. plantarum*. The range of the inhibitory zones is (16±0.04–23±0.05 mm). Also, Hashem and Rehab (2021) used an agar well diffusion assay to evaluate the antibacterial efficacies of 41 Lactobacilli supernatants against 5 MDR *E. coli* isolated. The average zone of inhibition diameters of the *E. coli* isolated were less than 9 millimeters when exposed to any of the Lactobacilli CFSs. About 22 (54%) out of 41 different CFSs of the tested Lactobacilli isolates exhibited the greatest inhibitory effects against the *E. coli* isolated that were of concern, having inhibitory zone diameters between 15 and 18 mm. The effectiveness of these 22 different *Lactobacillus* CFSs as antibiofilm agents was evaluated.

5.6. Antagonism activity of probiotics against Uropathogenic *E. coli*

The antagonist activity of CFS of probiotics against UPEC isolates was evaluated utilizing the well diffusion method. The results of the inhibition zone on Uropathogenic *E. coli* are reported in (Figure 4.**9**). The range of inhibition done diameter was (12.1-17.2 mm). The highest diameter of the inhibition zone was documented at (17.2 mm) for *L. casei* against UPEC, while the lowest inhibition zone diameter (mm) was noted for *S. thermophilus* (12.1 mm).

The inhibitory activity of CFs from Lactobacilli were performed by Naderi *et al.* (2014) against isolated UPEC and was measured utilizing the agar well diffusion method. These CFs were derived from Lactobacilli. After choosing strains that are isolated with a broad range of antibacterial resistance as well as common *Lactobacillus* species, like *L. acidophilus* ATCC 3456, *L. casei* ATCC 39392 as well as *L. rhamnosus* ATCC 7469, the activity of antagonists was assessed utilizing the well diffusion technique. There was no evidence of antagonistic action on the part of lactobacilli strains opposed to *K.*

pneumoniae, *Enterococcus* species, or *Enterobacter* species. Only *E. coli* strains resistant to eight or nine different antibiotics exhibited an antibacterial impact from the CFs of probiotic lactobacilli when tested using the well diffusion method. *L. casei* produced an inhibitory zone for *E. coli* that was the largest. To verify these findings, ten strains of *E. coli* resistant to 8 or 9 different antimicrobial drugs were tested for the minimal inhibitory concentrations of lactobacilli cultured supernatants. The *L. casei* ATCC 39392 supernatant was more efficient since its MIC was lesser than that of the other two species of bacteria. Additionally, an implicit association existed among the pH values that were measured as a reaction to the generation of acid and the antibacterial activity of the probiotics utilized in the study.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

1. The main family of enzyme genes found in *E. coli* isolates found was indicated by the $bla_{TEM.}$

2. The findings presented here demonstrate that the LAB we selected are potential strains for probiotic utilize.

3. These types of probiotics reveal antagonist activity against UPEC infectious *in vitro* and recommend that these probiotics could be effectively utilized to avoid UTIs.

4. According to the results, the significant rate of ESBL-producing UPEC isolates indicates the require for infection control policies to stop the further spread of resistant strains.

6.2. Recommendations

1. Find the relationship among antibiotic-resistant and ESBL genes in UPEC.

2. Detection of a correlation between the ESBL genes and biofilm formation among isolated UPEC.

3. Determine the effect of LAB probiotics on ESBL gene expression of UPEC.

4. Using experiment animals to evaluate the *in vivo* potential of LAB probiotics on UPEC.

APPENDICES

Appendix 1. Laboratory report for *Escherichia coli* identification using VITEK 2 system GN card.

bioMérieux Customer:
System #:

Laboratory Report

Printed Dec 13, 2021 16:26 CST
Autoprint

Patient Name: manal162737, manal
Isolate: manal162737-1 (To be reviewed)

Patient ID: manal162737

Card Type: GN Bar Code: 2411607403275374 Testing Instrument: 000019E20389 (17980)
Card Type: AST-N326 Bar Code: 7661729403247978 Testing Instrument: 000019E20389 (17980)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0405610444006610
Organism Quantity:

Selected Organism: Escherichia coli

Comments:	

Identification Information	**Card:**	GN	**Lot Number:**	2411607403	**Expires:**	Apr 21, 2022 13:00 CDT
	Completed:	Dec 13, 2021 11:13 CST	**Status:**	Final	**Analysis Time:**	3.87 hours
Organism Origin	VITEK 2					
Selected Organism	99% Probability		**Escherichia coli**			
	Bionumber:	0405610444006610			**Confidence:**	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages:						
Contraindicating Typical Biopattern(s)						

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Phenotypic

Therapeutic Interpretation Guideline: PHENOTYPIC
AES Parameter Last Modified: Sep 2, 2020 09:02 CDT

Page 1 of 2

Appendix 2. Laboratory report of antimicrobial susceptibility test using VITEK 2 system AST-N326 card.

bioMérieux Customer: | Laboratory Report | Printed Dec 13, 2021 16:26 CS
System #: | | Autoprin

Patient Name: manal162737, manal — Patient ID: manal162737
Isolate: manal162737-1 (To be reviewed)

Card Type: GN Bar Code: 2411607403275374 Testing Instrument: 000019E20389 (17980)
Card Type: AST-N326 Bar Code: 7661729403247978 Testing Instrument: 000019E20389 (17980)
Setup Technologist: Laboratory Administrator(Labadmin)

Bionumber: 0405610444006610
Organism Quantity: **Selected Organism: Escherichia coli**

Susceptibility Information	Card:	AST-N326	Lot Number:	7661729403	Expires:	Aug 21, 2022 13:00 CDT
	Completed:	Dec 13, 2021 16:26 CST	Status:	Final	Analysis Time:	9.10 hours

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Piperacillin	8	*R	Netilmicin	<= 1	S
Piperacillin/Tazobactam	<= 4	S	Tobramycin	<= 1	S
Ceftazidime	2	*R	Ciprofloxacin	0.5	S
Cefepime	<= 0.12	*R	Levofloxacin	1	S
Aztreonam	<= 1	*R	Tetracycline	<= 1	S
Imipenem	<= 0.25	S	Tigecycline	<= 0.5	S
Meropenem	<= 0.25	S	Colistin		
Amikacin	<= 2	S	Trimethoprim/Sulfamethoxazole	<= 20	S
Gentamicin	<= 1	S			

+= Deduced drug *= AES modified **= User modified

AES Findings:	Last Modified: Sep 2, 2020 09:02 CDT	Parameter Set: Global CLSI-based+Phenotypic
Confidence Level:	Consistent	

Installed VITEK 2 Systems Version: 08.01
MIC Interpretation Guideline: Global CLSI-based
AES Parameter Set Name: Global CLSI-based+Phenotypic

Therapeutic Interpretation Guideline: PHENOTYPIC
AES Parameter Last Modified: Sep 2, 2020 09:02 CDT

Page 2 of 2

Appendix 3. Acceptance letter of the manuscript in *Diyala Journal of Medicine*.

Manuscript Code No: DJM2023/7200102
No: 720
Date: 20/2/2023

Letter of Acceptance

Title: Molecular detection of blaTEM, blaSHV, and blaCTX-M genes among Uropathogenic Escherichia coli isolated from cases with urinary tract infection in Erbil city-Iraq

Author(s): Kharman K Ahmed, Aumed A Hawezy

This is to certify that the above mentioned manuscript has been accepted for publication in Diyala Journal of Medicine. This work is currently citable by using the Digital Object Identifier (DOI). The authors are responsible for the content of this Accepted Article.

Date of Received: 2/1/2023
Date of Acceptance: 20/2/2023

Editor Manage
Dr. Anfal Shakir Motib

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پوختە

هەوکردنی ڕێڕەوی میز بەناوبانگترین جۆری نەخۆشییە بەکتریایە دەستنیشانکراوەکانە و زۆرترین بەکتریا کە بەرپرسن لە هەوکردنی بۆری میز بریتین لە بەکتریای چیلکەیی کۆلای. زۆرترین بەرگری بۆ بەکتریا گرام نێگەتیڤەکان بەرامبەر بە دژەزیندەییەکانی بێتا-لاکتام بە تایبەتی لە بەکتریای چیلکەیی کۆلایدا بریتین لەئەنزیمی بێتا-لاکتامازەکان. لە ئێستادا، ژمارەی ئەو نەخۆشانەی کە تووشی بەکتریای چیلکەیی کۆلای کە هۆکاری دروستکردنی بێتا-لاکتاماز سپێکتریمی درێژکراوەن بەرز بووەوە و بە کێشەیەکی تەندروستی سەرەکی لە ئاستی جیهانیدا دادەنرێت. ئەم توێژینەوەیە ئامانجی هەڵسەنگاندنی چالاکیی دژە میکرۆبی بەکتریا تایبەتەکانی ترشی لاکتیک دژی بەکتریای چیلکەیی کۆلای بۆ خۆپاراستن و چارەسەرکردنی هەوکردنی ڕێڕەوی میز.

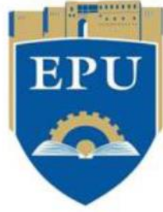
توێژینەوەکەمان پێکهاتبوو لە کۆکردنەوەی ٥٤ نمونەی میزی ناوەڕاست لە نەخۆشانی تووشبوو بە نیشانەکانی هەوکردنی ڕێڕەوی میز، لە گروپی تەمەنی جیاواز، لە بەشی دەرەوەی نەخۆشخانە لە نەخۆشخانەکانی هەولێر لە ماوەی ١ ی تشرینی یەکەمی ٢٠٢١ تا ١ ی نیسانی ٢٠٢٢ بۆ جیاکردنەوە و ناسینەوەی بەکتریای چیلکەیی کۆلای. دابەشبوونی جیاکراوەکان لە نێوان ڕەگەزەکاندا بەشێوەیەک بو کە ڕەگەزی مێ ٣٣ جیاکراوە (٦١.١١%) وە ڕێژەیەکی زیاتری هەوکردنی ڕێڕەوی میزیان هەیە لە چاو ڕەگەزی نێر کە ٢١ جیاکراوە (٣٨.٨٩%) بوو.

کاتێک کە ئامادەیی دژەزیندەیی تاقیکرایەوە لە دژی جیاکراوەکانی بەکتریای چیلکەیی کۆلای بە بەکارهێنانی پانێڵی VITEK 2 (AST). هەر یەکێک لە جیاکراوەکان بەرگری فرەیان هەبووە، لە کارەماندا جیاکراوەکان بەرزترین ڕێژەی بەرگرییان بەرامبەر بە سێفتازیدایم (٧٧.٧٨%)، سیفیپیم (٧٥.٩٣%)، سیپرۆفلۆکساسین (٧٠.٣٧%)، پیپەراسیلین (٦٨.٥٢%)، لە کاتێکدا جیاکراوەکان بەرزترین ڕێژەی هەستیاریان هەبوو بۆ مێرۆپینیم (٨٨.٨٩%)، ئیمیپێنم (٨٥.١٩%)، ئەمیکاسین (٨٣.٣٣%)، نێتیلمیسین (٧٧.٧٨%). سەرەڕای ئەوەش، دۆزینەوەکان دەریانخست کە هەندێک بەرگری دژە زیندەیی لە بەکتریای چیلکەیی کۆلای گەشەی کردووە. بۆیە تێگەیشتن لە هۆکاری بەکتریا و شێوازەکانی ئامادەیی لە جیاکراوەکان پێویستە بۆ دەستنیشانکردنی هەر گۆڕانکارییەکی ئەگەری.

جینەکانی *bla*$_{CTX}$، *bla*$_{TEM}$، *bla*$_{SHV}$ لە بە بەکارهێنانی ئامێری PCR پشکنینیان بۆ کرا بۆ دیاریکردنی سێ فۆرمی جیاوازی ئەنزیمی بێتا-لاکتامازەکان. بۆ هەموو جیاکراوەکانی بەکتریای چیلکەیی کۆلای ناسێنرانەوە بە بەکارهێنانی جینی 16S rRNA (قەبارەی ئەمپلیکۆن 797 bps) بە ڕێژەی (١٠٠%). لەم تاقیکردنەوەیەدا تۆمار کرا کە *bla*$_{SHV}$ لە ٤١ جیاکراوە (٧٥.٤%) لە جیاکراوەکانی بەکتریای چیلکەیی کۆلای هەڵگری ئەم جینەن، لە کاتێکدا جینی *bla*$_{TEM}$ کە زۆرترین

ئەنجامەکەی دەریخستووە کە لە ٤٢ جیاکراوە (%٧٧.٧) ئەم جینە بوونی هەیە، بەڵام جینی bla_{CTX} تەنها لە ٢٦ جیاکراوە (%٤٨.١٥) هەبووە.

گشت جیاکراوەکانی بەکتریای چیلکەیی کۆلای (٥٤ جیاکراوە) لەلایەن ٤ جۆری جیاوازی بەکتریای پرۆبایۆتیکەوە خرایە ژێر چالاکیی دژەگەشە کردن. ئەنجامەکان دەریانخست کە زۆربەی جۆرەکانی پرۆبایۆتیک چالاکیی ڕێگریبیان هەیە لە دژی گەشەکردنی جیاکراوەکانی بەکتریای چیلکەیی کۆلای کە تاقیکراونەتەوە، لەگەڵ تیرەی ناوچەی ڕێگریکردن لە گەشە کردن لە زۆرترین ئاستی خۆیدا (١٧.٢ ملم) کاتێک پرۆبایۆتیکی لاکتۆباسیلۆس کاسی بەکارهێنرا، لە کاتێکدا سترێپتۆکۆکۆس تەرمۆفیلەکان ناوچەی ڕێگریکردنیان هەبووە بە تیرەی (١٢.١) ملم و چالاکیی ڕێگریکەری کەمتری لە دژی جیاکراوەکانی بەکتریای چیلکەیی کۆلای نیشان دا.



زانكۆی پۆلیتەكنیكی هەولێر
ERBIL POLYTECHNIC UNIVERSITY

دیاریكردنی گەردیلەیی بەكتریای چیلكەیی كۆلای لە نەخۆشی میزەڵدان و بەكارهێنانی پرۆبایۆتیكە جیاوازەكان بۆ ڕێگریكردن لە گەشەكردنیان لە ناو تیوبی تاقیگەیی

نامەیەكە

پێشكەش بە ئەنجومەنی كۆلێژی تەكنیكی تەندروستی و پزیشكی هەولێر لە زانكۆی پۆلیتەكنیكی هەولێر كراوە وەك بەشێك لە مەرجەكانی بەدەستهێنانی بڕوانامەی ماستەر لە شیكاری نەخۆشیەكان

لەلایەن

خەرمان كاك احمد احمد
بەكالۆریۆس لە تەكنەلۆژیای تاقیگەی پزیشكی

بە سەرپەرشتی

پرۆفیسۆری یاریدەدەر م. ئومێد ئەرشەد حەوێزی
مایكرۆبایۆلۆجی پزیشكی

هەولێر، كوردستان
٢٠٢٣

خەرمانان ٢٧٢٣ | المُحَرَّم ١٤٤٥ | تەمووز ٢٠٢٣